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The Status of Dwarfed Populations of Short-Horned Lizards (*Phrynosoma hernandesi*) and Great Plains Toads (*Anaxyrus cognatus*) in the San Luis Valley, Colorado

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THE STATUS OF DWARFED POPULATIONS OF SHORT-HORNED LIZARDS (*PHRYNOSOMA HERNANDESI*) AND GREAT PLAINS TOADS (*ANAXYRUS COGNATUS*) IN THE SAN LUIS VALLEY, COLORADO

by

Megan E. Lahti

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Biology

Approved:

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UTAH STATE UNIVERSITY
Logan, Utah

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ABSTRACT

The Status of Dwarfed Populations of Short-horned Lizards (*Phrynosoma hernandesi*)
and Great Plains Toads (*Anaxyrus cognatus*) in the San Luis Valley, Colorado.

by

Megan E. Lahti, Doctor of Philosophy

Utah State University, 2010

Major Professor: Dr. Edmund D. Brodie Jr.
Department: Biology

The San Luis Valley is a large valley formation in Colorado surrounded on either side by mountain ranges exceeding 4,267 m. Within the Valley, two of the 14 amphibian and reptile species are dwarfed: the short-horned lizard (*Phrynosoma hernandesi*) and the Great Plains toad (*Anaxyrus cognatus*). Since its initial reporting in 1968 and confirmation in 1981, no research further investigating this dwarfism has been conducted. I collected morphological measurements to determine the extent and patterns of dwarfism of both species. I then investigated the genetics of both species using mitochondrial DNA to determine whether they are genetically distinct, their colonization histories within the Valley, and whether the Valley functions as a reproductive barrier. Lastly, I report life/natural-history data to determine the effects of dwarfism. *Phrynosoma hernandesi* and *A. cognatus* were significantly dwarfed and showed an increase in sexual size dimorphism compared to populations surrounding the Valley. Valley populations of *P. hernandesi* show high amounts of genetic divergence from populations surrounding the Valley while *A. cognatus* shows minimal genetic variation throughout its range. Based on the variable distribution of genetic variation in the Valley, historic climate patterns, and fossil records, there are two most likely colonization histories for *P. hernandesi*: 1.) populations colonized the Valley during a singular event and have since diverged or 2.) populations colonized the Valley during two events that correlate with the two warm, dry periods within the last 0.8 MYA. Dwarfed *P. hernandesi* consumed diets similar to populations outside the Valley although there is local variation in the diversity of prey items consumed. *Phrynosoma hernandesi* at Zapata Ranch showed annual variation in body size and morphology while population dynamics correlate with the timing of preciptation. Also, females show a reduced reproductive output, producing fewer neonates but of equal size to non-dwarfed neonates. Collectively, findings from this study suggest that Valley populations represent unique taxa and should be considered for further genetic study to determine their taxonomic and conservation status.

(199 pages)

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In Stebbin's Western Field Guide to Reptiles and Amphibians, a short sentence at the end of the *Phrynosoma hernandesi* species account reads, "Population of small (dwarfed) individuals inhabits San Luis Valley, s. Colo." The day I came across this is the day I chose my research project. I would like to thank Dr. Edmund Brodie Jr., "Doc," for supporting my admittedly biased interest in this project, with the understanding that if nothing came from it, then "there's always newts and garter snakes." I would also like to thank Doc for his many words of wisdom and guidance over the past 4 years. I have learned that few words can say many things.

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Megan E. Lahti

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CHAPTER 1

INTRODUCTION

Body size is among the most important of determinants of an organism's function and ecological role and is dictated by a combination of environmental and genetic factors (Peters, 1983; Hanken and Wake, 1993). Environmental dwarfism is a shift in body size as a result of an organism's habitat and examples include island species that are limited in size by prey availability or the presence of a competitor (MacArthur and Wilson, 1967). Genetic dwarfism is a shift in body size as a result of genetic variability that is influenced by and a product of ontogeny and phylogeny (Gould, 1966; Endler, 1977). Regardless of the underlying mechanisms, body size reduction has direct impacts on the performance, physiology, ecology, life/natural-history, and behavior of an organism (Hanken and Wake, 1993; Garland and Losos, 1994). For instance, smaller organisms generally have higher metabolic rates and faster heating and cooling capacities due to an increase in surface area (Calder, 1984; Schmidt-Nielsen, 1984). Similarly, the cost of body size for reproductive output can include fewer and/or smaller offspring.

Intraspecific divergence in body size can be facilitated by biotic and/or abiotic barriers that result in reproductive isolation (MacArthur and Wilson, 1967). Island biogeography is the most prevalent study system for reproductively isolated systems, including most notably Darwin's finches on the Galapagos islands and *Anolis* lizards in the Caribbean islands. On mainland ecosystems, dwarfism is minimal simply because reproductive barriers are typically lacking; however, for species specialized to a local habitat or with low mobility across the landscape isolation, is possible.

Evolutionary histories are often intertwined with geologic formations, particularly if the geology forms a reproductive barrier, and can provide insight to the history of the formation of a novel lineage.

The San Luis Valley (the Valley) is located in south-central Colorado and north-central New Mexico (Fig. 1-1). The Valley is currently formed by mountain ranges exceeding 4,267 m along the eastern (Sangre de Cristo mountain range) and western boundaries (San Juan mountain range) and is the origin for the Rio Grande River. Within the Valley, two species are dwarfed: *Phrynosoma hernandesi* (short-horned lizard) and *Anaxyrus cognatus* (Great Plains toad) (Fig. 1-2). Although they are sympatric with other reptiles and amphibians in the Valley, dwarfism is only reported in these two species (Hahn, 1968). The Valley potentially represents a reproductive barrier for these two species and understanding the geologic history of the Valley's formation in conjunction with the genetic structure across the landscape will provide a better understanding the occurrence of these dwarfed populations.

The San Luis Valley is an extensional fault-bounded basin within the Rio Grande Rift, a geologic feature that resulted from the spreading of the earth's lithosphere and subsequent formation of rift basins that began during the Miocene (~26-5 MYA) (Kluth and Schaftenaar, 1994). Since the late Oligocene (~20-23 MYA), sediments primarily eroded from the San Juan Mountains have been filling the Valley floor to a maximum depth of 6,400 m (Kluth and Schaftenaar, 1994). Currently, the Valley spans a maximum of 240 km length and 7,120 km width and mountain ranges exceeding 4,267 m form the boundary of this expansive basin.

Fig. 1-1. The San Luis Valley (the Valley), Colorado. The Valley formed in response to rifting during the Miocene (~30 MYA) and mountain ranges exceeding 4,267 m currently form the Valley’s boundary to the east (Sangre de Cristo mountain range) and west (San Juan mountain range). The Rio Grand River headwaters originate within the Valley.

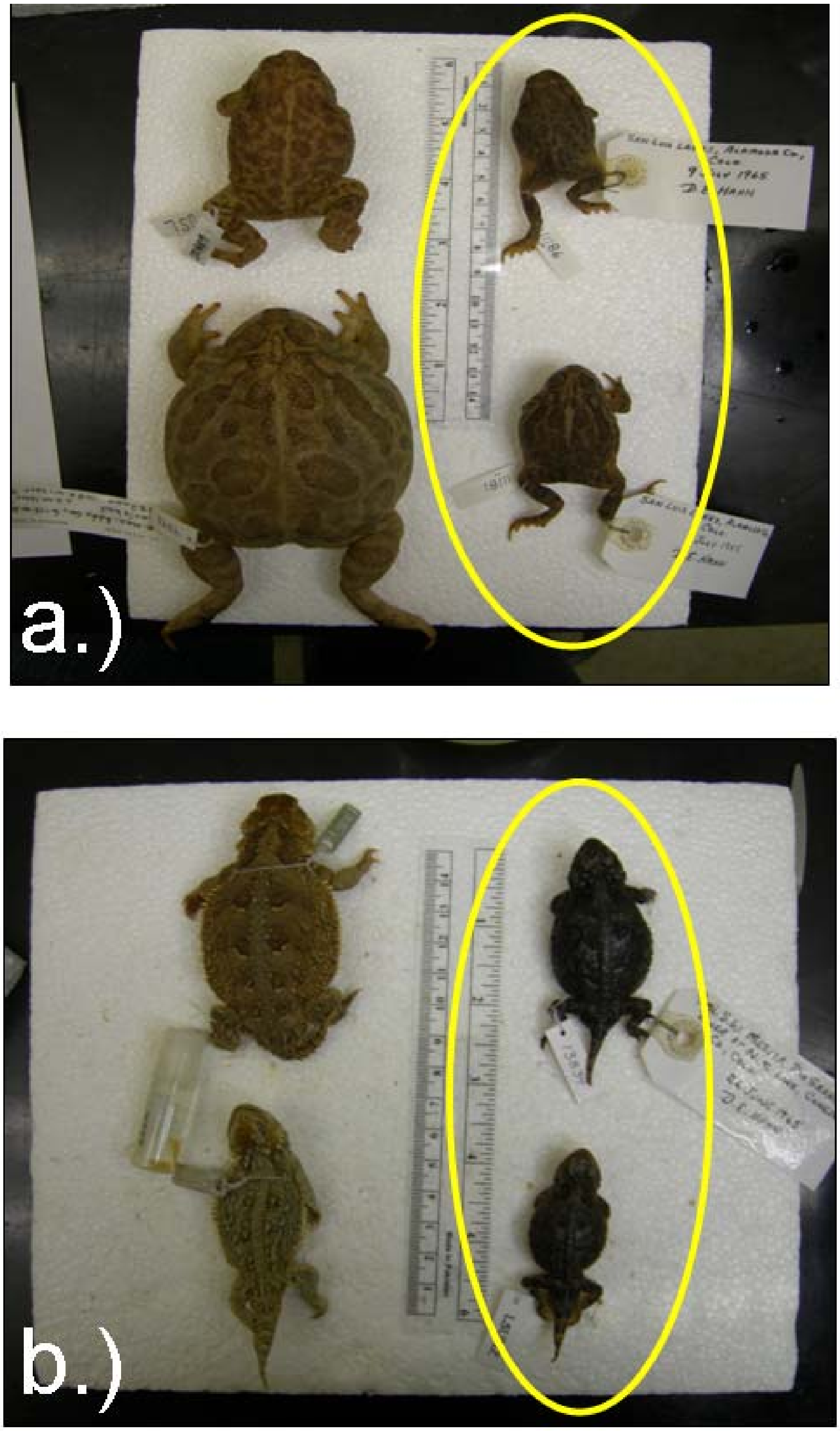

Fig. 1-2. Within the Valley, a.) *A. cognatus* and b.) *P. hernandesi* are dwarfed (outlined in yellow) although no studies have investigated this dwarfism since its initial reporting in the late 1960s.

Within the Valley, there are 5 physiographic provinces based on unique geology and topography (Upson, 1939). The Valley is a high-elevation (2,300 m) alpine desert that consists of a variety of habitats and ecosystems including stable and active sand dunes, grassland, woodland, wetland, riparian, playa, and shrub-land. The ecology and status of most populations of species within the Valley are inferred from populations outside the Valley, and it is possible that many species within the Valley have adapted to their unique habitat.

Although there is a plethora of research conducted on numerous taxa, one issue has persisted throughout science: lack of basic data, such as natural history information. Often, species are presumed to be of a certain conservation status though this determination is based on minimal data. For instance, the Riverside fairy shrimp (*Streptocephalus woottoni*) has been listed as Federally Endangered since 1993, though basic population data on its distribution and status throughout its range is lacking (USFWS, 2008). Although neither *P. hernandesi* or *A. cognatus* is considered for statewide or federal conservation, Valley populations are unique because of their dwarfism and should be investigated to better assess their status. Similarly, Valley populations of *P. hernandesi* and *A. cognatus* provide the opportunity for many research questions aimed at understanding the effects of dwarfism on natural/ife-history variables, the genetic correlation of dwarfism, and potentially the effects of isolation within a mainland system.

There is an obvious phylogenetic constraint on body size in *P. hernandesi* as they belong to a dwarfing lineage, having overall reduced body forms and horn lengths (Sherbrooke, 2003). Horned lizards within the dwarfing lineage (*P.*

hernandesi, P. douglasii, P. ditmarsi, and *P. orbiculare*) occupy more non-traditional habitats than the other horned lizards including high-elevations and more northerly latitudes instead of true deserts. *Phrynosoma hernandesi* generally experiences a reduced growing season and have a longer and more harsh hibernation period compared to its counterparts in the desert southwest. Similarly, *P. hernandesi* occupies a wide range of habitats and occurs across a high latitudinal gradients (nearly 50° latitude in southern Alberta, Canada to 23° latitude in southern Durango, Mexico) and elevational gradients (from approximately 270 m to 3,200 m) throughout its range (Sherbrooke, 2003; Stebbins, 2003). Common to many reptile species, *P. hernandesi* shows clinal variation in body size, becoming smaller towards the northern portion of its range extension.

Discordance among datasets still remains the prevailing issue with horned lizard phylogenetic reconstruction. Among the 17 currently recognized horned lizard species, *P. hernandesi,* like *P. cornutum,* has undergone multiple revisions and little consensus of its taxonomic status, largely because it is the most widespread species and shows great morphological, life/natural-history variation throughout its range (Smith, 1946; Reeve, 1952; Zamudio, 1996). Though, unlike *P. hernandesi, A. cognatus* shows minimal variation in its morphological (dorsal color and pattern) and life/natural-history variation throughout its range (Lanoo, 2005).

I investigated the morphology, genetics, and life-natural-history information for dwarfed populations of *P. hernandesi* and *A. cognatus* within the San Luis Valley. Since the initial reporting in 1968 (Hahn, 1968) and confirmation in 1981 (Hammerson, 1981), no studies have further investigated the extent or degree of body size reduction in these presumably isolated populations (Hahn, 1968; Hammerson, 1981). In Chapter 2, I used both museum and live specimens to measure morphological features of *A. cognatus* and *P. hernandesi* to determine the degree and patterns of dwarfism including sexual size dimorphism (SSD). Both *P. hernandesi* and *A. cognatus* showed significant dwarfism compared to their non-Valley counterparts; lizards are 28-29% smaller and toads are 34% smaller than populations surrounding the Valley. There are also distinct patterns of dwarfism among both species including an increase in sexual size dimorphism.

The localized dwarfism seen in Valley populations of *P. hernandesi* and *A. cognatus* is potentially indicative of a unique evolutionary history within the Valley. In Chapter 3, I use mitochondrial DNA gene to test whether Valley populations of *P. hernandesi* (ND4) and *A. cognatus* (16S/tRNA) show any genetic distinction compared to populations surrounding the Valley, whether the genetic diversity correlates with the geology of the Valley, and the colonization history within the Valley. Valley lizards form a distinct clade that includes populations adjacent to the southern end of the Valley. The mountains surrounding the Valley form a geologic barrier to *P. hernandesi* outside the Valley; Valley lizards are 7.08-8.50% divergent from populations at Mosca Pass that are 16 km from the nearest Valley population. Genetic variation across the landscape shows that *P. hernandesi* haplotypes correlate with the 5 physiographic regions in the Valley. Genetic distribution over the landscape, in conjunction with the geology and historic climate of the Valley, suggests two most likely colonization events: 1.) a singular colonization event originating from the southern end of the Valley and subsequent divergence or 2.) two major colonization events during the initial (0.82-0.74 MYA) and most recent (0.74-current) warm, dry climate cycles. Genetic data from this study further clarifies a distinct boundary between two of the previously recognized subspecies, *P.h.ornatissimum* and *P.h.brevirostre*, along the eastern edge of the Valley. *Anaxyrus cognatus* also show divergence in their genetic structure (0.92-1.19%) although small sample sizes preclude any likely scenarios regarding their colonization history. The variable genetic findings among populations of *P. hernandesi* underscore the importance of re-evaluating the subspecific designation of this species and potentially *A. cognatus*.

Body size, as well as genetic architecture, is inherently correlated with the life/natural-history of an organism. The life/natural-history data, in combination with morphological and genetic data, are used in conjunction in Chapter 4 to provide a better understanding of the Valley lizard populations and their current taxonomic status. I first investigate the occurrence of lizards throughout and surrounding the Valley to determine their current distribution. Second, I report the diets of Valley *P. hernandesi* and compare this information to populations surrounding the Valley. Finally, I report population demographics and other life/natural-history data for a local population of *P. hernandesi* at Zapata Ranch from 2007-2009. Overall, there is great similarity to *P. hernandesi* distribution as previously reported and lizards are most abundant in the stabilized sand dune habitat, although population densities within this habitat varied two-fold across years. *Phrynosoma hernandesi* shows significant variation in diet across localities, age/sex classes (neonate, adult male, adult female), and years (2007-2009). The Zapata Ranch population consumes mostly ants (58.3-92.2% of prey items) in proportions similar to populations throughout its range. Ontogenetic variation in diet occurs in the Valley, where neonates consume almost exclusively ants (90.1-98.7%) while adults decrease proportion of ants (80.9-93.2%) and instead increase consumption of beetles (3.8-14.3%). No juveniles were observed at Zapata Ranch during the demographic surveys, suggesting that neonates quickly mature to adults within their second full year of life. Horned lizards in general reproduce within 2-3 years from birth, a reproductive strategy common for short-lived species. Valley females have compensated for a reduced body size by producing fewer offspring of similar size to non-dwarfed populations. Likewise, there is a positive relationship between body size and reproductive output to the timing of precipitation; in 2009 when lizard densities were highest (2.3-3.8 times greater than other years), a majority of rainfall occurred in late spring. Based on morphological, molecular, and natural/ life-history data, Valley populations of *P. hernandesi* appear to have a unique evolutionary history compared to surrounding populations. The results from this research further validating the importance of re-assessing the status of *P. hernandesi* and possibly *A. cognatus*, both within the Valley and throughout its range.

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CHAPTER 2

MORPHOLOGICAL VARIATION AMONG DWARFED POPULATIONS OF *PHRYNOSOMA HERNANDESI* AND *ANAXYRUS COGNATUS* IN THE SAN LUIS VALLEY, COLORADO

INTRODUCTION

Body size among populations is known to vary over latitude and elevation gradients (e.g. Bergmann's rule [larger bodies evolve in colder climates], Bergmann, 1847; Allen's Rule [appendages shorten in colder climates], Allen, 1877). This phenomenon is well-studied in island ecosystems where populations typically undergo body size reduction or gigantism (e.g. Lomolino, 2005; Meiri, 2007). In non-insular systems, isolation is more difficult to achieve since populations are more contiguous over the landscape/habitat, facilitating migration; however, there is still a great degree of body size variation within species.

Most commonly, latitude and/or elevation account for variation in body sizes. Other variables known to contribute to body size among non-isolated populations include varying resource availability, environmental conditions, selective pressures, and genetic mutations (Hobday, 1994; Mendelson, 1998; Sutter et al., 2007). Though, most populations show a gradual cline in body size variation throughout the landscape (elevation, latitude). In reptiles and amphibians, there is a common trend for species to have a negative correlation in body size with elevation and latitude (e.g. Ashton and Feldman, 2003). Regardless of the underlying cause, that an organism would respond to its environment by altering body size shows that this response is necessitated, or in other words, that there is a net advantage to doing so. Even though there are physiological limitations on the amount an organism can shift its size, size shift inherently creates an allometric shift because the portions of scaling become inequivalent (Calder, 1984; Schmidt-Nielsen, 1984).

Allometric principles have been well-studied in a variety of taxa and there are often trends common to many organisms. Sexual size dimorphism (SSD) is the difference between adult male and female forms and represents the various selective pressures experienced by either sex (Zamudio, 1996). Most taxa that increase in body size (over evolutionary time scales) show a decrease in degree of SSD when that SSD is female-biased. Conversely, when the males are the larger sex, SSD increases with increasing body size, a principle is known as Rensch’s rule. Sexual size dimorphism has been the focus of many life-history studies (e.g. Fitch, 1978; Berry and Shine, 1980; Cox et al., 2003), and is a popular topic because there are numerous scenarios, thus making it a complex topic.

In this chapter, I present morphological data for two dwarfed species within the San Luis Valley, *P. hernandesi* and *A. cognatus*, using both museum and live specimens. First, I determine whether populations occurring within the Valley are significantly dwarfed compared to populations surrounding the Valley by describing the degree of dwarfism using various morphological features. Second, I compare the patterns of dwarfism present in Valley populations to surrounding populations for a suite of morphological features (allometric shift). Third, I investigate SSD to determine whether dwarfed Valley populations have maintained female-biased SSD.

METHODS

Specimens

Museum and live *P. hernandesi* and *A. cognatus* specimens from Louisiana State University Museum of Natural Science and University of Michigan Museum of Zoology were used to collect morphometric measurements. In total, 92 lizard and 103 toad adult and subadult museum specimens were measured. Specimens from both within the Valley as well as adjacent populations surrounding the Valley were included. Measurements from live specimens were obtained from May-August 2007 and 2008 and August 2009. In total, 145 live lizards (all age classes) and 139 live toads (adults and subadults only) were measured and analyzed.

Measurements

I used digital calipers (0.001 mm accuracy) (Fred V. Fowler Co., Inc., Newton, MA) to measure specimens. Lizard horn length was measured using a dissecting scope at 3X magnification with an occipital micrometer (= 0.001mm). All measurements were taken from the right side of the body unless damage occurred or that part was missing. I performed all measurements to reduce measurement error. A total of 19 morphological features from museum *P. hernandesi*, 5 features from live *P. hernandesi,* 16 features from museum *A. cognatus,* and 6 features from live *A. cognatus* were measured. The morphological features measured on live specimens were determined based on my capacity to accurately measure the feature on a live specimen and based on informative features from museum specimens. The morphological features measured, including abbreviations and definitions, are found in Appendix A. Most measurements were conducted following Mendelson (1998) for *A. cognatus* and Meyers et al. (2006) for *P. hernandesi*.

Analysis

Descriptive statistics and linear regressions were used to describe overall differences in morphology among and within both localities and sexes. An Anderson Darling Normality Test was performed on body size (snout-vent length; SVL) for museum lizards and toads. All data were log transformed to normalize the data distribution prior to analysis.

To determine whether morphological differences among sexes (adults only) and localities were significant, multivariate analysis of covariance (MANCOVA) using RegWQ was conducted using SAS Statistical Software (proc GLM; vers. 9.1, SAS Institute Inc., Cary, NC). I also used this analysis to determine whether Valley populations are of similar body proportions compared to subadults of equivalent size outside the Valley. The RegWQ analysis is a multiple pairwise comparisons test that detects for inequalities among means. Adjusted *P*-values are used to interpret the results to account for the high number of morphological variables analyzed. Model robustness was calculated using Wilk's Lambda, which calculates the proportion of variance among multiple datasets that is not explained by an effect. Using the adjusted *P*-values to indicate true significance is a conservative measure. Thus, results for both uncorrected ($P < 0.05$) and corrected ($P < 0.03125$) *P*-values are presented. An analysis of covariance (ANCOVA) was used to determine whether body size is significantly different among taxa both within and outside the Valley, using mass as a covariate.

RESULTS

Phrynosoma hernandesi

The size class distribution in outside females shows a normal distribution (Anderson Darling Normality Test: P = 0.978, AD-value = 0.132, N = 33) where body size is greatest around 80-84.5 mm (Figs. 2-1 and 2-2). In contrast, most outside males are 65-69.5 mm SVL although this distribution does not significantly deviate from normality (Anderson Darling Normality Test: AD-value = 0.544, N = 8, P = 0.110). Valley female lizards show a rapid decrease in the number of larger lizards (Anderson Darling Normality Test: AD-value = 0.946, N = 23, P = 0.014); most female lizards are in the smallest size class (50-54.5 mm) and the number of females in successively higher size classes tapers smoothly. In contrast, most Valley males show a clumped distribution of body size at 40-49.5 mm SVL and the distribution of larger-sized males is relatively even. Males in the Valley do not significantly deviate from a normal distribution (Anderson Darling Normality Test: AD-value = 0.562, N = 14, P = 0.118).

Outside populations of both sexes are significantly larger than Valley populations, and males of both localities are significantly smaller than their female counterparts. Based on SVL, Valley females are 71.9% as large as outside females and Valley males are 70.6% as large as outside males. Box plot distributions of all measured morphological features (n = 18) standardized to SVL for adult male and female *P. hernandesi* within and outside the Valley are presented in Fig. 2-3.

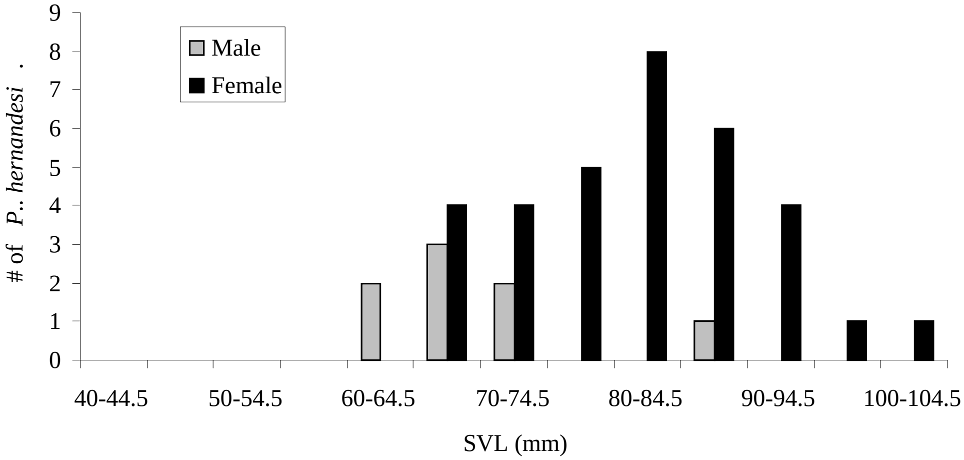


Fig. 2-1. Body size (SVL) distribution of adult male and female *P. hernandesi* outside the Valley. Both males and females show a normal distribution in body size (*P* > 0.05) and females are larger than males.

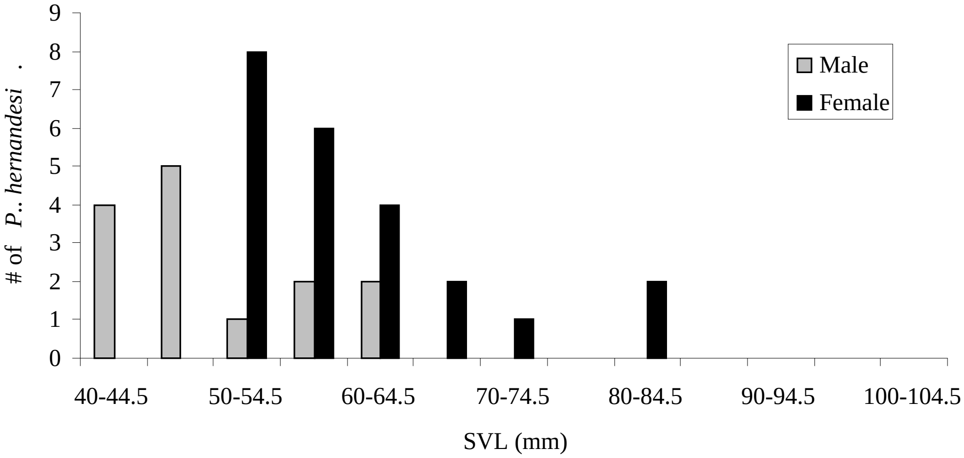


Fig. 2-2. Body size (SVL) distribution of adult male and female *P. hernandesi* within the Valley. Females have a skewed distribution (*P* < 0.05) while males are mostly of smaller size but the distribution does not deviate from normality (*P* > 0.05).

Since sample sizes for *P. hernandesi* within and surrounding the Valley are

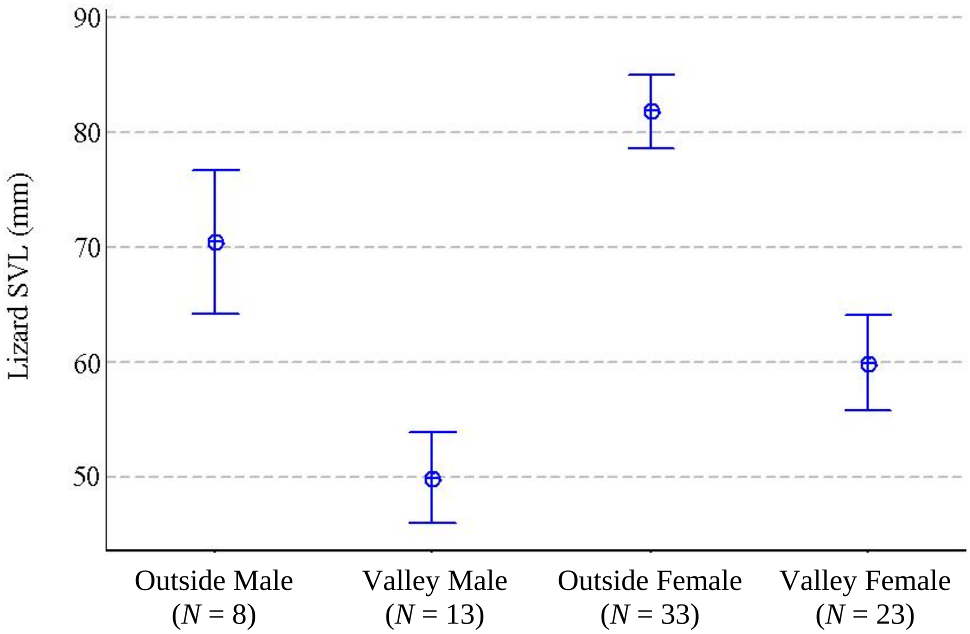


Fig. 2-3. Interval plot showing 95% CI for body size among adult male and female *P. hernandesi* within and outside the Valley. Valley *P. hernandesi* are ~30% smaller than populations outside the Valley.

limited (*N* = 7 outside, 12 Valley) in comparison to the number of morphological variables being analyzed (*N* = 18) and the MANCOVA glm model test was insignificant (*P* = 0.1418), a reduced morphological dataset of nine features was used to determine allometric shifts among males. Morphological features that were highly covariate were selected for removal from analysis. With the dataset composed of 9 features, the MANCOVA glm model was significant (*P* = 0.03). Because there is a larger dataset for females (*N* = 29 outside, 21 Valley) and the model is significant with all variables included (*P* = 0.0006), I used the entire dataset for analysis of females.

Valley populations of both males and females were significantly different for all 9 morphological features compared to outside males and females, respectively (Tables 2-1 and 2-2, Fig. 2-4a-r). Valley populations are proportionately smaller for all features, which primarily associate with limb length and head shape. Thus, there are substantial allometric shifts among both sexes. Interestingly, Valley populations of males and female have different body proportions than populations outside the Valley of equal sizes (MANCOVA RegWQ: males: $F_{11,10} = 4.39$, $P = 0.0134$; females: $F_{11,17} = 4.20$, $P = 0.0134$; Table 2-3). In both males and females, most differences in body proportions are associated with limb length and head morphology, where Valley populations are proportionally smaller for all feature.

Horn width and length are not disproportionate among Valley females compared to outside females (MANCOVA RegWQ: $F_{4,44} = 0.80$, $P = 0.5344$) or Valley males compared to outside males (MANCOVA RegWQ: $F_{4,13} = 1.14$, $P = 0.3813$) when covariated with SVL. For all traits except occipital spine distal length, outside females are significantly different in size proportion than Valley females ($P < 0.03125$; Table 2-2). Outside females are larger for all significant features except occipital spine distal width.

Valley *P. hernandesi* have increased SSD compared to outside populations (Tables 2-4 and 2-5). Both populations are sexually dimorphic for 7 of the 18 features measured that are associated with head shape and locomotion, primarily of the hind limbs. For these seven features (head width, head length, head shield width, femur length, tibia length, radius length), the SSD is in favor of females. Valley populations are dimorphic for an additional 7 morphological features associated with

Table 2-1. MANCOVA using RegWQ for adult male museum *P. hernandesi* showing allometric shifts among Valley populations compared to outside populations.

Morphological Feature	Grouping Mean Outside ($N = 7$)	Grouping Mean Valley ($N = 12$)	Critical Range	Significance
Tail Length	3.630	3.225	0.090	*
Head Width	2.994	2.651	0.044	*
Head Length	2.785	2.493	0.036	*
Eye-nose Distance	1.388	1.098	0.065	*
Femur Length	3.734	3.341	0.042	*
Tibia Length	2.731	2.301	0.071	*
Longest Hindfoot Toe	2.246	1.662	0.174	*
Humerus Length	2.835	2.437	0.066	*
Radius Length	2.448	2.038	0.065	*

* $P < 0.05$

Table 2-2. MANCOVA using RegWQ for adult female museum *P. hernandesi* showing allometric shifts among Valley populations compared to outside populations.

Morphological Feature	Grouping Mean Valley ($N = 21$)	Grouping Mean Outside ($N = 29$)	Critical Range	Significance
Tail Length	3.145	3.521	0.081	*
Head Width	2.785	3.101	0.044	*
Head Length	2.589	2.872	0.030	*
Head Shield Width	2.295	2.534	0.029	*
Eye-nose Distance	1.180	1.439	0.046	*
Internasal Distance	1.212	1.410	0.054	*
Tympanum Height	0.954	1.206	0.073	*
Femur Length	3.472	3.823	0.031	*
Tibia Length	2.428	2.838	0.036	*
Foot Length	2.552	2.890	0.036	*
Longest Hindfoot Toe	1.790	2.197	0.055	*
Humerus Length	2.542	2.914	0.059	*
Radius Length	2.177	2.585	0.042	*
Hand Length	2.263	2.581	0.056	*
Occipital Spine Medial Length	0.431	0.773	0.192	*
Occipital Spine Medial Width	0.446	0.827	0.178	*
Occipital Spine Distal Length	0.660	0.759	0.159	
Occipital Spine Distal Width	2.277	1.868	0.149	*

* $P < 0.03125$

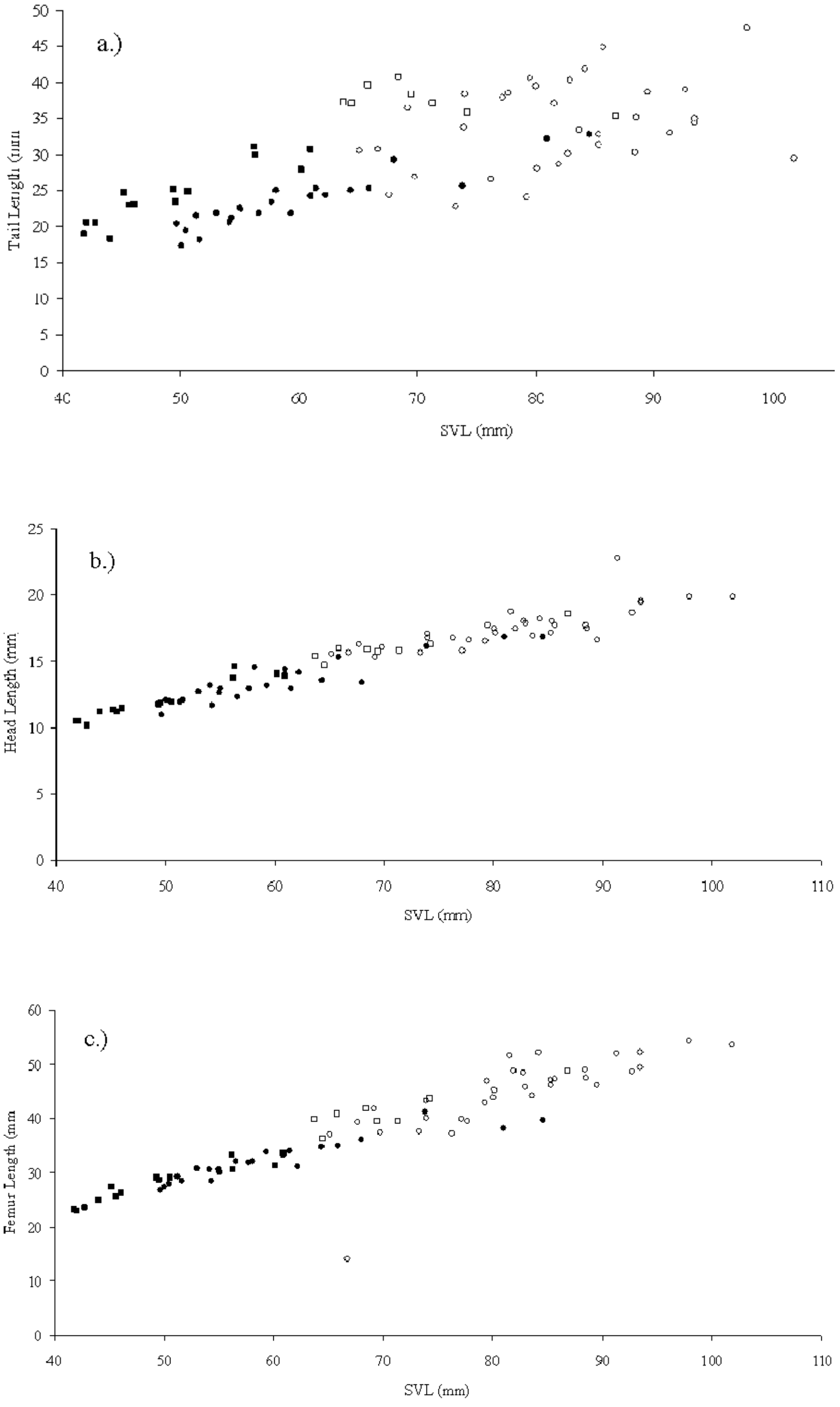


Fig. 2-4a-r. Morphological variation of *P. hernandesi* adult museum specimens outside and within the Valley. □ = outside male (*N* = 8), ○ = outside female (*N* = 14), ■ = Valley male (*N* = 32), ● = Valley female (*N* = 33). Valley populations of both sexes are proportionately smaller for all features than outside the Valley.

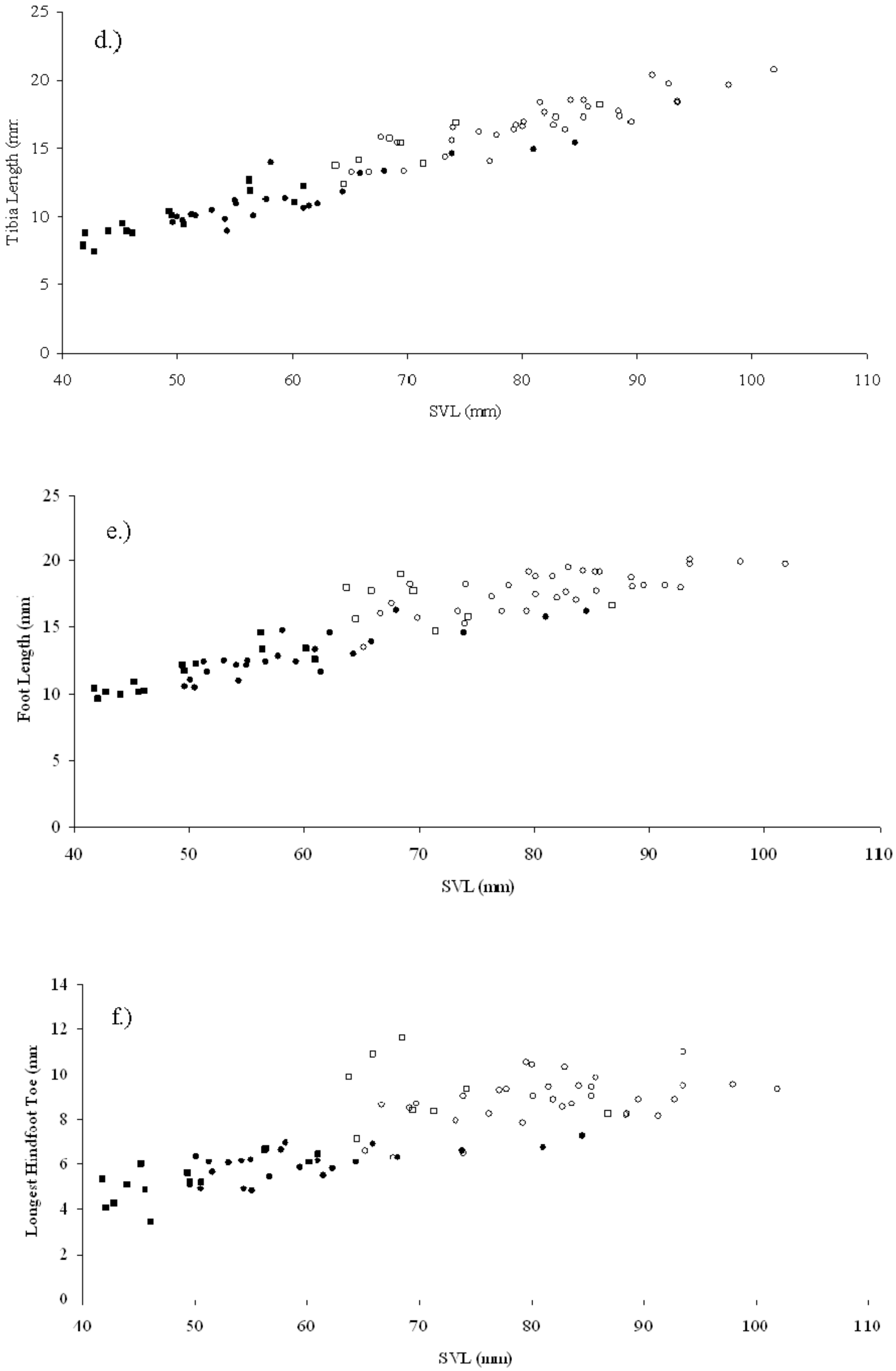


Fig. 2-4a-r continued.

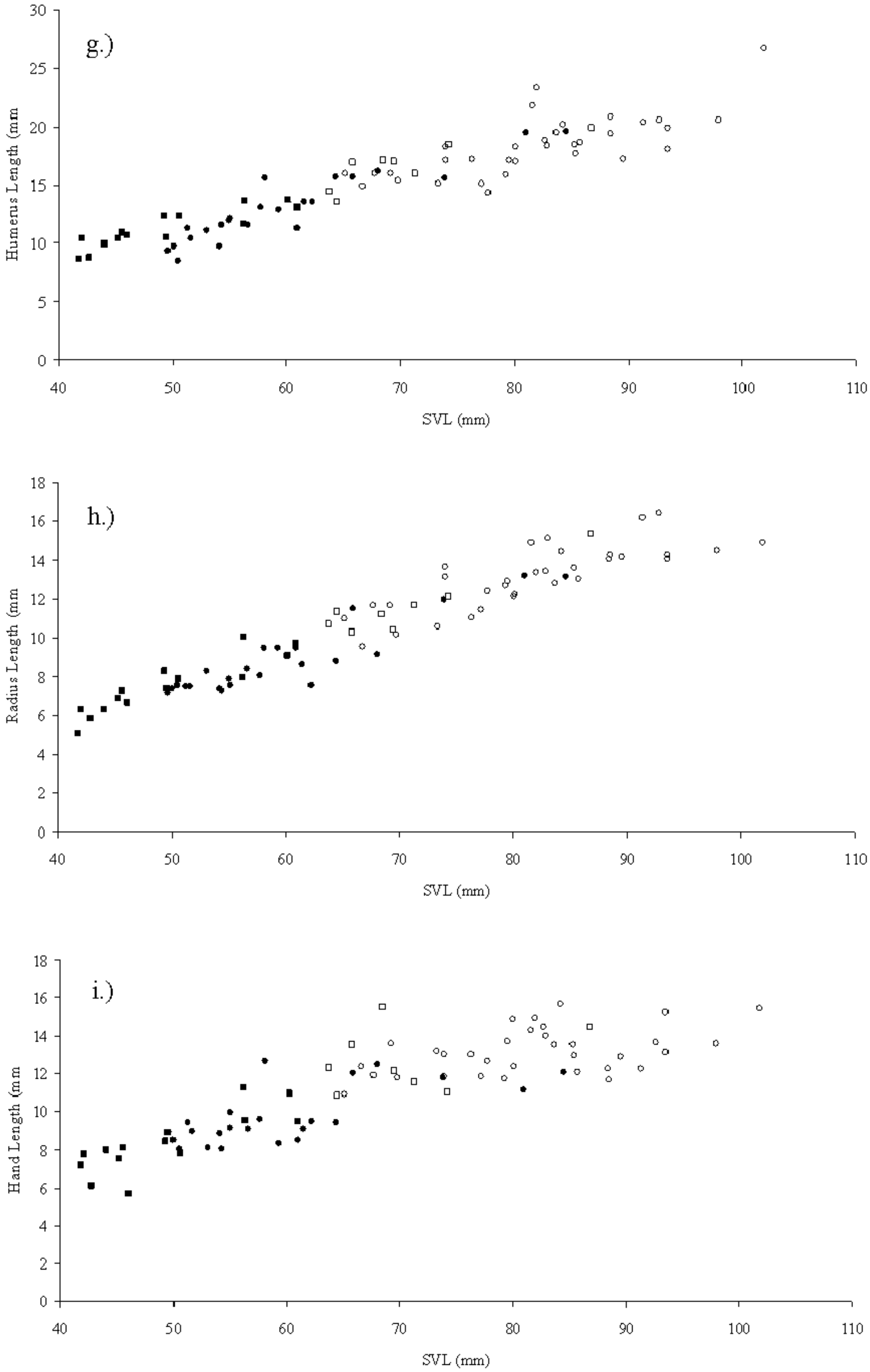


Fig.2-4a-r continued.

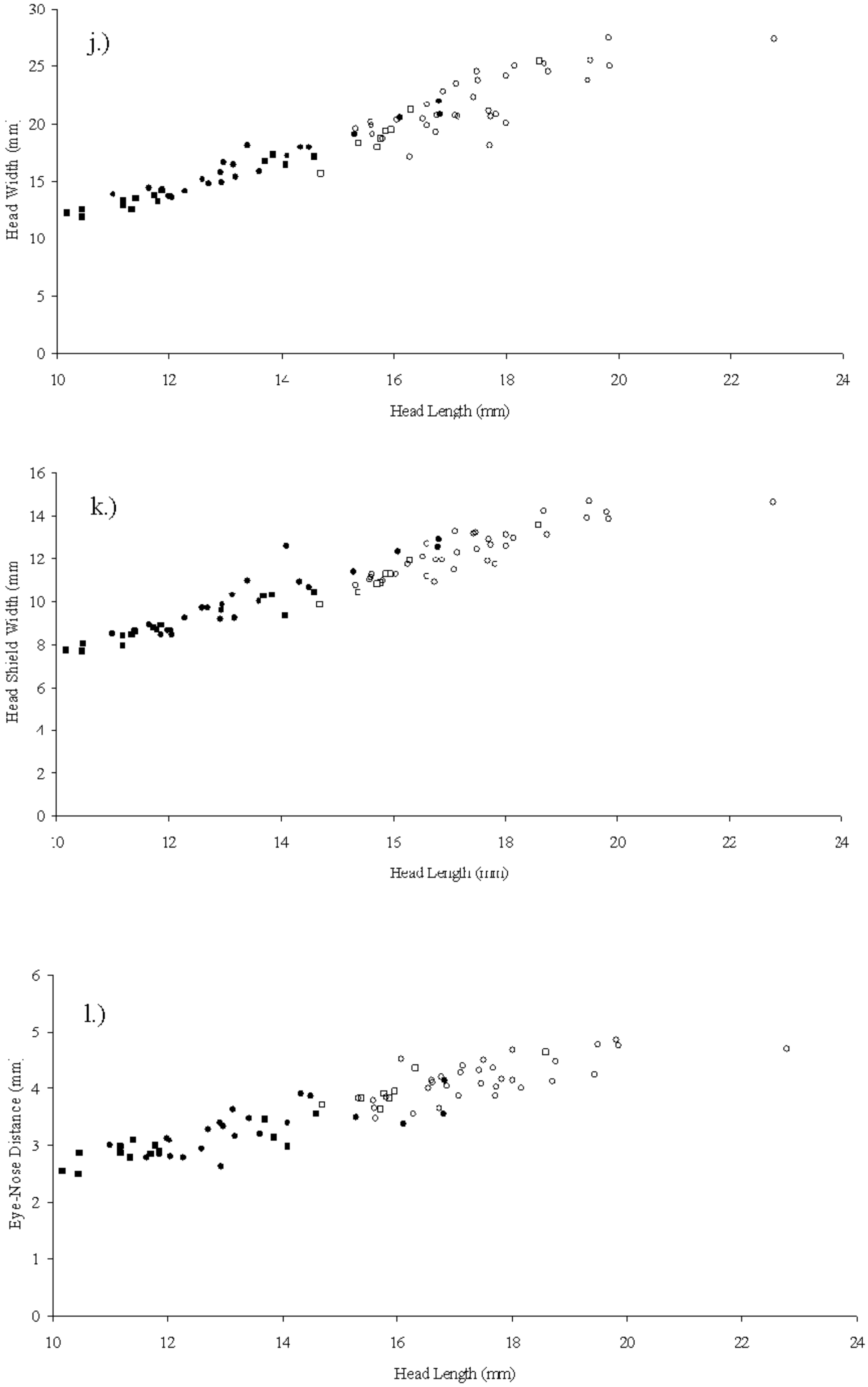


Fig.2-4a-r continued.

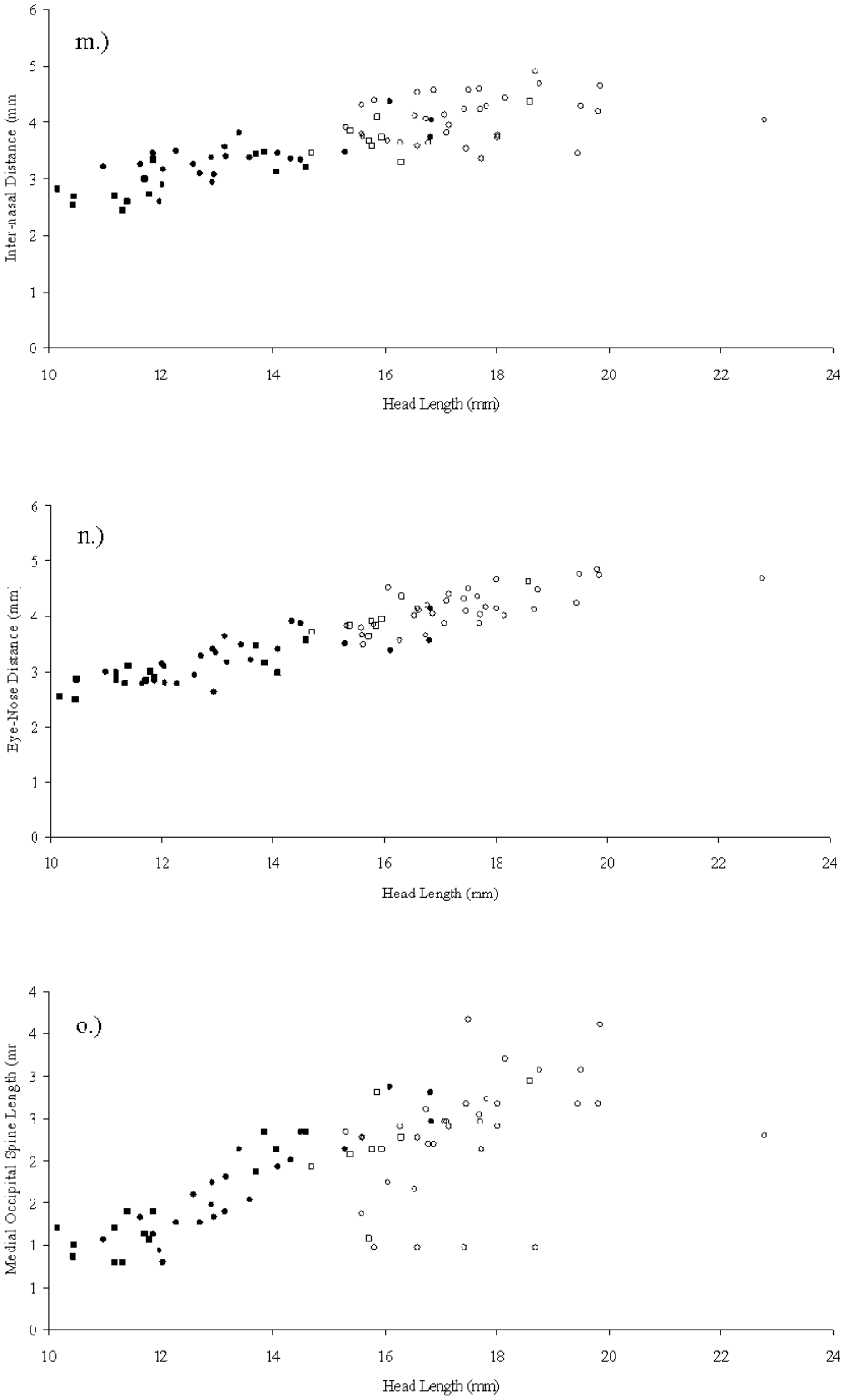


Fig.2-4a-r continued.

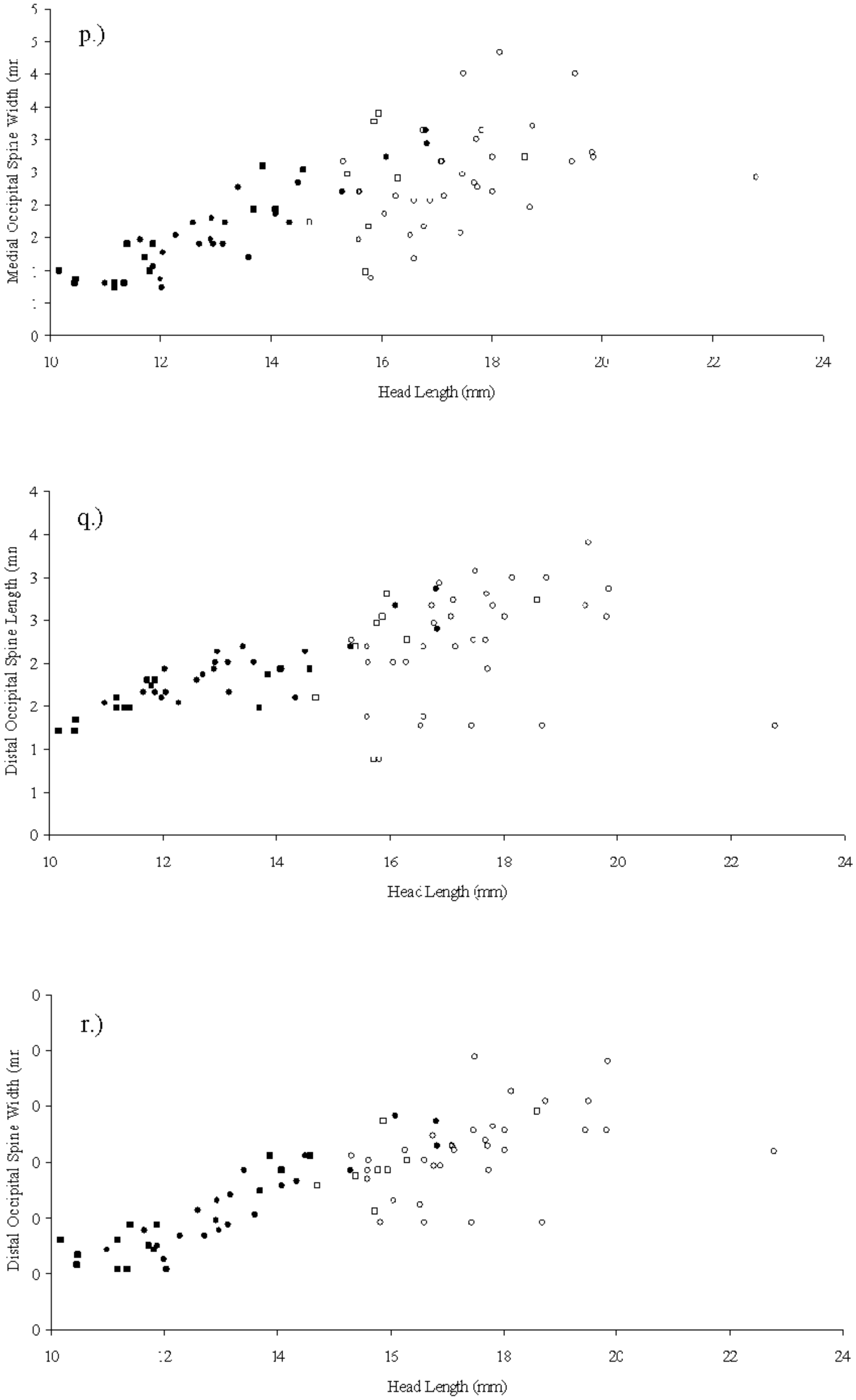


Fig. 2-4a-r continued.

Table 2-3. MANCOVA using RegWQ for adult Valley *P. hernandesi* compared to subadults of equal sizes outside the Valley.

	Male				Female			
Morphological Feature	Grouping Mean Outside Subadults (*N* = 11)	Grouping Mean Valley Adults (*N* = 12)	Critical Range	Significance	Grouping Mean Outside Subadults (*N* = 21)	Grouping Mean Valley Adults (*N* = 9)	Critical Range	Significance
Tail Length	3.255	3.225	0.0675		3.145	3.298	0.072	*
Head Width	2.693	2.651	0.0461		2.785	2.842	0.047	*
Head Length	2.579	2.493	0.0276	**	2.589	2.678	0.038	*
Head Shield Width	2.213	2.191	0.0369		2.296	2.330	0.044	
Eye-Nose Distance	1.158	1.098	0.0559	*	1.180	1.230	0.074	
Femur Length	3.414	3.341	0.0467	**	3.456	3.472	0.153	
Tibia Length	2.400	2.301	0.0585	**	2.428	2.562	0.064	*
Longest Hindfoot Toe	1.922	1.663	0.1075	**	1.790	2.007	0.092	*
Humerus Length	2.442	2.437	0.0758		2.542	2.624	0.081	*
Radius Length	2.119	2.038	0.0749	*	2.177	2.280	0.069	*
Inter-nasal Distance	1.126	1.079	0.0951		1.212	1.247	0.066	

* $P < 0.05$

** $P < 0.004$

Table 2-4. MANCOVA using RegWQ for outside museum *P. hernandesi* showing SSD among adult males and females.

Morphological Feature	Grouping Mean Female (*N* = 29)	Grouping Mean Male (*N* = 7)	Critical Range	Significance
Tail Length	3.520	3.630	0.220	
Head Width	3.100	2.990	0.110	*
Head Length	2.870	2.780	0.070	**
Head Shield Width	2.530	2.430	0.060	**
Eye-nose Distance	1.440	1.390	0.080	
Internasal Distance	1.410	1.330	0.140	
Tympanum Height	1.210	1.100	0.180	
Femur Length	3.820	3.730	0.080	**
Tibia Length	2.840	2.730	0.070	**
Foot Length	2.890	2.830	0.100	
Longest Hindfoot Toe	2.250	2.200	0.150	
Humerus Length	2.910	2.840	0.130	
Radius Length	2.590	2.450	0.100	**
Hand Length	2.580	2.550	0.130	
Occipital Spine Medial Length	0.780	0.750	0.500	
Occipital Spine Medial Width	0.830	0.810	0.500	
Occipital Spine Distal Length	0.760	0.760	0.490	
Occipital Spine Distal Width	1.870	1.900	0.330	

* $P < 0.05$
** $P < 0.03$

Table 2-5. MANCOVA using RegWQ for Valley museum *P. hernandesi* showing SSD among adult males and females.

Morphological Feature	Grouping Mean Female (*N* = 21)	Grouping Mean Male (*N* = 12)	Critical Range	Significance
Tail Length	3.140	3.220	0.070	**
Head Width	2.790	2.990	0.060	**
Head Length	2.590	2.780	0.050	**
Head Shield Width	2.300	2.430	0.060	**
Eye-nose Distance	1.180	1.390	0.100	*
Internasal Distance	1.210	1.330	0.090	**
Tympanum Height	0.950	1.100	0.120	**
Femur Length	3.470	3.730	0.050	**
Tibia Length	2.430	2.730	0.090	**
Foot Length	2.550	2.830	0.080	**
Longest Hindfoot Toe	1.790	2.200	0.150	*
Humerus Length	2.540	2.840	0.110	*
Radius Length	2.180	2.450	0.080	**
Hand Length	2.260	2.550	0.140	**
Occipital Spine Medial Length	0.430	0.750	0.240	
Occipital Spine Medial Width	0.450	0.810	0.260	
Occipital Spine Distal Length	0.660	0.760	0.110	**
Occipital Spine Distal Width	2.280	2.370	0.240	

* $P < 0.05$

** $P < 0.03$

a greater divergence in head shape and locomotion of front and hind limbs compared to the outside populations (tail length, eye-nose distance, internasal distance, tympanum height, foot length, longest hindfoot toe, humerus length; Fig. 2-5). Again, SSD is female-biased for all features except tail length, which is relatively longer in populations of Valley males.

Growth slopes for Valley populations vary depending on age and sex class (Fig. 2-6). In dwarfed *P. hernandesi*, males have a small range in body sizes (16 mm range, $N = 36$) and tend to increase size at a rate higher than they increase mass ($Y = 0.323x - 8.384$). In contrast, dwarfed females show two-fold greater range in body size compared to males (35 mm range, $N = 64$) and have a higher rate of mass gain ($Y = 0.587x - 20.033$); however, dwarfed females gain mass at a lower rate compared to non-dwarfed females ($Y = 0.756x - 28.979$) and non-dwarfed females show the highest range in body size variations overall (43 mm range, $N = 16$). Non-dwarfed males also have a size range nearly double that of dwarfed males (37 mm range, $N = 10$) and a higher mass gain ($Y = 0.562x - 20.200$). Thus, both dwarfed males and females have higher masses at a given body size than outside males and females, respectively; nearly all dwarfed males are heavier than outside males at a specific body size. And, dwarfed females are heaver than outside females at a specific body size up to approximately 50 mm SVL.

Live dwarfed females are significantly larger in body size (SVL) than dwarfed males (ANCOVA; $F_{1,59} = 40.5$, $P < 0.0001$) but not disproportionate when covaried with mass (ANCOVA; $F_{1,59} = 3.26$, $P = 0.076$). Live males outside the Valley are significantly larger for all four morphological features (tail length, head length, head

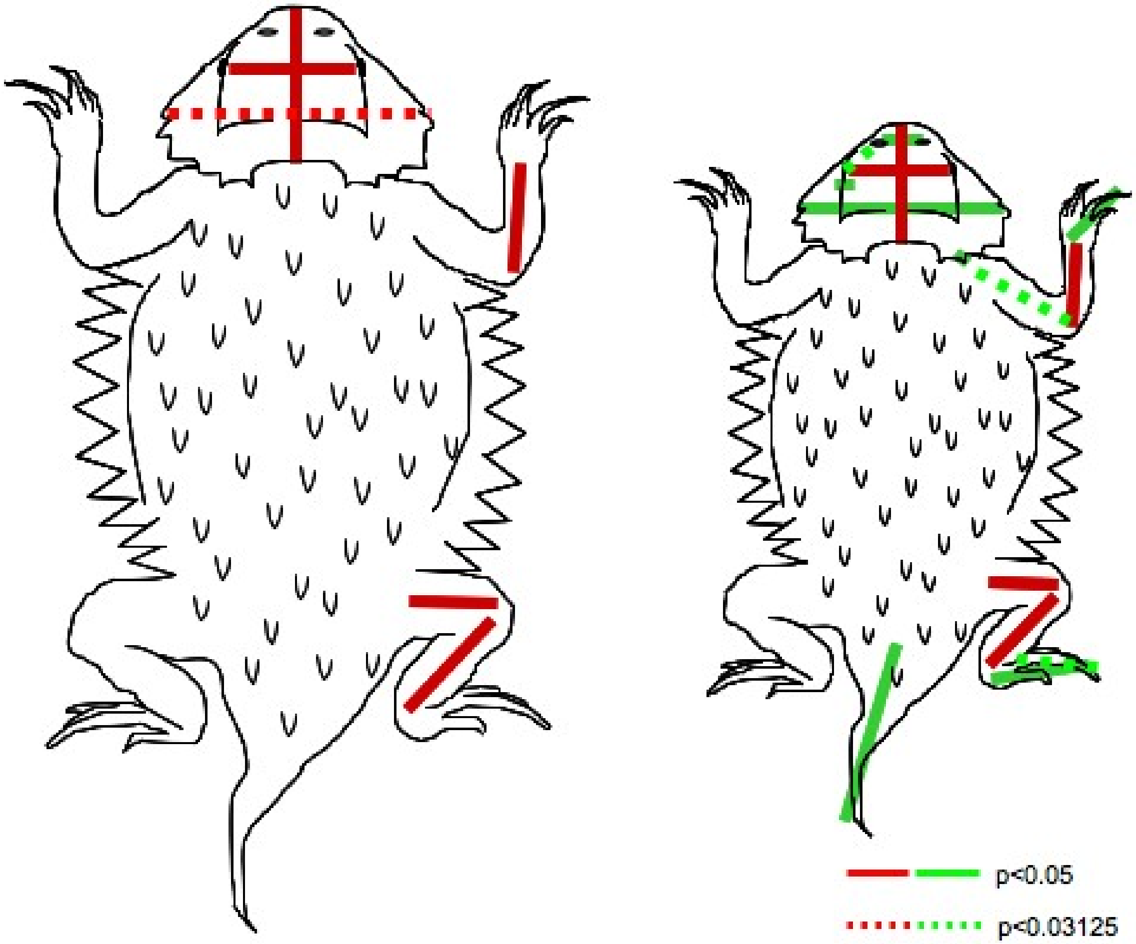


Fig. 2-5. SSD is increased among dwarfed populations of *P. hernandesi*. Red indicates features that *P. hernandesi* show SSD for both outside (left) and within (right) the Valley. Green indicates additional features for which Valley *P. hernandesi* sexually dimorphic.

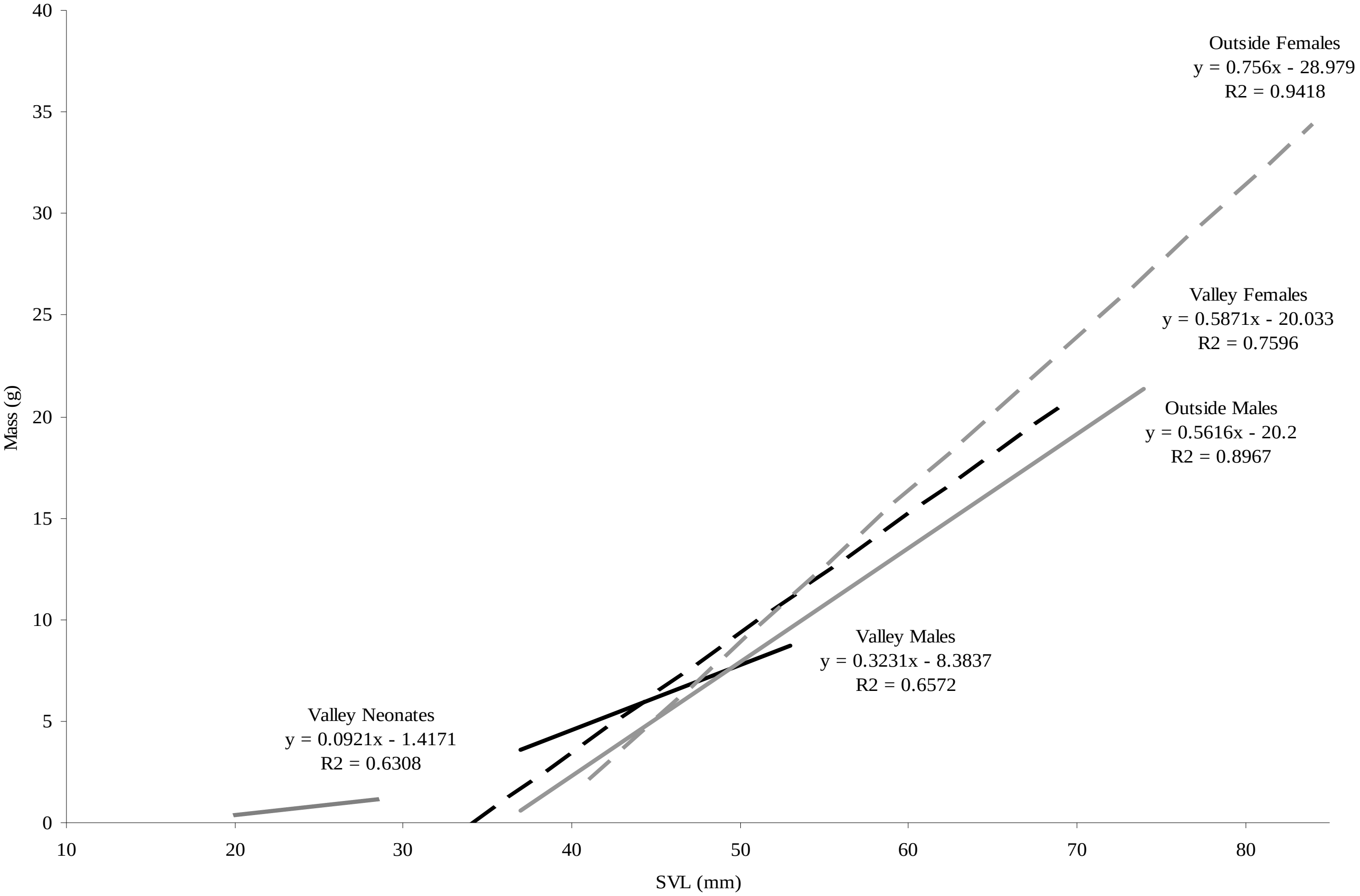


Fig. 2-6. Growth slopes for live *P. hernandesi* within and outside the Valley

shield width, femur length) compared to Valley males (Fig. 2-7a-e) while females outside the Valley are significantly larger for all four morphological features except tail length compared to Valley females (Fig. 2-8a-e; Table 2-6). *Phrynosoma hernandesi* outside the Valley are sexually dimorphic for tail length and femur length in favor or males (Table 2-7) while Valley lizards are sexually dimorphic for head length, shield width, and femur length in favor of females.

Anaxyrus cognatus

The size class distribution in outside females shows a normal distribution ranging from 56-96 mm (Anderson Darling Normality Test: AD-value = 0.22, N = 16, P = 0.802) although body size does not peak at a particular size class (Fig. 2-9 and 2-10). In contrast, male sizes increase gradually from 50-54.5 mm and peak at 75-79.5 mm, with few toads being larger in size and having a distribution that is non-normal (Anderson Darling Normality Test: AD-value = 0.83, N = 46, P = 0.030). Valley female and male *A. cognatus* are all found within a small range of size classes and the distribution pattern does not deviate from normality, particularly among males (Anderson Darling Normality Test: AD-value = 0.505, N = 20, P = 0.179 and AD-value = 0.235, N = 24, P = 0.766, respectively).

Anaxyrus cognatus of both sexes outside the Valley are significantly larger than Valley populations. Both males and females within the Valley are only 66.0% as large as males and females outside the Valley (based on SVL; Fig. 2-11). Both outside and Valley males are on average smaller than their female counterparts; however, this relationship is not significant when covariated with mass.

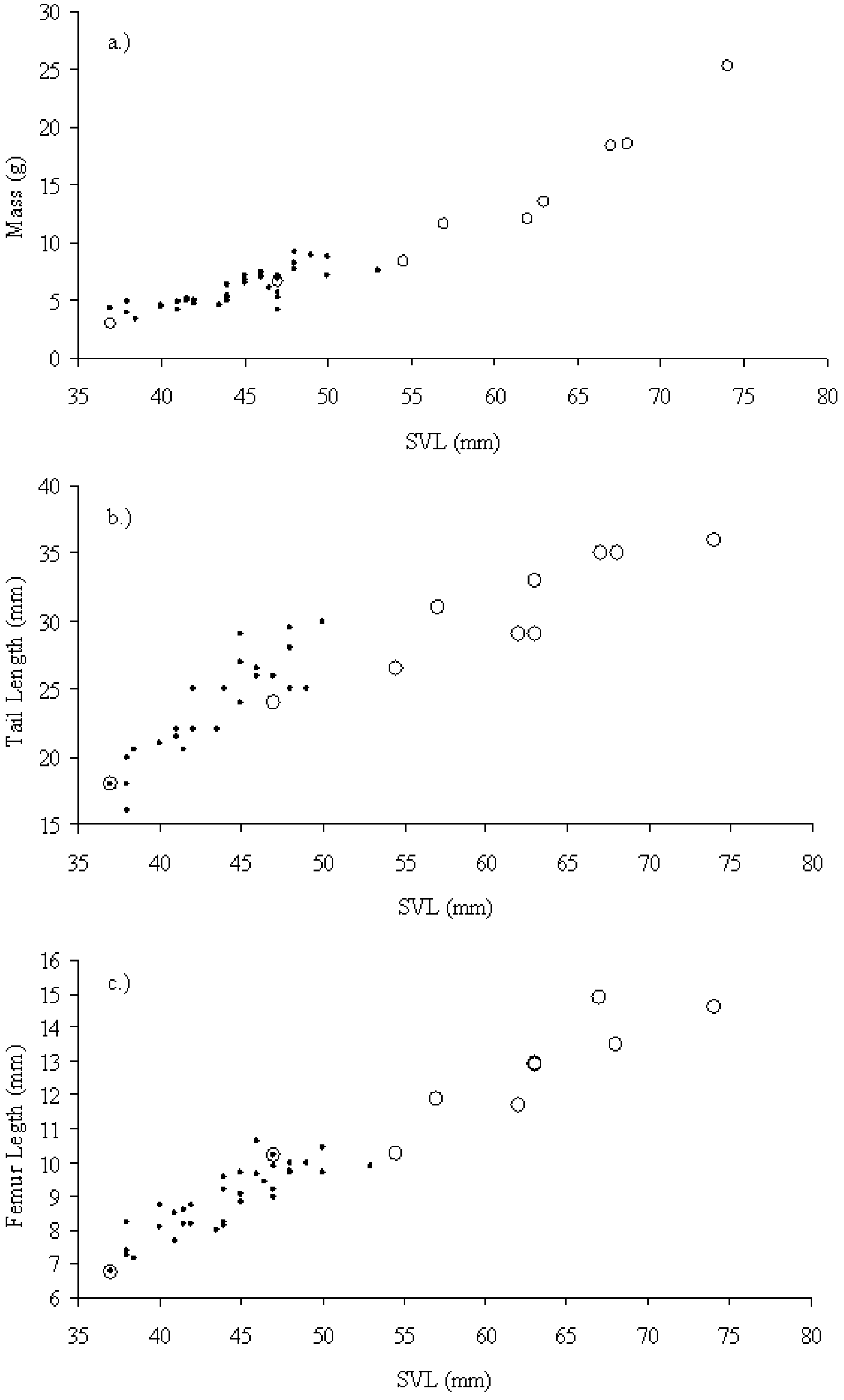


Fig. 2-7a-e. Distribution of morphological measurements for live adult male *P. hernandesi* within and outside the Valley. ○ = outside (n = 10), ● = Valley (n = 24). Valley females are relatively smaller for all body proportions than outside the Valley.

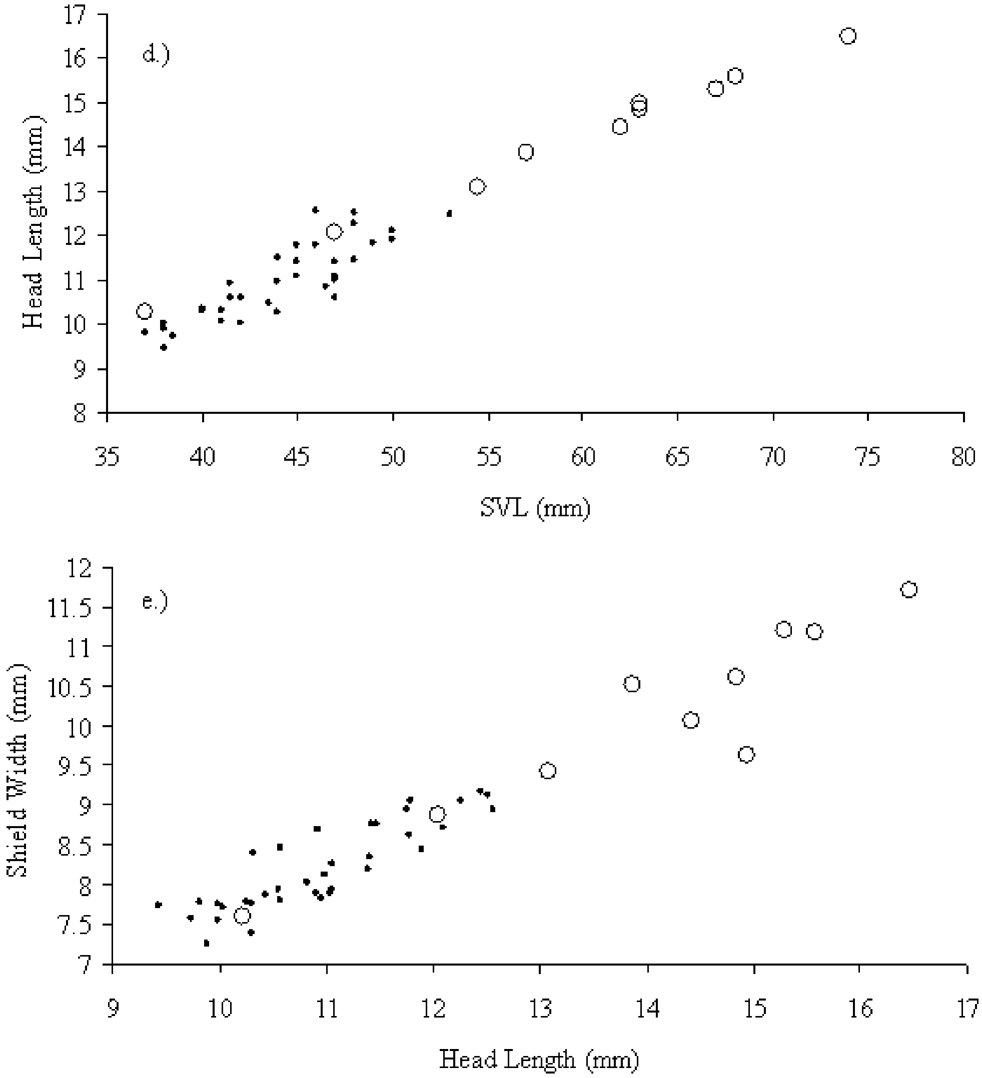


Fig. 2-7a-e. continued.

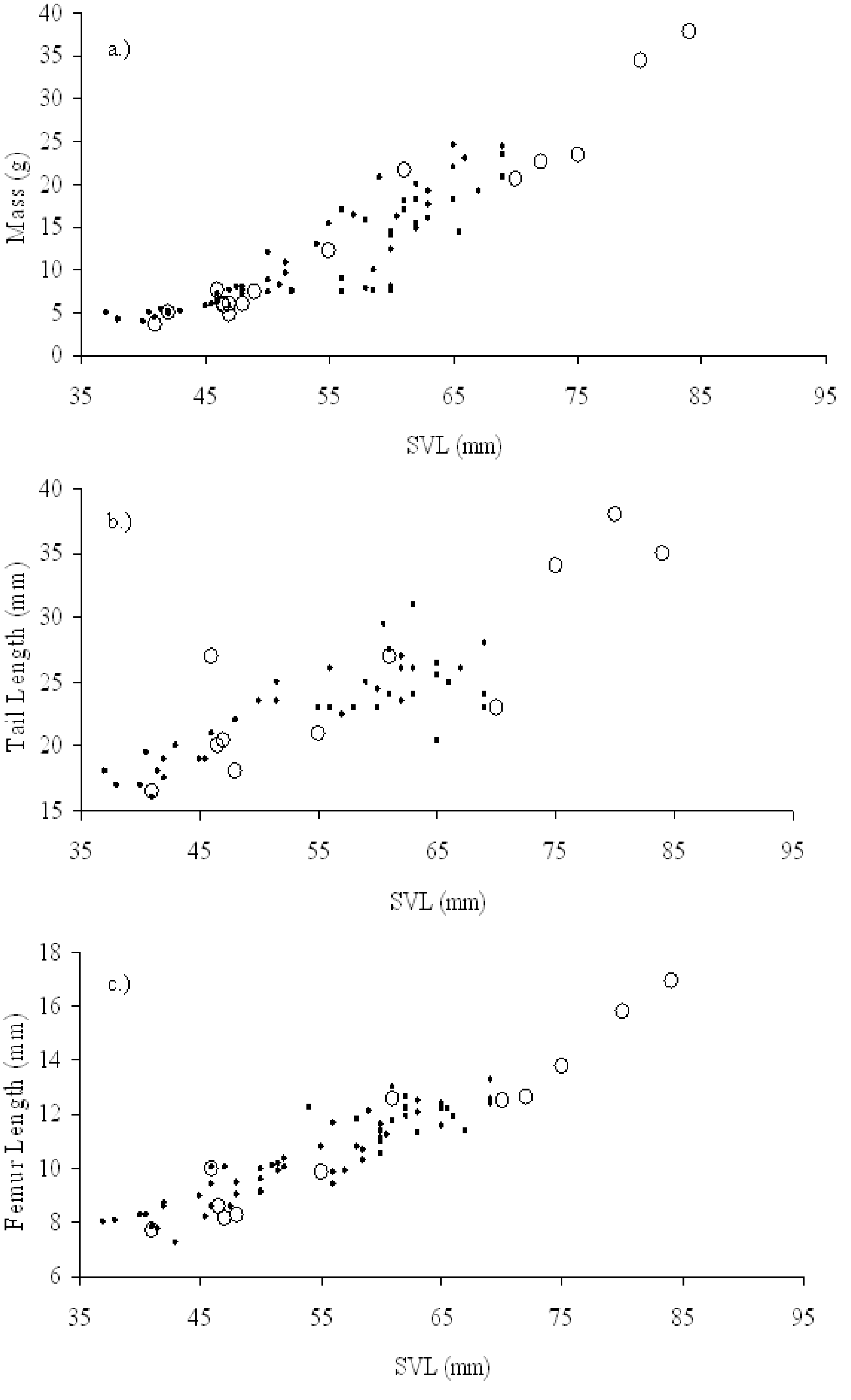


Fig. 2-8a-e. Distribution of morphological measurements for live adult female *P. hernandesi* within and outside the Valley. ○ = outside (n = 15), ● = Valley (n = 41). Valley males are relatively smaller for all body proportions than outside the Valley.

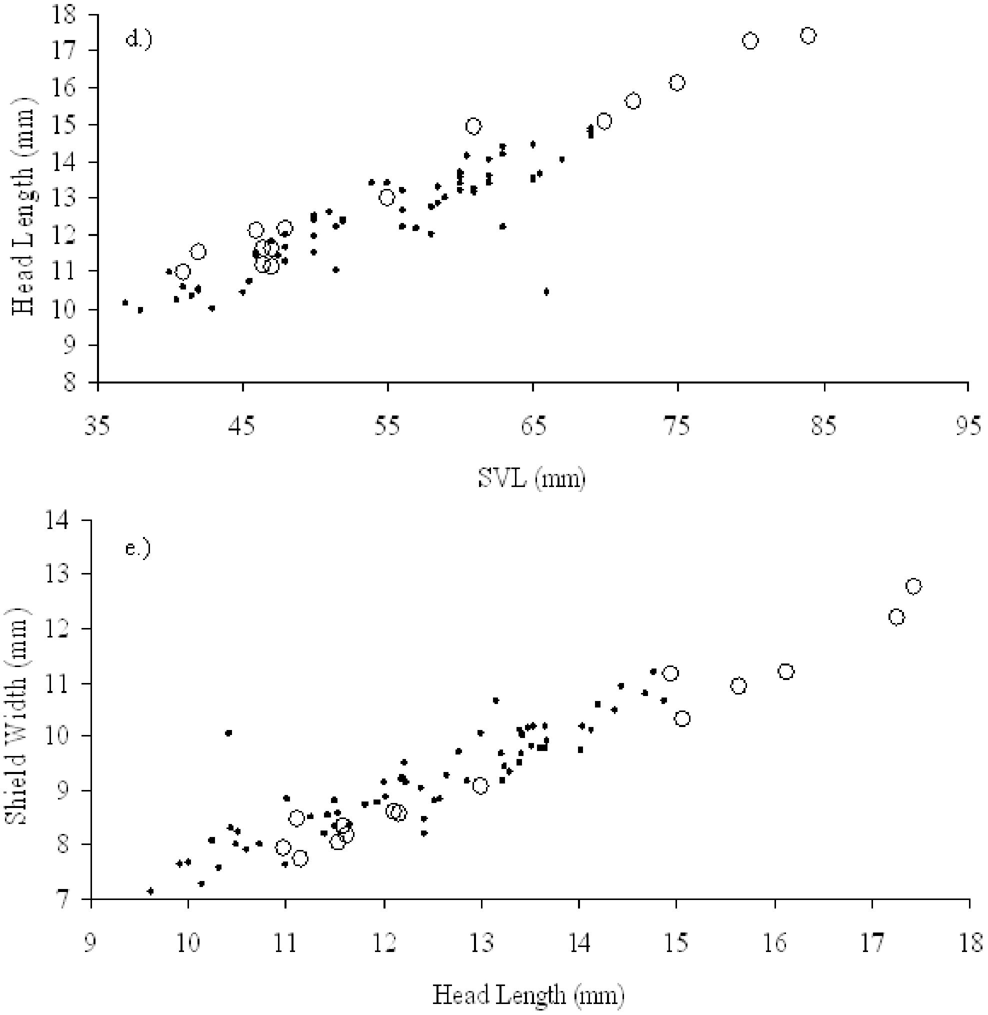


Fig. 2-8a-e continued.

Table 2-6. MANCOVA using RegWQ for live male and female *P. hernandesi* showing allometric shifts among Valley populations compared to outside populations.

	Male				Female			
Morphological Feature	Grouping Mean Outside (*N* = 10)	Grouping Mean Valley (*N* = 34)	Critical Range	Significance	Grouping Mean Outside (*N* = 11)	Grouping Mean Valley (*N* = 60)	Critical Range	Significance
Tail Length	3.371	2.426	0.714	*	3.000	2.332	0.681	
Head Length	2.636	2.397	0.027	*	2.634	2.524	0.034	*
Head Shield Width	2.303	2.109	0.031	*	2.298	2.226	0.024	*
Femur Length	2.460	2.183	0.041	*	2.408	2.348	0.042	*

* $P < 0.05$

Table 2-7. MANCOVA using RegWQ for live *P. hernandesi* showing SSD among adult males and females both within and outside the Valley.

	Outside				Valley			
Morphological Feature	Grouping Mean Female (*N* = 10)	Grouping Mean Male (*N* = 10)	Critical Range	Significance	Grouping Mean Female (*N* = 60)	Grouping Mean Male (*N* = 34)	Critical Range	Significance
Tail Length	3.191	3.370	0.082	*	2.426	2.332	0.469	
Head Length	2.622	2.636	0.023		2.524	2.397	0.022	*
Head Shield Width	2.289	2.303	0.042		2.226	2.109	0.016	*
Femur Length	2.396	2.460	0.056	*	2.348	2.183	0.026	*

* $P < 0.05$

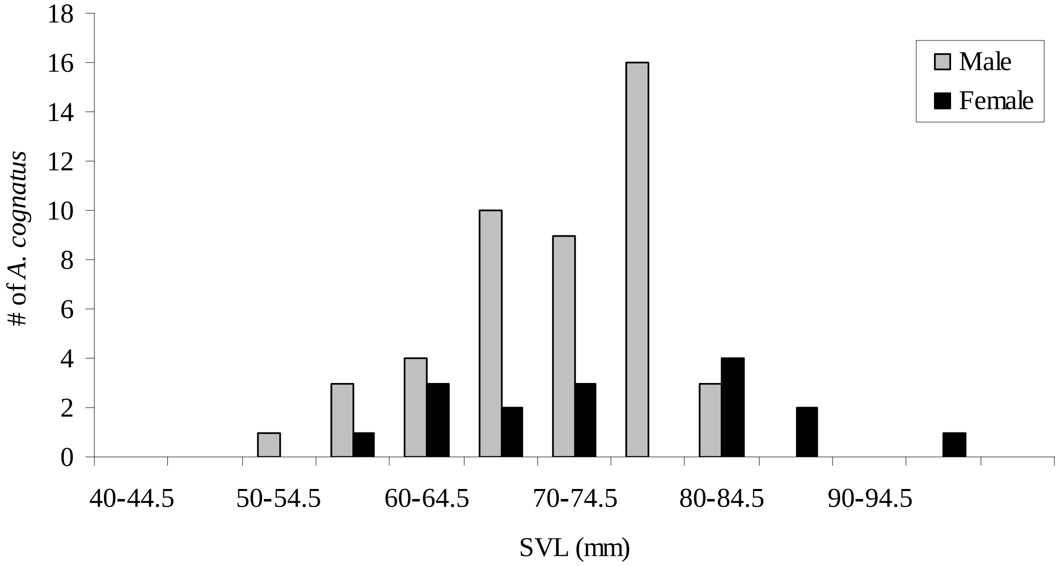


Fig. 2-9. Body size (SVL) distribution of adult male and female *A. cognatus* outside the Valley. Females show a normal distribution in body size ($P > 0.05$) while males show an uneven distribution ($P < 0.05$).

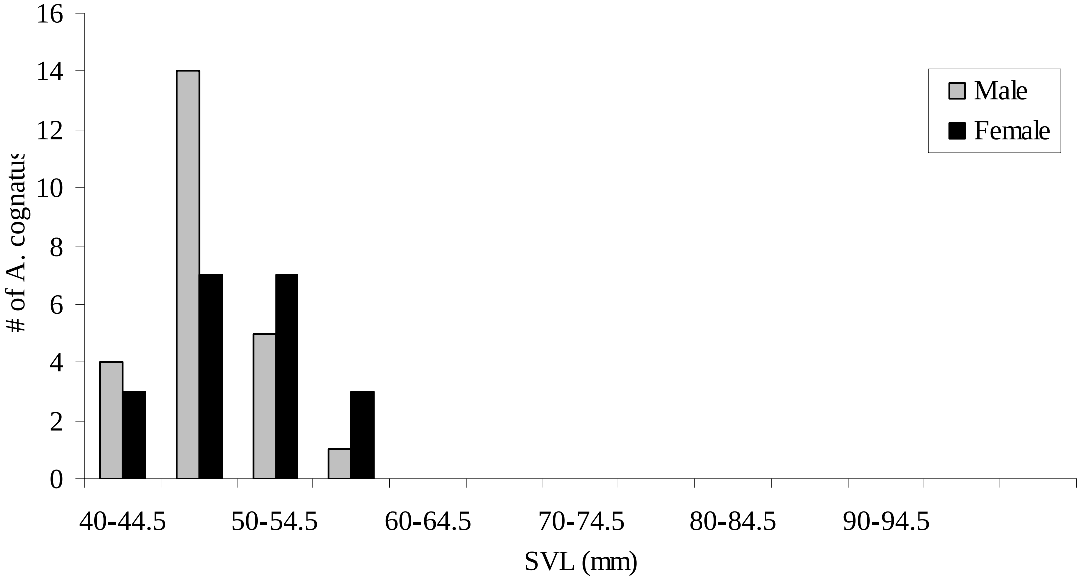


Fig. 2-10. Body size (SVL) distribution of adult male ($N = 20$) and female ($N = 24$) *A. cognatus* within the Valley. Adult Valley *A. cognatus* show a more clumped distribution in body size than populations outside the Valley, though distributions for both sexes are normal ($P > 0.05$)

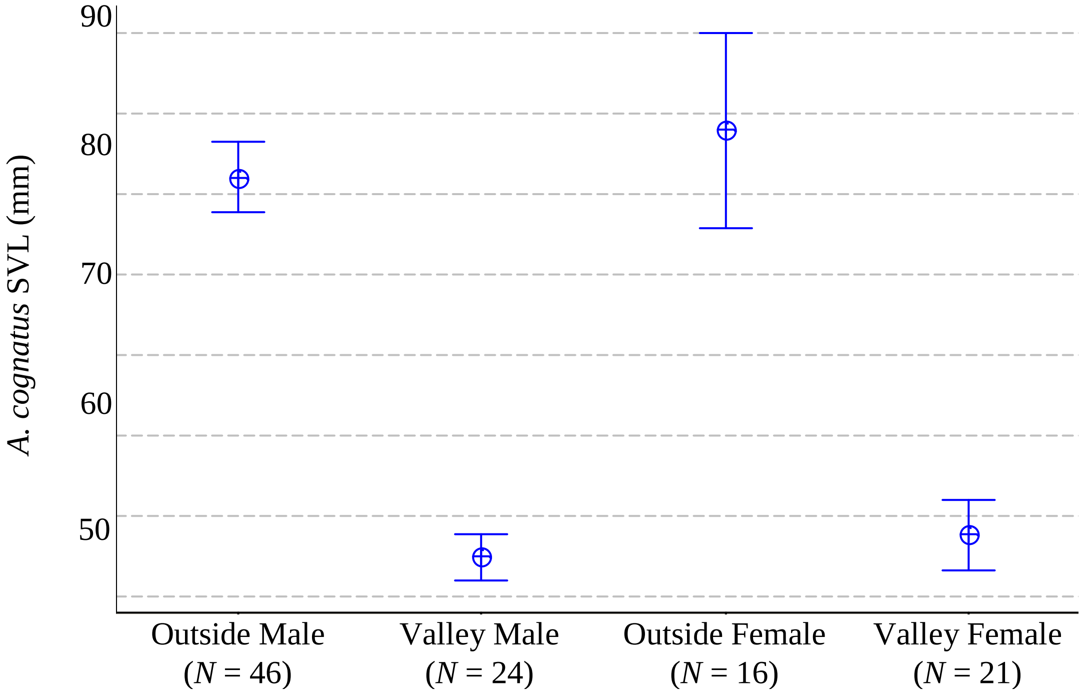


Fig. 2-11. Interval plot showing 95% CI for body size among adult male and female *A. cognatus* within and outside the Valley. Valley males and females are ~35% smaller than populations outside the Valley.

Both male and female populations within the Valley are disproportionately dwarfed for all 16 morphological features compared to outside male and female populations, respectively (Tables 2-8 and 2-9). As with the lizards, there are numerous allometric shifts among dwarfed *A. cognatus*; both males and females show significant deviations in proportions for all measured morphological features. These features correspond to locomotion (front and rear limb lengths), head dimensions (width, length), and other adaptive body parts including paratoid and tubercule shape. Based on features measured in both *P. hernandesi* and *A. cognatus,* body size reduction has had a more drastic shift in body form for *A. cognatus* than *P. hernandesi*. Compared to similarly sized individuals outside the Valley, *A. cognatus*

Table 2-8. MANCOVA using RegWQ for male museum *A. cognatus* showing allometric shifts among Valley populations compared to outside populations.

Morphological Feature	Grouping Mean Outside (*N* = 44)	Grouping Mean Valley (*N* = 24)	Critical Range	Significance
Head Width	3.350	2.920	0.035	**
Head Length	2.960	2.580	0.020	**
Occipital Distance	2.346	2.090	0.027	**
Eye-nose Distance	1.320	0.960	0.046	**
Internasal Distance	1.460	1.210	0.040	**
Tympanum Height	1.640	1.260	0.057	**
Tympanum Width	1.390	1.040	0.052	**
Femur Length	3.350	2.880	0.035	**
Tibia Length	3.310	2.850	0.018	**
Foot Length	3.780	3.360	0.024	**
Humerus Length	3.130	2.720	0.027	**
Radius Length	2.930	2.420	0.037	**
Hand Length	2.870	2.520	0.033	**
Paratoid Length	2.440	1.970	0.040	**
Paratoid Width	1.870	1.460	0.051	**
Tubercule Width	1.710	1.270	0.089	**

** $P < 0.03$

Table 2-9. MANCOVA using RegWQ for female museum *A. cognatus* showing allometric shifts among Valley populations compared to outside populations.

Morphological Feature	Grouping Mean Outside ($N = 15$)	Grouping Mean Valley ($N = 18$)	Critical Range	Significance
Head Width	3.360	2.990	0.065	**
Head Length	2.990	2.620	0.053	**
Occipital Distance	2.360	2.150	0.052	**
Eye-nose Distance	1.310	1.010	0.067	**
Internasal Distance	1.500	1.250	0.061	**
Tympanum Height	1.620	1.310	0.106	**
Tympanum Width	1.420	1.090	0.082	**
Femur Length	3.380	2.950	0.081	**
Tibia Length	3.340	2.900	0.076	**
Foot Length	3.770	3.410	0.068	**
Humerus Length	3.160	2.760	0.065	**
Radius Length	2.910	2.440	0.124	**
Hand Length	2.860	2.570	0.072	**
Paratoid Length	2.510	2.060	0.071	**
Paratoid Width	1.880	1.600	0.090	**
Tubercule Width	1.740	1.390	0.095	**

** $P < 0.03$

in the Valley is uniquely proportioned (MANCOVA RegWQ: males: $F_{14,5} = 31.44$, $P = 0.001$; females: $F_{14,11} = 3.09$, $P = 0.033$); Valley males show significant morphological divergence in 13 of the 14 morphological features and Valley females show morphological divergence for 10 of the 14 features (Fig. 2-12a-m).

Within the Valley, female and male *A. cognatus* are sexually dimorphic for all measurements associated with head (both shape and eye orbit) and paratoids in addition to femur length, humerus length, hand length, and tubercule width (Fig. 2-13; Tables 2-10 and 2-11). For all features, SSD is female-biased. Outside populations are reduced in the degree of sexual size dimorphism compared to Valley populations, showing SSD for only head length, paratoid length, and rear limb morphologies (Tables 2-11 and 2-12). The trend of increased in SSD among dwarfed *A. cognatus* parallels the increase in female-biased SSD among dwarfed *P. hernandesi*, particularly for head shape. Body size variation among Valley male and female *A. cognatus* is not significant (ANOVA: $F_{1,38} = 0.31$, $P = 0.579$) until covariated with mass (MANCOVA: $F_{1,137} = 4.03$, $P = 0.047$), where dwarfed females are significantly larger. In spite of the increased SSD in Valley populations, adult males and females show minimal variation in their respective growth slopes (Female: $Y = 10.191x-30.818$, Male: $Y = 11.122x-36.241$; Fig. 2-14). At smaller sizes, adult females are only slightly greater in mass than adult males and near 55 mm SVL, females and males have nearly identical masses for a particular body size. Data for live adult or toadlet *A. cognatus* outside the Valley are not available.

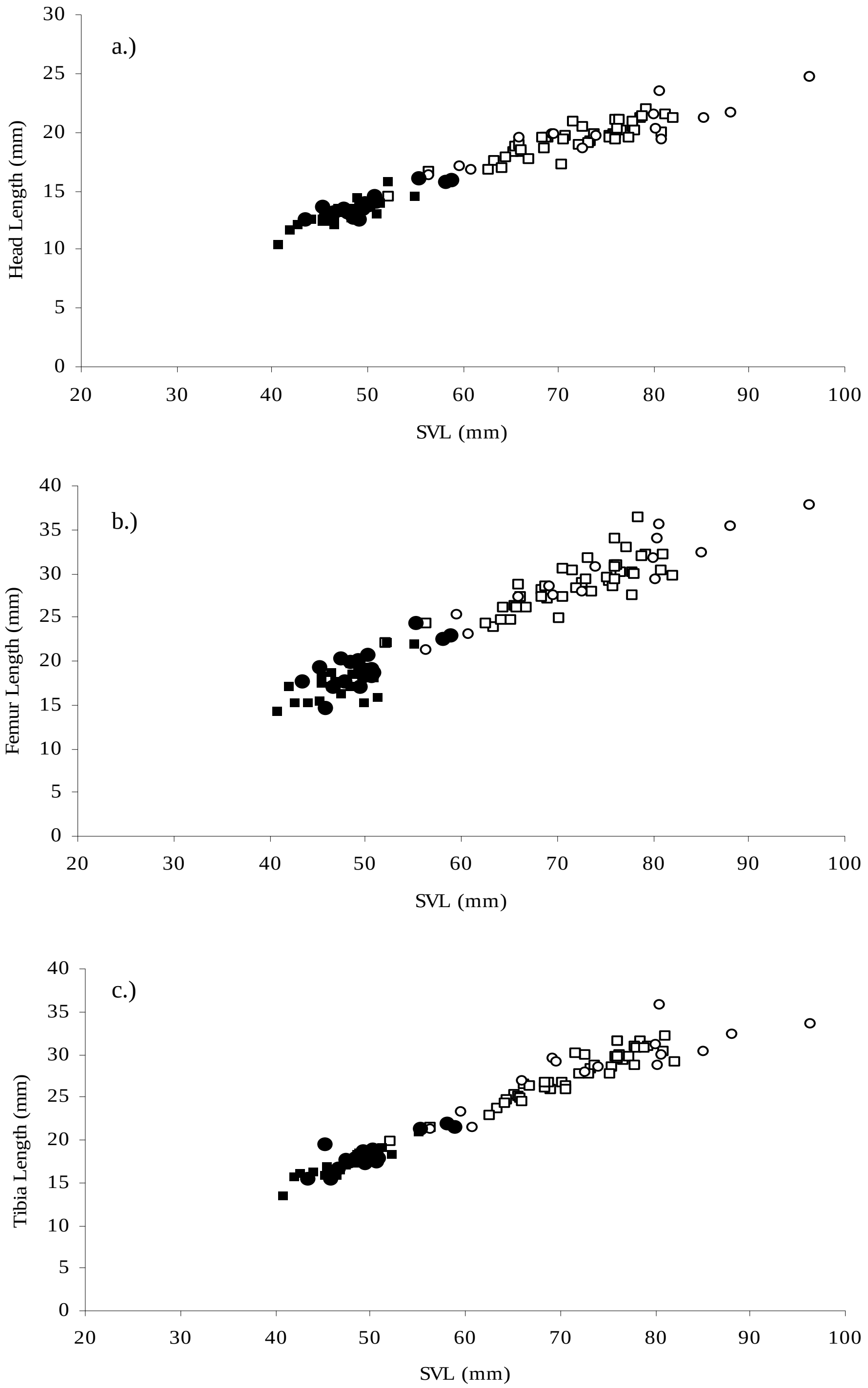
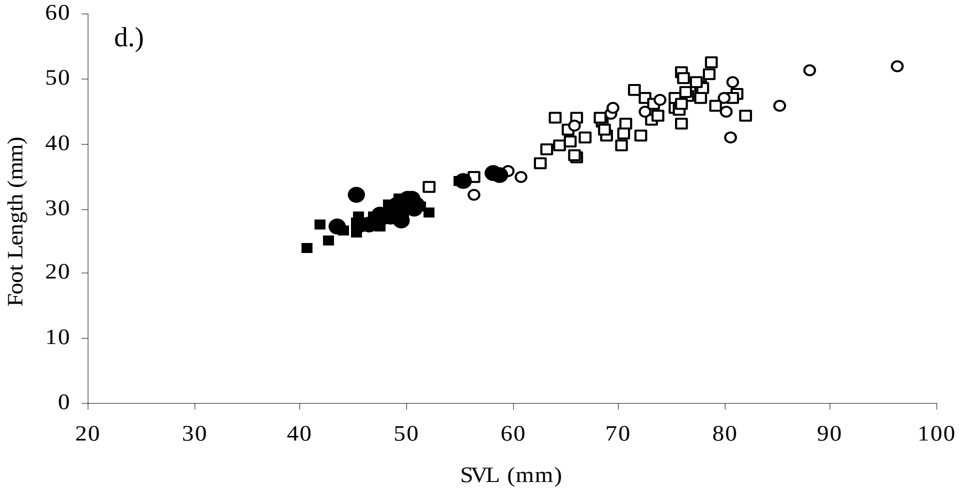
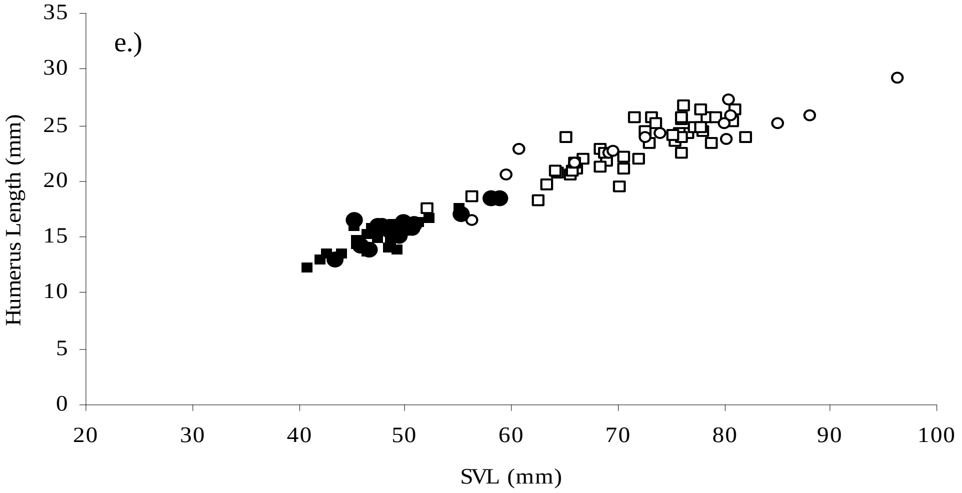
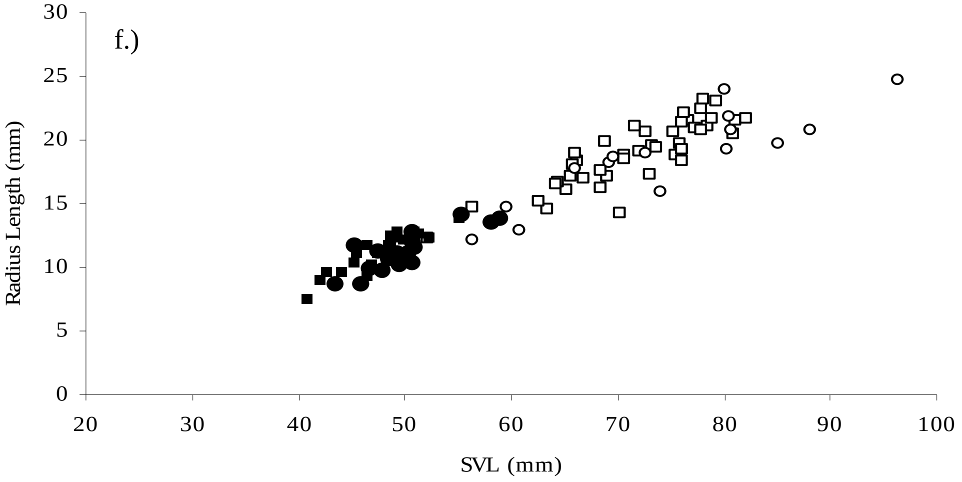


Fig. 2-12a-m Morphological variation of adult *A. cognatus* museum specimens outside and within the Valley. □ = outside male (*N* = 44), ○ = outside female (*N* = 15), ■ = Valley male (*N* = 24), ● = Valley female (*N* = 18). *Anaxyrus cognatus* shows substantial allometric shifts in body proportions and males and females are proportionally smaller for all measured features.







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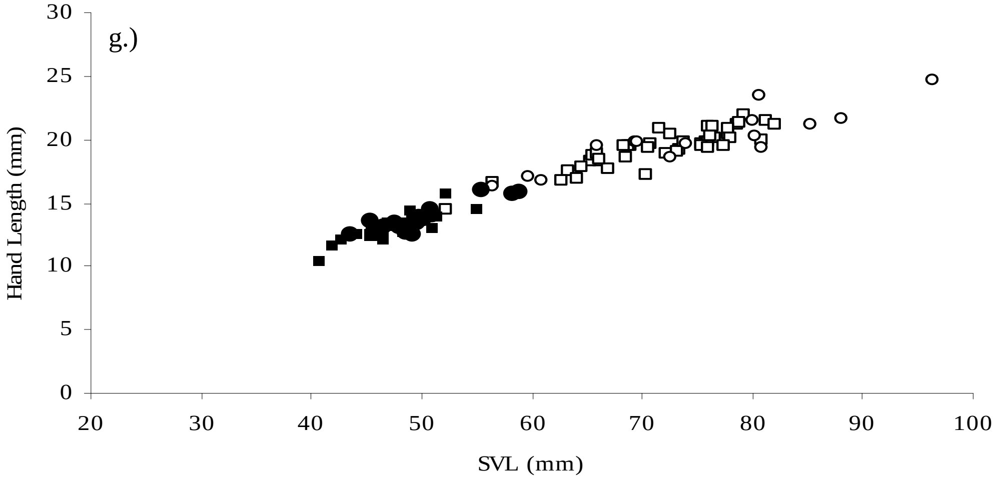

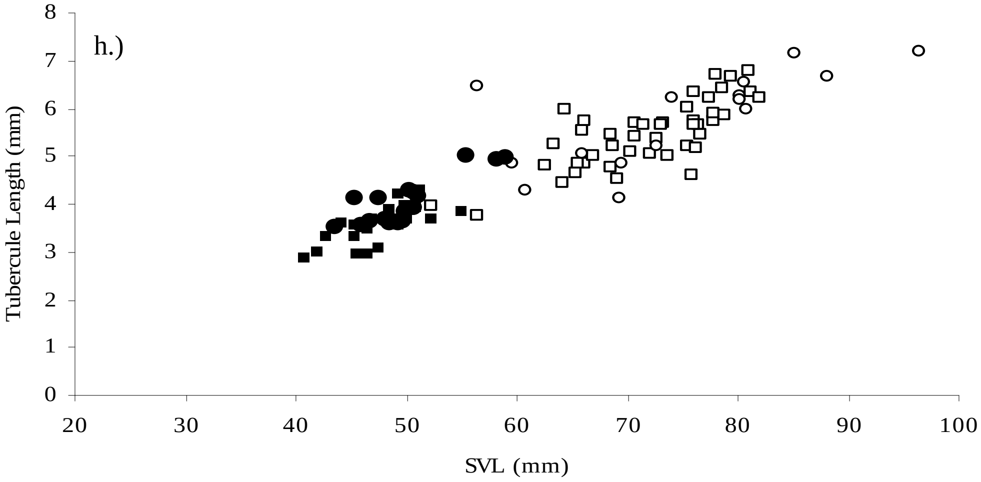

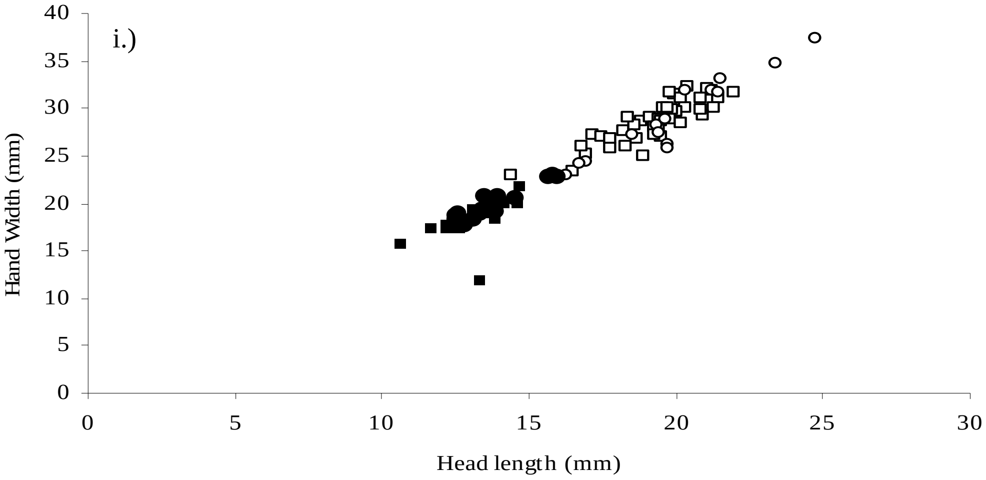

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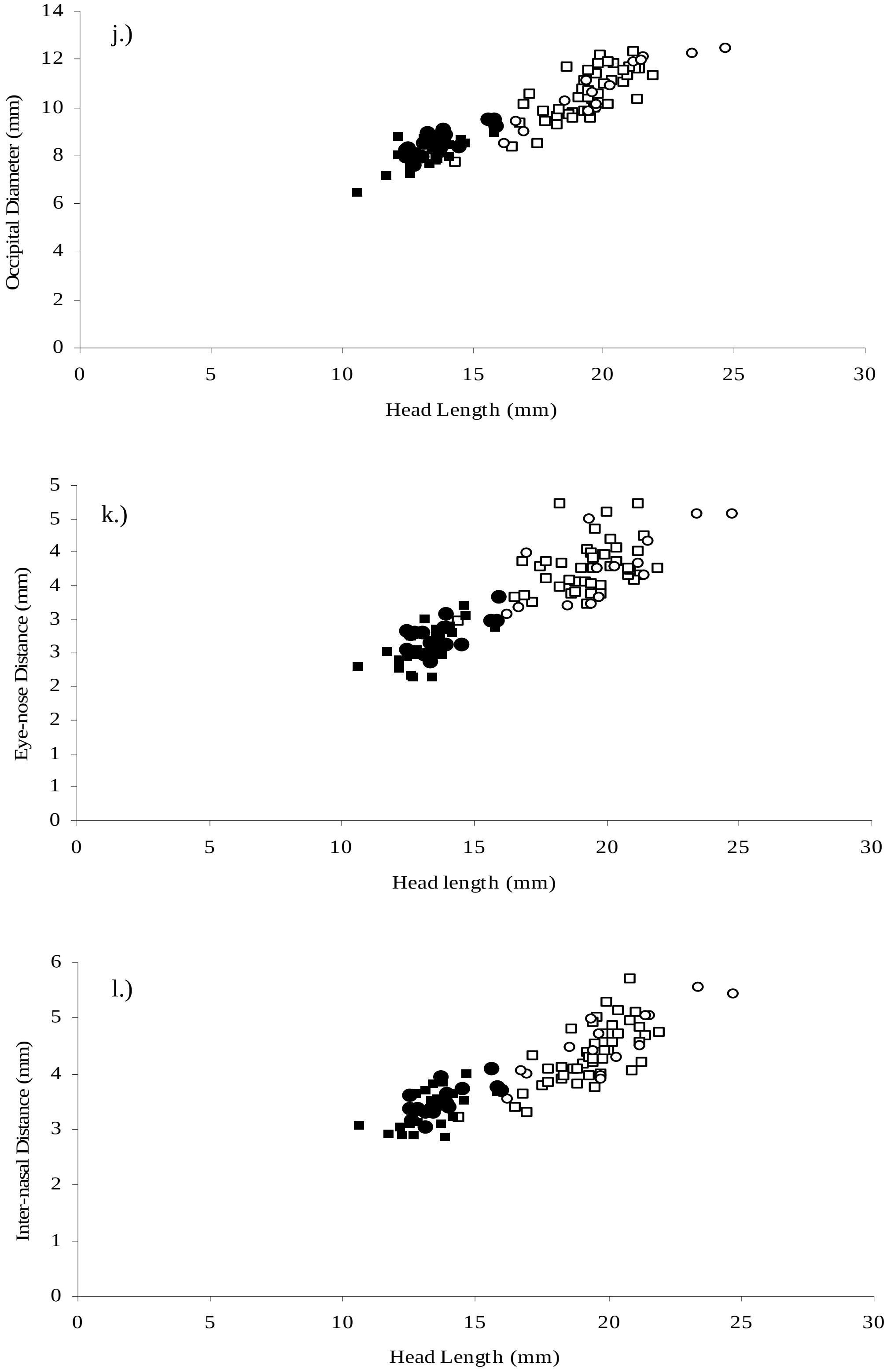

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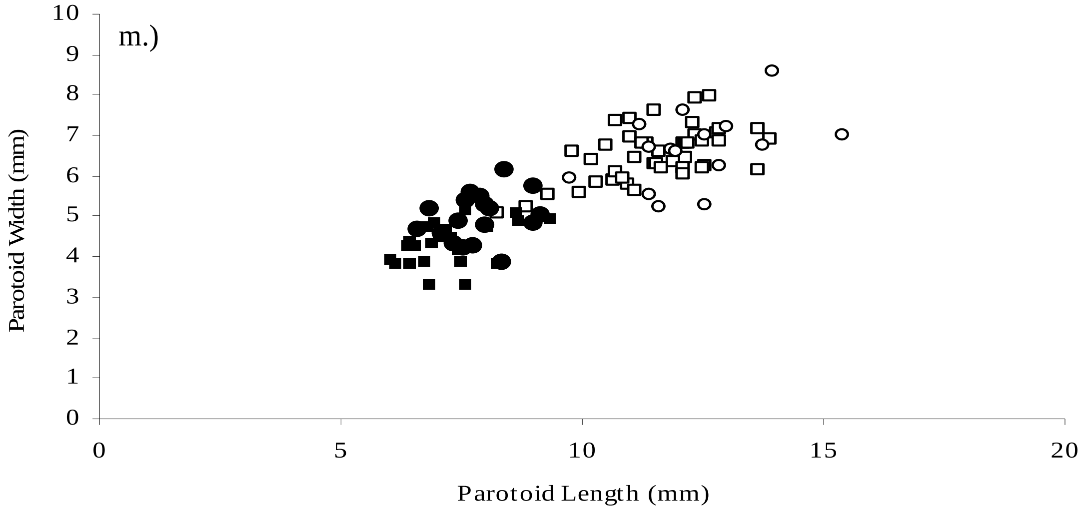

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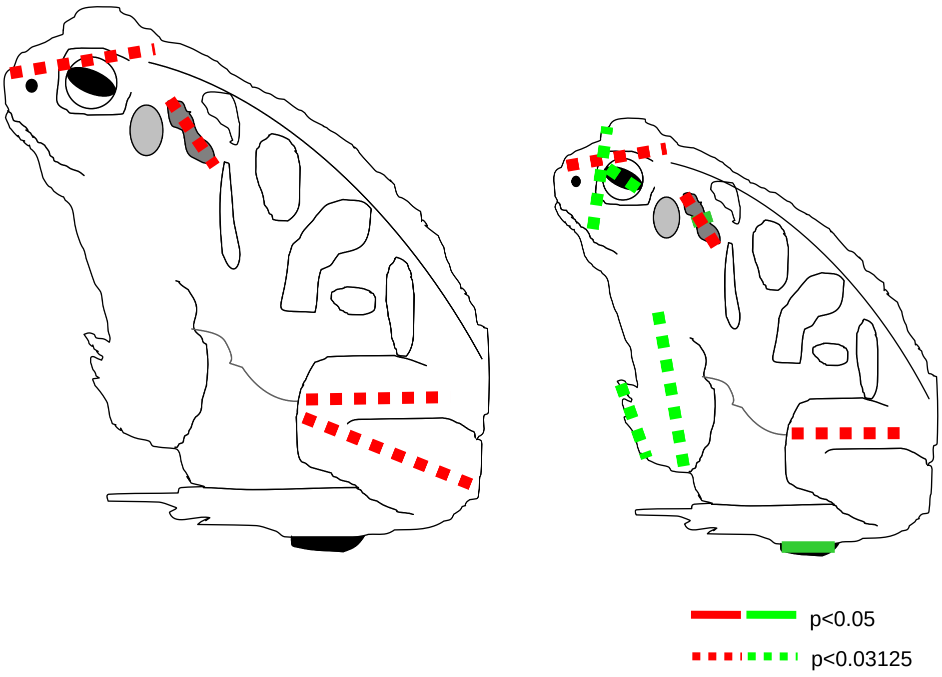

Fig. 2-13. SSD is increased among dwarfed populations of *A. cognatus*. Red indicates features that *A. cognatus* show SSD for both outside (left) and within (right) the Valley. Green indicates additional features for which Valley *A. cognatus* are sexually dimorphic.

Table 2-10. MANCOVA using RegWQ for adult Valley *A. cognatus* compared to subadults of equal sizes outside the Valley.

	Male				Female			
	Grouping Mean Outside SA	Grouping Mean Valley A			Grouping Mean Outside SA	Grouping Mean Valley A		
Morphological Feature	(*N* = 3)	(*N* = 18)	Critical Range	Significance	(*N* = 3)	(*N* = 24)	Critical Range	Significance
Head Width	2.986	2.796	0.108	**	3.050	2.919	0.126	*
Head Length	2.622	2.442	0.093	**	2.667	2.576	0.051	**
Occipital Distance	2.145	1.842	0.088	**	2.091	2.001	0.083	*
Eye-Nose distance	1.155	1.012	0.097	*	1.115	0.957	0.114	*
Inter-nasal Distance	1.250	1.128	0.090	*	1.212	1.177	0.097	
Femur Length	2.949	2.779	0.152	*	3.014	2.880	0.113	*
Tibia Length	2.896	2.693	0.138	*	2.972	2.851	0.049	**
Foot Length	3.411	3.190	0.113	**	3.441	3.362	0.046	**
Humerus Length	2.758	2.412	0.138	**	2.715	2.711	0.069	
Radius Length	2.445	2.201	0.244		2.584	2.422	0.085	**
Hand Length	2.572	2.163	0.110	**	2.583	2.518	0.084	
Parotoid Length	2.060	1.800	0.123	**	2.118	1.965	0.124	*
Parotoid Width	1.595	1.305	0.164	**	1.493	1.463	0.185	
Tubercule Length	1.389	1.202	0.152	*	1.404	1.274	0.097	*

* $P < 0.05$

** $P < 0.003$

Table 2-11. MANCOVA using RegWQ for outside museum *A. cognatus* showing SSD among adult males and females.

Morphological Feature	Grouping Mean Female ($N = 15$)	Grouping Mean Male ($N = 44$)	Critical Range	Significance
Head Width	3.360	3.350	0.026	
Head Length	2.990	2.960	0.024	*
Occipital Distance	2.362	2.350	0.030	
Eye-nose Distance	1.310	1.320	0.055	
Internasal Distance	1.500	1.460	0.048	
Tympanum Height	1.620	1.640	0.074	
Tympanum Width	1.420	1.390	0.068	
Femur Length	3.380	3.350	0.033	*
Tibia Length	3.340	3.310	0.029	*
Foot Length	3.770	3.780	0.035	
Humerus Length	3.160	3.130	0.035	
Radius Length	2.910	2.930	0.047	
Hand Length	2.860	2.870	0.040	
Paratoid Length	2.510	2.440	0.050	*
Paratoid Width	1.880	1.870	0.059	
Tubercule Width	1.740	1.710	0.116	

* $P < 0.05$

Table 2-12. MANCOVA using RegWQ for Valley museum *A. cognatus* showing SSD among adult males and females.

Morphological Feature	Grouping Mean Female ($N = 18$)	Grouping Mean Male ($N = 24$)	Critical Range	Significance
Head Width	2.990	2.920	0.064	*
Head Length	2.620	2.580	0.043	*
Occipital Distance	2.150	2.090	0.041	*
Eye-nose Distance	1.010	0.960	0.059	
Internasal Distance	1.250	1.210	0.053	
Tympanum Height	1.310	1.260	0.087	
Tympanum Width	1.090	1.040	0.075	
Femur Length	2.950	2.880	0.069	*
Tibia Length	2.900	2.850	0.054	
Foot Length	3.410	3.360	0.045	
Humerus Length	2.760	2.720	0.050	*
Radius Length	2.440	2.420	0.095	
Hand Length	2.570	2.520	0.052	*
Paratoid Length	2.060	1.970	0.067	*
Paratoid Width	1.600	1.460	0.076	**
Tubercule Width	1.390	1.270	0.065	**

* $P < 0.05$

** $P < 0.03$

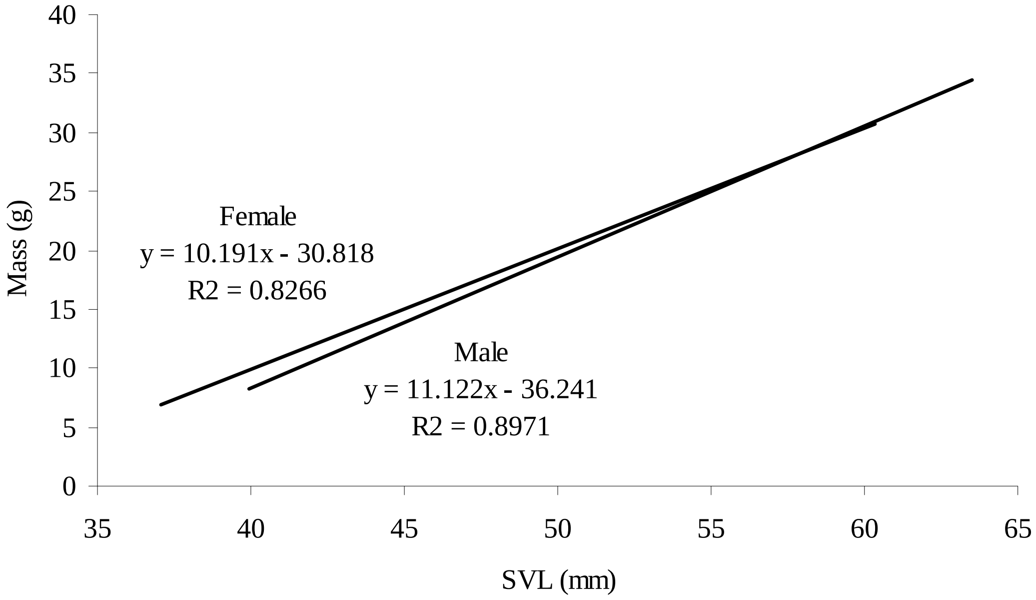

Fig. 2-14. Growth slope for adult *A. cognatus* within the Valley. Male and female *A. cognatus* show similar body size and mass proportions, especially above 55 mm SVL.

DISCUSSION

In concordance with Hahn's (1968) initial reporting of dwarfism, Valley populations of *P. hernandesi* and *A. cognatus* were found to be significantly dwarfed in this study. That male and female *P. hernandesi* are dwarfed the same percent in the Valley compared to outside populations suggests that the selective pressures influencing overall body size are experienced similarly by all Valley populations of *P. hernandesi* and *A. cognatus*, regardless of sex. Additionally, that both species are dwarfed nearly the same percent is curious. Because size differences are typically a result of both genetic differentiation and plastic response to local environmental conditions, there is (or a potential for) a wide variety of growth patterns among and within taxa. What is unknown is whether the dwarfing response of either species within the Valley is due to genetic variation from their non-dwarfed counterparts, differential environmental pressures within the Valley (plasticity), or a combination of these two variables. Regardless of the underlying cause(s), both sexes experience this overall body size shift nearly to the same degree and either scenario implicates that body size reduction is not sex-dependent.

A shift in body size or limb proportions has direct consequences on the performance of an organism. Limb length, for instance, correlates greatly with body size and can vary greatly across taxa, within populations, and among individuals (Garland and Losos, 1994). Lizards typically show negative allometry in hindlimb length (e.g. Pounds et al., 1983; Garland, 1985; Marsh, 1988). Limb length affects the capacity of lizard performance such as sprint speed, climbing ability, mobility, thermoregulation, and territorial behaviors (i.e. push-up displays). In *Callisaurus draconoides* (zebra-tailed lizard), long legs allow the lizard to increase its overall body height when in an elevated posture. This increase also functions to reduce heat load during temperature extremes and reduce the risk of predation (Muth, 1977). Having long legs is considered critical for the zebra tailed lizard since it occupies relatively open microhabitats.

For dwarfed *P. hernandesi* and *A. cognatus*, limb ratios with a differential allometry from outside populations can be expected to provide some advantage specific to within the Valley. Lizards have occupied the Valley approximately from at least 0.7-0.74 MYA while toads have occupied the Valley approximately from at least 0.8 MYA and based on data from the fossil records, both species were known to be dwarfed throughout these time periods (Rogers, 1987; Rogers et al., 1995; Rogers et al., 2000). This historic evidence further implicates that dwarfism in short-horned lizards and Great Plains toads within the Valley provides some advantage.

Dwarfed populations of *P. hernandesi* and *A. cognatus* show a drastic shift in body form compared to their non-dwarfed counterparts, suggesting that dwarfism has affected the overall form of both species. Scaling in organisms is commonly accompanied by proportional shifts in order for that feature to maintain its function. Similarly, it can be expected that a shorter head length will correspond to a shorter eye-nose distance because of both phylogenetic constraints and constraints on the organism's bauplan. Dwarfed females of both species have relatively smaller morphological features compared to their non-dwarfed counterparts, many of which are associated with locomotion. Females are potentially released from some (or have a new) selection pressure in the Valley that outside females do not experience. For instance, activity levels among dwarfed populations might have been reduced such that the pressure to maintain mobility levels equivalent to outside populations is not necessary.

In amphibians, neoteny occurs in many species, primarily aquatic amphibians that have indirect development (i.e. *Taricha torosa*, California Newt) while neoteny is less common or absent in other taxa (Stebbins and Cohen, 1995). Although dwarfed, Valley populations of *P. hernandesi* and *A. cognatus* are not juvenile forms of non-dwarfed, subadult populations outside the Valley. That Valley populations are not neotenic versions of their taxa further indicates the unique body form of Valley populations, particularly for *A. cognatus*.

There does not seem to be a clear trend in horn morphology within or among populations of *P. hernandesi*. Outside populations show minimal SSD for horn shape, while Valley populations show increased dimorphism. Though, horn length among dwarfed and non-dwarfed females shows disproportionate variation. The lack of a clear pattern in horn morphology suggests geomorpohometric analysis of horn shape might be more informative at revealing any pattern.

Geomorpohometric analysis is an effective measure to discern 3-D features because it allows for quantification of differences unaccountable in simple linear measurements (Blanckenhorn et al., 2009). Leaché et al. (2009) were able to quantify the visual discrepancy in horn morphology of *P. coronatum* (coast horned lizard) using geomorphometric analyses that was previously never realized. This analysis provided support in differences among significant groupings of populations into species throughout the range of *P. coronatum* and further supports evolutionary histories evidenced in genetics. However, horn morphology is difficult to perform in *P. hernandesi*, particularly Valley populations, as horn length is so greatly reduced. This difficulty was noted by Hahn (1968) when trying to distinguish Valley lizards as belonging to *P.d. hernandesi* or *P.d. ornatissimum*, the previously-recognized subspecies. Regardless, it is clear that in this study, horn morphology has been modified in Valley populations.

Sexual size dimorphism has been a key topic of interest in biology because it spans such a great number of taxa, has profound evolutionary implications, and can be highly variable, both within and among species (Fairbairn et al., 2009). Sexual size dimorphism is prevalent among horned lizards and when present, is always female-biased (Sherbrooke, 2003; Stebbins, 2003). Many Bufonids are also known to be sexually dimorphic, which is often in favor of females (Stebbins, 2003). In many taxa where females are the larger sex, SSD decreases with an increasing body size. Conversly, SSD increases when males are the larger sex. This relationship is known as Rensch's Rule. Although present in a wide array of taxa from plants to animals, not all species follow this trend; however, Rensch's rule is not thought to be the norm in insects, since only approximately half of insect orders show support of Rensch's rule (Blanckenhorn et al., 2009). Similar to insects, Valley populations of *P. hernandesi* and *A. cognatus* are exceptions to Rensch's rule.

Zamudio (1996) found that changes in SSD among lizards within the short-horned lizard clade (*P. hernandesi, P. douglasii, P. ditmarsi,* and *P. orbiculare*) correlate with evolutionary reductions in male size; because males become sexually mature at smaller sizes, female-biased SSD inherently increases. And, although female lizards do show body size shifts associated with reproductive output (clutch or litter sizes) in accordance with the fecundity advantage hypothesis (selection for larger females that confers a higher fecund advantage), changes in the amount of SSD among the lizard populations Zamudio (1996) studied are due to shifts in male body size. Supposing Valley males do sexually mature at a smaller size as evidenced previously (Zamudio, 1996), the narrow range in adult male size could be a result of selection for males that mature at smaller body sizes.

Additionally, Valley populations of *P. hernandesi* retain all of the SSD features found among outside populations but also show a large increase in SSD for all additional morphological features measured. Dwarfed females appear to be most dimorphic for features associated with locomotion and head morphology. And, SSD is in favor of females for all features except tail length. Sexual size dimorphism of locomotor features is typically biased towards males, since males traverse the landscape in search of females for mating, endure intraspecific combats for mate competition, battle for territory, and encounter more predators because of their increased movement activities (Stark et al., 2005). It is interesting that in the Valley, dwarfed females are favored over dwarfed males for the morphological features typically associated with locomotion. It is possible that the limb proportions observed actually do not increase the performance of dwarfed females compared to dwarfed males, but no performance trials of these features were conducted.

Morphological divergence can result from a shift in habitat, diet, reproductive role, or environmental variable(s) that impose selection on a group of organisms (e.g. Langerhans et al., 2003; Bermer et al., 2008; Fairbairn et al., 2009). Male fitness is often determined by the ability of males to encounter and mate with females, a measure which is based off of locomotor ability (Ghiselin, 1974; e.g. Blanckenhorn et al., 1995). On the other hand, females having larger abdomens are often favored, a measure based off of fecundity, or number/size of offspring (Goodman et al., 2009). Thus, selection becomes counterbalanced since the gain of locomotor capacity is inversely correlated with abdomen size. Such features under more direct selection are more pronounced among males and females than features less important to sex (i.e. structural features such as neck length).

Degree of SSD is also known to vary in response to environmental conditions (Teder and Tammaru, 2005); SSD increases when environmental conditions approach the optimum and body size of the larger sex (typically females) increases. Thus, when SSD is female-biased, variability in female body size is greater; the larger sex typically responds more strongly to shifts in environmental conditions and therefore deviates a greater amount from its optimal size than the opposite sex (Blanckenhorn et al., 2009). Sex-based constraints are different for males versus females, such that conditions allowing for an optimal body size in one sex may hinder or provide no advantage towards the optimum for the opposite sex. For instance, Berry and Shine (1980) found the direction of SSD in 76 taxa correlates with habitat type and that increased body sizes lead to increases in SSD; however, other studies have found no correlation between body size and dimorphism (Gibbons and Lovich, 1990). This lack of correlation was attributed to size at maturity based on selective pressures (sexual and natural). Similarly, SSD might be present simply due to phylogenetic inertia since SSD is deeply rooted in the radiation of horned lizards (Zamudio, 1996).

As with dwarfed *P. hernandesi*, dwarfed *A. cognatus* exhibit greater SSD than their non-dwarfed counterparts; however, the amount of SSD present in either population is reduced when a conservative statistical correction factor is applied. The application of this correction factor is arguable, but I present it to retain a conservative measure. Regardless, dwarfed *A. cognatus* do show an increase in SSD that is contrary to Rensch’s rule.

Although female *A. cognatus* are still larger in overall size than males, the lack in SSD might be attributable to varying life histories of males compared to females. The life history of *A. cognatus* varies from *P. hernandesi*, largely in the role of the female during reproductive activity and associated life-history characteristics.

For example, significantly larger body cavity (and therefore body size; Goodman et al., 2009) is not necessarily as critical for *A. cognatus* compared to *P. hernandesi*, since *A. cognatus* produces eggs that only temporarily develop within the female. And, supposing dwarf females have adapted their reproductive strategies by producing fewer eggs, smaller eggs, or retaining the eggs for a shorter developmental period, the degree of SSD in overall body size could further be reduced since selection for larger body size would be reduced.

Growth slopes are important because they show the point at which sexes start investing more energy into mass gain versus growth gain. This knowledge can provide evidence towards causes underlying SSD or body size in organisms. Females and males are expected to follow different trajectories, largely because of the different reproductive investments made by each sex; females must increase body size to support developing fetuses which often correlates with having a larger mass while males often cease growth upon sexual maturity to maximize their reproductive potential (at least in species where the male is the smaller sex and therefore a lack of competition for body size) (Zamudio, 1998). Females are overall larger and heavier than males both within and outside the Valley, a trend common to taxa with larger females. Dwarfed *P. hernandesi* of both sexes are heavier than their non-dwarfed counterparts at a particular size, suggesting that there is a higher investment into mass than size in the Valley for both sexes.

Neonates are often under great selective pressure their first winter season, particularly if they must endure long overwintering periods (e.g. Vitt, 1974). To improve their chances of survival, neonates should gain a higher mass relative to size.

Energetic reserves will be greater (since they are usually born relatively late in the activity season) and they will have a higher surface area to volume ratio where they respond more slowly to rapid climate shifts (larger organisms retain their heat longer, and thus take longer to cool; Schmidt-Nielsen, 1984). Having a higher mass is thought to increase survival chances for neonates, particularly in harsh environments and/or periods of long overwintering (Vitt, 2000). In the Valley, nighttime freezing begins as early as October and remains as late as May (see Chapter 3). To survive the harsh Valley winters, neonates would be expected to have a high mass gain to size gain ratio; however, Valley neonates show the opposite; mass gain from the time of birth (mid-July to late-August) to the onset of overwintering (mid-September to mid-October; typically during the onset of freezing nighttime temperatures) is minimal. In fact, adult *P. hernandesi* have a higher mass gain than neonates which is not expected. This is a curious finding and contradicts my expected observation.

Live *P. hernandesi* and *A. cognatus* show many of the same allometric trends as museum specimens. Overall, dwarfed males and females have relatively smaller morphological features than outside males and females, respectively. Dwarfed lizard and toads also maintain SSD observed in outside lizards but gain SSD for additional features. Again, because males and females of both species are dwarfed nearly the same degree, the selective force(s) acting upon either species is/are currently are experienced equally by both sexes.

While not all morphological features show the same statistical significance as museum specimens, measurements on live specimens could simply be different and/or there could be a greater degree in measurement error with live *P. hernandesi*.

Museum specimens are known to shrink upon preservation and there is variability in shrinkage based upon age, type of preservation, storage conditions, amount of tissue subject to shrinking, type of body part (a heavily calcified/bony skull versus a fatty abdomen) and physical condition prior to preservation (i.e. dessication of road kill specimens versus euthanasia after multiple days in captivity without food) (e.g. Bart et al., 2009). The variability in measurement conditions for live and museum specimens is not considered critical to the results, since museum and live specimens were compared separately.

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CHAPTER 3

GENETIC VARIATION AMONG DWARFED POPULATIONS OF *PHRYNOSOMA HERNANDESI* AND *ANAXYRUS COGNATUS* IN THE SAN LUIS VALLEY, COLORADO

INTRODUCTION

Phylogenetics enable the reconstruction of evolutionary time scales to reveal evolutionary histories, interpret current relationships among taxa, and assess the evolutionary trajectory on which a species is headed (Felsenstein, 2003). When assessing a species for such variation, differentiation in morphology, behavior, physiology, and/or life/natural-history of organisms can be indicative of a unique evolutionary history. Though, this differentiation can be confounded by plasticity which typically occurs at a more local and smaller time scale, such as varying body sizes over elevational and latitudinal gradients. A unique evolutionary history only exists when there are underlying differences in the genetic structure, such as mutations, transversions, and substitutions. Often, there is an interplay of environmental and genetic interactions that contributes to differentiation among a group of organisms.

One environmental mechanism that often promotes increased rates of species diversification is topographic complexity, such as mountain ranges, island systems, and rivers (e.g. MacArthur and Wilson, 1967; Barraclough et al., 1998; Volger et al., 1998). For instance, relict populations of pupfish that once belonged to a larger lake system known as Manly Lake, have been separately evolving in localized pools throughout the former lake bed for approximately 60,000 yrs (Duvernell and Turner, 1988; Echelle and Dowling, 1992). Accounting for the evolution of a species via geologic history of the landscape is important because topography is directly associated with climate and habitat, and all three variables can either limit or facilitate species evolution (i.e. migration capacity and occurrence across the landscape).

The San Luis Valley is a unique land form inhabited by dwarfed populations of *P. hernandesi* and *A. cognatus*. Dwarfism among both species is prevalent though it is not known whether populations of either species are genetically distinct or isolated within the Valley. Understanding the history of the Valley formation allows inferences to be made regarding its role in the evolution, colonization history, and taxonomic identity of both species.

The San Luis Valley is an extensional fault-bounded basin that formed over the last approximately 30 MYA in response to rifting. Uplift of the surrounding fault footwall blocks produced high mountain ranges exceeding 4,300 m peak elevation (Tweto, 1979; Kluth and Schaftenaar, 1994). As the rift evolved, sediments eroded from the surrounding mountain ranges have filled the basin to depths of 6,000-9,000 m (Davis and Keller, 1978; Tweto, 1979). Today, there are 5 physiographic subdivisions within the Valley defined by unique topography and geology (Upson, 1939; Fig. 3-1): 1.) Alamosa basin (flat-lying deposits over feet 6,400 km thick that constitutes the majority of the northern half of the Valley), 2.) San Luis hills (a rugged, nearly continuous hill formation 800-1,600 m relief that traverses east to west just below the middle of the Valley), 3.) Taos plateau (extends from the San Luis hills 100 km south into New Mexico and contains broadly-rolling, highly-eroded hills that

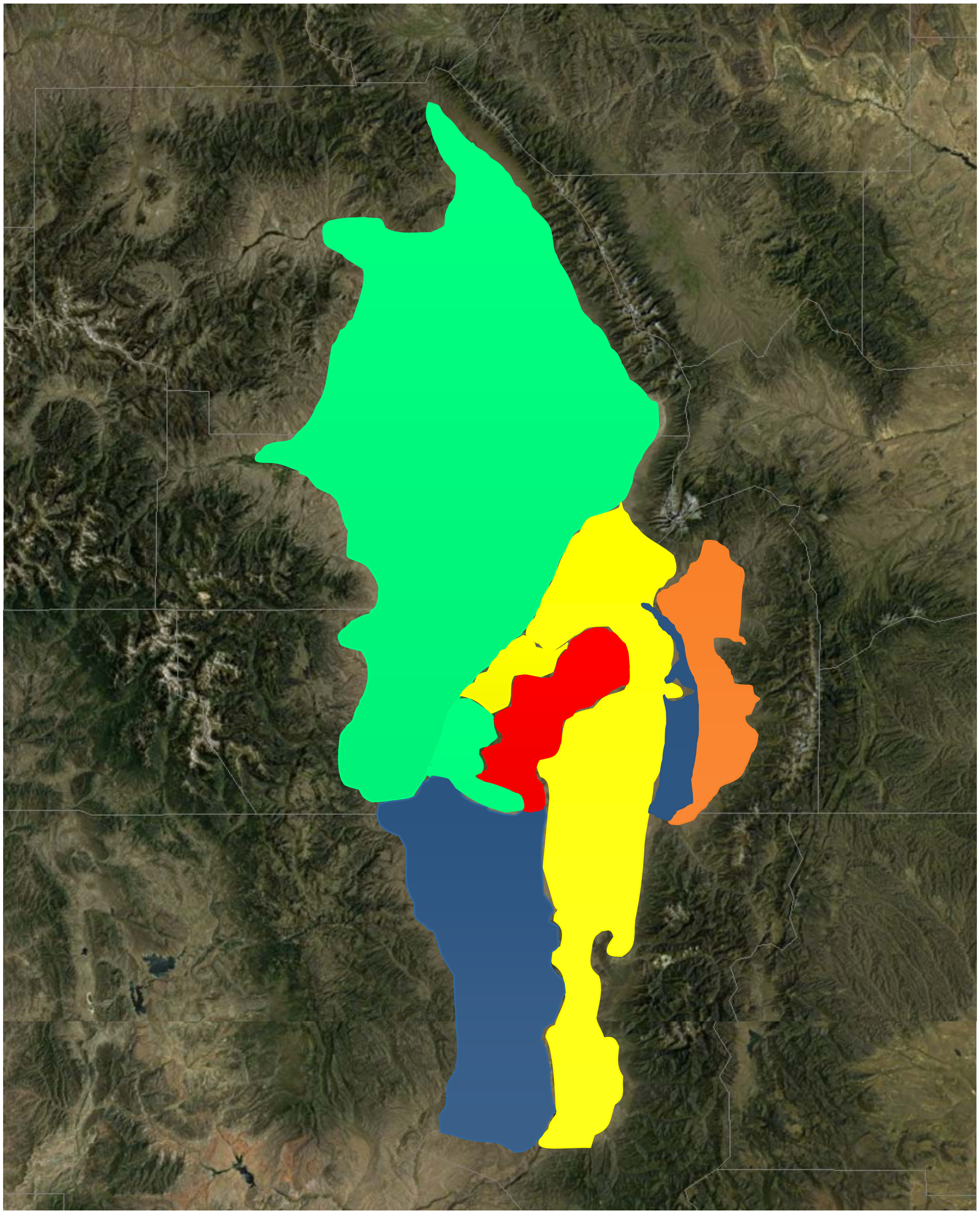


Fig. 3-1. Physiographic subdivisions within the Valley based on unique geology and topography (adapted from Upson, 1939). Green = Alamosa formation, yellow = Costilla plains, red = San Luis Hills, blue = Taos plateau, orange = Culebra reentrant.

rest above the plateau), 4.) Costilla plains (a 30 m deep floodplain terrace east of the Taos plateau), and 5.) Culebra reentrant (a region along the eastern portion of the Valley with a lot of dissection from geologic activity). Both *P. hernandesi* and *A. cognatus* inhabit primarily the Alamosa Basin subregion (Hammerson, 1999; Degenhardt et al., 1996). Since the Valley forms a closed system north of the New Mexico border, many species are potentially confined within the Valley. There are at least 14 endemic species and subspecies known in the Valley, including insects, plants, and mammals (Armstrong, 1971; Fitzgerald et al., 1994; Riley, 1998). Although genetic data are lacking for many plant and animal species within the Valley, it is likely that many species, particularly those with limited dispersal capacity and endemic species, have unique genetic diversities as a result of genetic drift or selection (MacArthur and Wilson, 1967).

Within the Valley, *P. hernandesi* and *A. cognatus* show morphological discontinuity in both overall body size and allometric scaling (Chapter 2), indicating that there is potentially a genetic component associated with the dwarfism. Given the geologic history of the Valley, the presence of other endemic species and subspecies within the Valley, and the life/natural-history of both species (i.e. dispersal and migration capacities, habitat requirements), it is likely that there is at least some genetic sub-structuring unique among Valley populations of *P. hernandesi* and *A. cognatus*. Additionally, it is likely that the genetic sub-structuring, if present, corresponds with the formation of the Valley's five physiographic provinces.

I test whether there are any underlying genetic variations among Valley populations of *P. hernandesi* and *A. cognatus* using mitochondrial DNA. If there is a unique genetic structure in Valley populations, then it is likely that they have historically been present in the Valley, since fossil records indicate the presence of dwarfed populations of *P. hernandesi* and *A. cognatus* up to 0.8 MYA (Rogers et al., 1985; Rogers, 1987; Rogers et al., 2000). Given the geologic formation of the Valley, I expect both species to show genetic divergence from populations surrounding the Valley, suggesting that the Valley functions as a reproductive barrier.

METHODS

Population Sampling

Phrynosoma hernandesi and *A. cognatus* were collected throughout the San Luis Valley and surrounding area from May to August in 2007 and 2008 (Table 3-1). Tissue was taken from live specimens either via tail clips (*P. hernandesi*) or toe clips (*A. cognatus*). Tissue samples were obtained for a total of 11 lizards from six populations within the Valley, 1 lizard from each of four populations surrounding the Valley, and 2 toads from two populations within the Valley (Table 3-1).

Molecular Data

Tissue samples were air-dried and stored in a freezer until return to the lab where samples were stored at -80°C. Total genomic DNA was extracted following the salting-out method adapted from Sunnucks and Hales (1996). Tissue samples were homogenized in 300µl of TNES buffer (ddH_2O, 1M Tris, 5M NaCl, 0.5M EDTA, and 10% SDS) with 7µl of Proteinase K (20µl/ml) and incubated at 55°C overnight. Then, 85µl of chilled 5M NaCl was added and the homogenate was then

Table 3-1. Collection localities and sample sizes for genetic samples of *P. hernandesi* and *A. cognatus*. UTM zone is 13N and datum is WGS84.

Species	Locality	Easting	Northing	Elevation (m)	# Samples
P. hernandesi	Zapata Ranch (Valley)	443361	4166208	2323	6
	Medano Ranch SE Corner (Valley)	443262	4169055	2299	1
	Alamosa NWR/hwy 160 (Valley)	437453	4143714	2293	1
	McIntyre Springs (Valley)	428300	4126559	2323	1
	Capulin BLM (Valley)	486415	4121035	2508	1
	Saguache (Valley)	486469	4108576	2490	1
	Mosca Pass (Outside)	459468	4176159	2905	1
	Forbes Trinchera (Outside)	467624	4143735	2700	1
	Walsenburg (Outside)	532172	4155332	1891	1
	Pawnee National Grasslands (Outside)	522269	4521935	1657	1
A. cognatus	San Luis Lakes (Valley)	435391	4169055	2350	1
	Blanca Wetlands (Valley)	441206	4159014	2303	1

vortexed and centrifuged (14,000 rpm) for 10 min. The supernatant was removed, 1 volume of chilled 100% EtOH was added, and centrifuged (14,000 rpm) for 10 min to pellet the DNA. The pellet was washed with another volume of chilled 70% EtOH and centrifuged (14,000 rpm) for 5 min to ensure removal of all debris. The resulting pellet was air-dried overnight and resuspended in 50µl of TE buffer. DNA samples were permanently stored at -80°C.

I used polymerase chain reaction (PCR) to amplify the ND4 and 16S/tRNA mitochondrial genes from *P. hernandesi* and *A. cognatus*, respectively. Mitochondrial DNA was used since any divergence among populations likely occurred more historically and both genes have previously been used to show divergence among taxa within the genera *Phrynosoma* (i.e. Zamudio et al., 1997; Leaché and McGuire, 2006) and *Anaxyrus* (i.e. Pauly et al., 2004). Primers ND4 and Leu were used to amplify the ~800-bp ND4 gene (Zamudio et al., 1997; Arévalo et al., 1994) and primers Bw16S-L and Bwlle-H were used to amplify the ~1200-bp 16S and partial tRNA gene (Kumazawa and Nishida, 1993; Masta et al., 2002). Primer sequences are listed in Table 3-2. Each PCR was carried out in a 50µl volume: 5.0µl of each primer (10mM), 5.0µl 10X buffer containing 25mM $MgCl_2$, 5.0µl 20mM dNTPs, 0.2µl Qiagen TAQ buffer, 28.8µl ddH_2O, and 1.0µl DNA template (1:10 dilution of DNA). For *P. hernandesi*, PCR conditions included denaturation at 93°C for 60s, annealing at 53.2°C for 30 sec, and extension at 72°C for 2 min for 35 cycles (Zamudio et al., 1997). For *A. cognatus*, PCR conditions included denaturation at 94°C for 30 sec, annealing at 54°C for 45 sec, and extension at 72°C for 75 sec for 35 cycles (Masta et al., 2002). PCR products were purified using Qiagen QIAquick PCR

Table 3-2. Primers used to sequence mtDNA in *A. cognatus* and *P. hernandesi*. Primer position refers to 5' primer end relative to the published *Xenopus laevis* mitochondrial genome (Roe et al., 1985).

Species	Primer	Gene Region	Xenopus Position	Source	Sequence
A. cognatus	Bw16S-L	16S	4604	Masta et al., 2002	5' ATT TTT TCT AGT ACG AAA GGA 3'
A. cognatus	B-Ile-H	tRNA^{Ile}- ND1	5779	Masta et al., 2002	5' GCACGT TTC CAT GAA ATT GGT GG 3'
P. hernandesi	ND4	ND4	12900	Arevalo et al., 1994	5' CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC 3'
P. hernandesi	Leu	ND4	13857	Arevalo et al., 1994	5' AC CAC GTT TAG GTT CAT TTT CAT TAC 3'

purification kit (Qiagen Inc., Valencia, CA) and quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Gene fragments for both forward and reverse directions were sequenced at Nevada Genomics Center (Reno, NV) using an ABI PRISM® 3730 DNA Analyzer.

Phylogenetic Analysis

Using Sequencher 4.2 (Gene Codes Corp, Ann Arbor, MI), I aligned and edited sequences within individuals using the forward and reverse sequence and across taxa by combining sequenced individuals with other *Phrynosoma* or *Anaxyrus* taxa. To conduct genus-wide analyses, I downloaded published mtDNA sequences for *P. hernandesi* and *A. cognatus* from GenBank (Table 3-3). Previous analyses of mtDNA sequences in both *P. hernandesi* and *A. cognatus* indicate these gene regions are not pseudogenes (Masta et al., 2002; Zamudio, 1996). A total of 908 base pairs was analyzed for *P. hernandesi* and a total of 1,402 base pairs was analyzed for *A. cognatus*.

I used MrModelTest to create nucleotide substitution models for *Phrynosoma* using a hierarchical likelihood ratio test (v3.04; Posada and Crandall, 1998). The goodness of fit for each model was tested using Akaike Information Criterion (AIC; Akaike, 1974; Posada and Buckley, 2004). The best-fit model for *P. hernandesi* was TVM+I+G (-lnL = 5460.66, K = 9, AIC = 10939.52). Base frequencies used as input parameters for the best-fit model were as follows: A = 0.3668, C = 0.2700, G = 0.0967, T = 0.2666, proportion of invariables sites (I) = 0.4809, and gamma distribution shape parameter = 1.642. The best-fit model for *A cognatus* was HKY (-

Table 3-3. GenBank Accession numbers and collection data for sequences used in this study.

Old Taxonomy	Current Taxonomy	GenBank #	Country	State	County	Source
P. ditmarsi	*P. ditmarsi*	PDU71591	Mexico	Sonora	-	Zamudio et al., 1997
P. ditmarsi	*P. ditmarsi*	PDU71595	Mexico	Sonora	-	Zamudio et al., 1997
P. ditmarsi	*P. ditmarsi*	AY141051.1	Mexico	Sonora	-	Hodges and Zamudio, 2004
P.d. brevirostre	*P. hernandesi*	PDU71571	USA	Colorado	Weld	Zamudio et al., 1997
P.d. brevirostre	*P. hernandesi*	AY141053.1	USA	Colorado	Weld	Hodges and Zamudio, 2004
P.d. brevirostre	*P. hernandesi*	PDU71569	USA	Arizona	Cochise	Zamudio et al., 1997
P.d. brevirostre	*P. hernandesi*	PDU71580	USA	Montana	Rosebud	Zamudio et al., 1997
P.d. brevirostre	*P. hernandesi*	PDU71564	USA	North Dakota	Sioux	Zamudio et al., 1997
P.d. brevirostre	*P. hernandesi*	PDU71585	Canada	Alberta	-	Zamudio et al., 1997
P.d. brevirostre	*P. hernandesi*	PDU71592	USA	Wyoming	Johnson	Zamudio et al., 1997
P.d. brevirostre	*P. hernandesi*	PDU71579	USA	South Dakota	Harding	Zamudio et al., 1997
P.d. brevirostre	*P. hernandesi*	PDU71586	Canada	Alberta	-	Zamudio et al., 1997
P.d. brevirostre	*P. hernandesi*	PDU71584	Canada	Alberta	-	Zamudio et al., 1997
P.d. douglassii	*P. douglasii*	PDU71573	USA	California	Siskiyou	Zamudio et al., 1997
P.d. douglassii	*P. douglasii*	AY141052.1	USA	California	Siskiyou	Hodges and Zamudio, 2004
P.d. douglassii	*P. douglasii*	PDU71567	USA	California	Siskiyou	Zamudio et al., 1997
P.d. douglassii	*P. douglasii*	PDU71593	USA	Oregon	Harney	Zamudio et al., 1997
P.d. douglassii	*P. douglasii*	PDU71581	USA	Oregon	Harney	Zamudio et al., 1997
P.d. douglassii	*P. douglasii*	PDU71583	USA	Oregon	Linn	Zamudio et al., 1997
P.d. douglassii	*P. douglasii*	PDU71570	USA	Washington	Kittitas	Zamudio et al., 1997
P.d. douglassii	*P. douglasii*	PDU71594	USA	Oregon	Morrow	Zamudio et al., 1997
P.d. douglassii	*P. douglasii*	PDU71582	USA	Washington	Lincoln	Zamudio et al., 1997
P.d. douglassii	*P. douglasii*	PDU71557	USA	Oregon	Bonneville	Zamudio et al., 1997

Table 3-3 Continued.

Old Taxonomy	Current Taxonomy	GenBank #	Country	State	County	Source
P.d. hernandesi	*P. hernandesi*	PDU71563	USA	Utah	Garfield	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71561	USA	Utah	Kane	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71597	USA	Utah	Kane	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71562	USA	Utah	Garfield	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71596	USA	Utah	Iron	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71551	USA	Arizona	Mohave	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71560	USA	Arizona	Coconino	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71565	USA	Arizona	Coconino	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71568	USA	Arizona	Gila	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71576	USA	Utah	Grand	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71588	USA	Arizona	Cochise	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71590	USA	Arizona	Cochise	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71589	USA	Arizona	Cochise	Zamudio et al., 1997
P.d. hernandesi	*P. hernandesi*	PDU71587	USA	Arizona	Cochise	Zamudio et al., 1997
P.d. ornatissimum	*P. hernandesi*	PDU71553	USA	New Mexico	Otero	Zamudio et al., 1997
P.d. ornatissimum	*P. hernandesi*	PDU71556	USA	New Mexico	Catron	Zamudio et al., 1997
P.d. ornatissimum	*P. hernandesi*	PDU71558	USA	Arizona	Apache	Zamudio et al., 1997
P.d. ornatissimum	*P. hernandesi*	PDU71559	USA	Arizona	Apache	Zamudio et al., 1997
P.d. ornatissimum	*P. hernandesi*	PDU71552	USA	New Mexico	Taos	Zamudio et al., 1997
P.d. ornatissimum	*P. hernandesi*	PDU71554	USA	New Mexico	Taos	Zamudio et al., 1997
P.d. ornatissimum	*P. hernandesi*	PDU71572	USA	New Mexico	Taos	Zamudio et al., 1997
P.d. ornatissimum	*P. hernandesi*	PDU71555	USA	New Mexico	Sandoval	Zamudio et al., 1997
P.d. ornatissimum	*P. hernandesi*	PDU71577	USA	Texas	Hudspeth	Zamudio et al., 1997

Table 3-3 continued.

Old Taxonomy	Current Taxonomy	GenBank #	Country	State	County	Source
P.d. ornatum	*P. hernandesi*	PDU71566	USA	Utah	Tooele	Zamudio et al., 1997
P.d. ornatum	*P. hernandesi*	PDU71578	USA	Nevada	Elko	Zamudio et al., 1997
P. orbiculare	*P. orbiculare*	POU71575	-	-	-	Zamudio et al., 1997
P. orbiculare	*P. orbiculare*	AY141056.1	-	-	-	Hodges and Zamudio, 2004
P. modestum	*P. modestum*	AY141055.1	-	-	-	Hodges and Zamudio, 2004
P. platyrhinos	*P. platyrhinos*	PPU71574	-	-	-	Zamudio et al., 1997
P. platyrhinos	*P. platyrhinos*	AY141057.1	-	-	-	Hodges and Zamudio, 2004
P. mcallii	*P. mcallii*	AY141054.1	-	-	-	Hodges and Zamudio, 2004
P. solare	*P. solare*	AY141058.1	-	-	-	Hodges and Zamudio, 2004
P. cornutum	*P. cornutum*	PCU66224	-	-	-	Zamudio et al., 1997
P. cornutum	*P. cornutum*	AY141049.1	-	-	-	Hodges and Zamudio, 2004
P. taurus	*P. taurus*	AY141059.1	-	-	-	Hodges and Zamudio, 2004
P. braconnieri	*P. braconnieri*	AY141060.1	-	-	-	Hodges and Zamudio, 2004
P. asio	*P. asio*	AY141048.1	-	-	-	Hodges and Zamudio, 2004
P. coronatum	*P. coronatum*	GQ464803	-	-	-	Leaché, 2010
P. coronatum	*P. coronatum*	AY141050	-	-	-	Leaché, 2010
A. cognatus	*A. cognatus*	AF462502.1	USA	Texas	Parmer	Masta et al., 2002
A. cognatus	*A. cognatus*	AF462503.1	USA	Kansas	Harper	Masta et al., 2002
A. cognatus	*A. cognatus*	AF462504.1	USA	California	San Bernardino	Masta et al., 2002

lnL = 5460.66, K = 9, AIC = 10939.52). Base frequencies used as input parameters for the best-fit model were as follows: A = 0.2837, C = 0.2462, G = 0.1389, T = 0.3312, proportion of invariables sites (I) = 0.0000, and gamma distribution shape parameter = equal rates for all sites. Sequence divergence among individuals was calculated using the uncorrected (“p”) distance matrix in PAUP4.

I used MrBayes (v3.1.2; Ronquist and Huelsenbeck, 2003) and BEAST (v1.5.4; Drummond and Rambaut, 2007) to infer phylogenetic relationships among *Phrynosoma* using the best-fit model. Both analyses were set to these parameters: four Markov Chain Monte Carlo (MCMC) chains (three heated and one cold) for 25 million generations (Drummond et al., 2002). Trees were sampled every 1,000 iterations from each MCMC chain. I removed the first 10% of posterior tree samples as burn-in since these initial trees typically have low likelihood values and then used Tracer (vers. 1.4.1; Rambaut and Drummond, 2003) to verify model parameters had reached stationary values. For BEAST, I used the Bayesian skyline plot to estimate historical population dynamics (Drummond et al., 2005). The Bayesian skyline plot accounts for errors inherent to phylogenetic reconstruction including stochastic error that creates a more confident estimate of posterior probabilities (Drummond et al., 2005). Additionally, the Bayesian skyline plot makes phylogenetic calculations using an uncorrelated lognormal clock model, which allows variation in evolutionary rates across branches, and is most appropriate since my data show an uncorrelated pattern (Drummond et al., 2005).

I used FigTree (vers.1.3.1; Rambaut, 2009) to display the 50% majority consensus trees. Nucleotide substitution models were selected using Akaike

Information Criterion (AIC; Akaike, 1974; Posada and Buckley, 2004) in MrModelTest (vers. 2.2; Kass & Raferty, 1995). I considered branches well-supported if the posterior probabilites were >0.95. Tree construction for either *P. hernandesi* or *A. cognatus* did not include an outgroup; *P. hernandesi* is nested within the short-horned lizard clade which is highly resolved while too few samples from other species within *Anaxyrus* were present to conduct a meaningful analysis.

RESULTS

Phrynosoma hernandesi

Bayesian analysis inferred strong support (>0.95 probability) for 12 nodes within the *P. hernandesi* clade while 11 nodes are unresolved (Fig. 3-2). There is strong support (0.99 probability) for one of the two major clades within *P. hernandesi* that includes lizards from northeast Nevada, Utah, northern Arizona, the eastern half of Colorado, and eastern New Mexico (Fig. 3-3). The second clade shows weak basal support (0.57 probability) but strong support for 2 of the 3 internal clades (Fig. 3-4). The first internal clade consists of *P. hernandesi* from southeast Arizona and northwest Texas. The second internal clade includes *P. hernandesi* from eastern Utah, North Dakota, South Dakota, Wyoming, Montana, and Alberta, Canada. The third internal clade includes *P. hernandesi* from north-central New Mexico that are basal to all populations of Valley populations.

Based on sequence divergence rate similarities among Valley populations of *P. hernandesi,* Valley populations show a genetic association with the 5 physiographic provinces within the Valley (Fig. 3-5); the four populations of *P.*

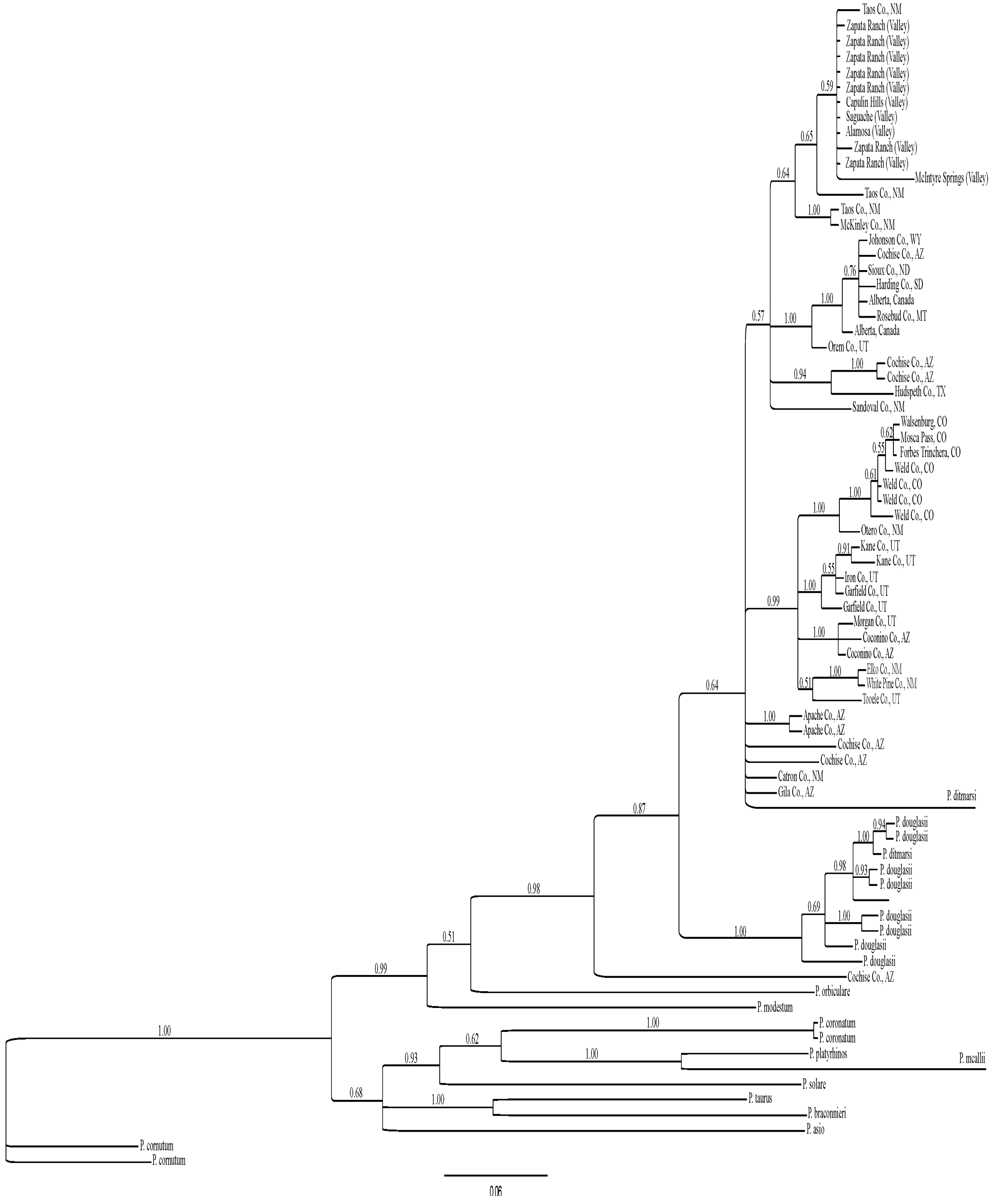
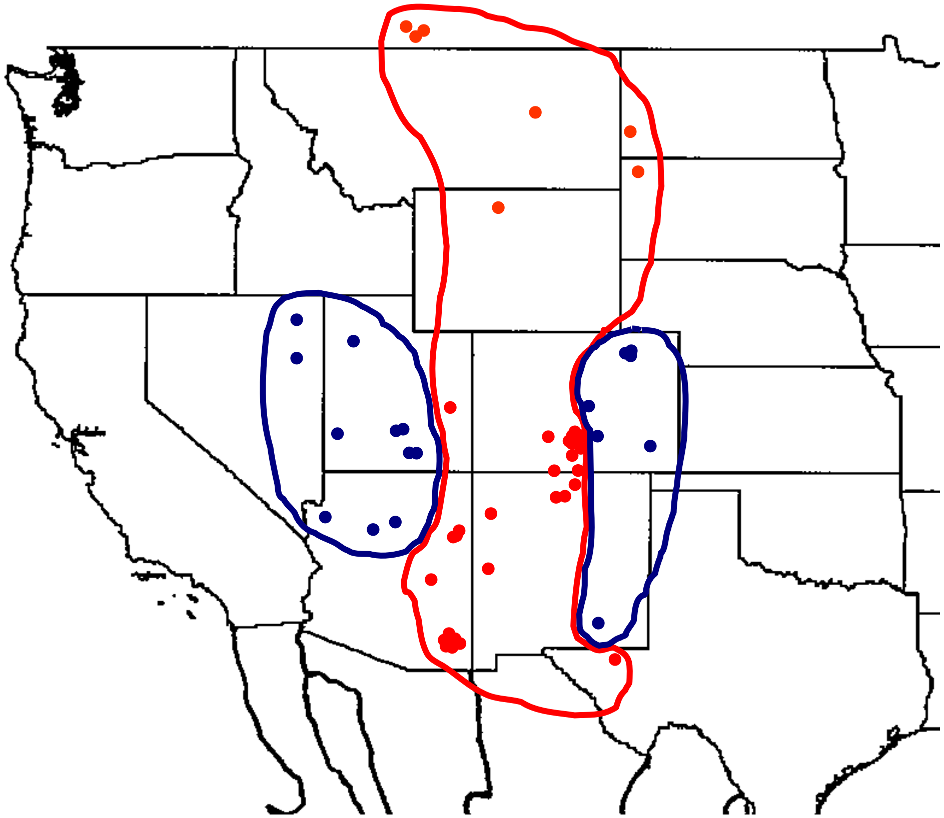
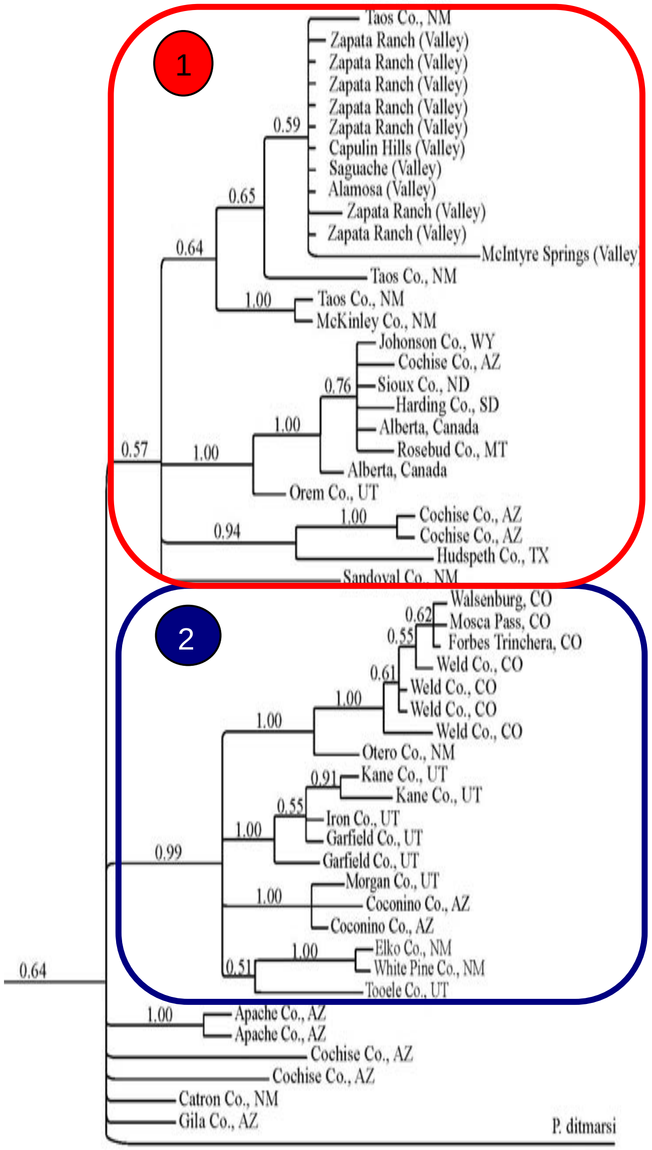


Fig. 3-2. Maximum likelihood tree using Bayesian inference for *P. hernandesi* using the ND4 gene including posterior probabilities. Branch length indicates divergence. Within the *P. hernandesi* clade, there are four main clades.

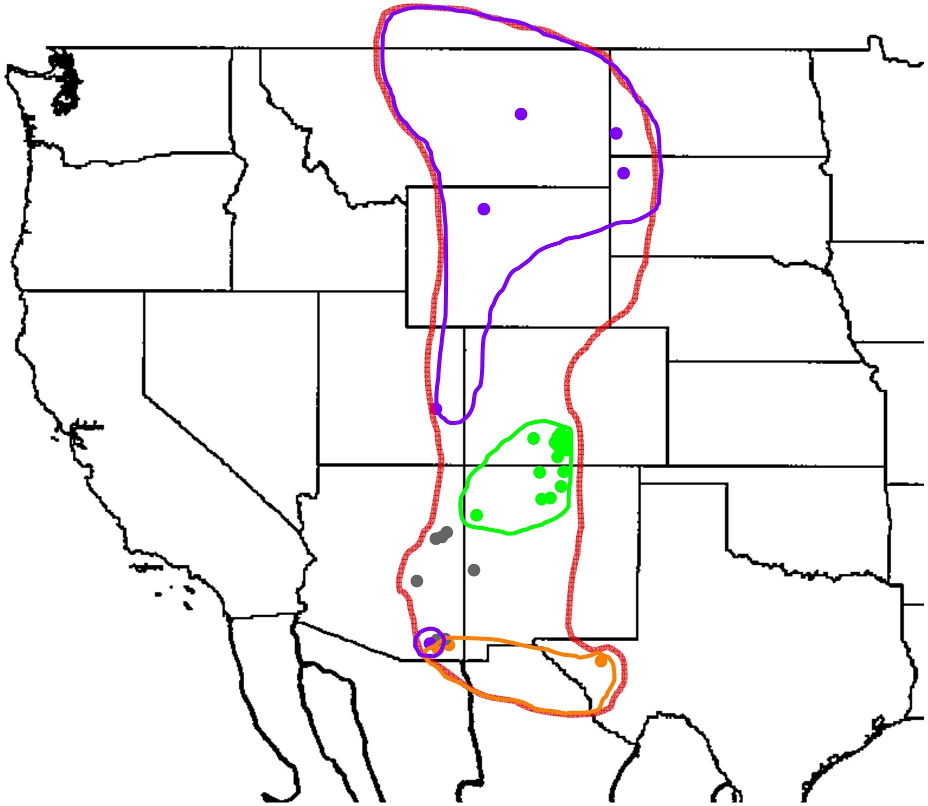
Fig. 3-3. Distribution of the two major *P. herandnesi* clades using the ND4 gene. The second clade that includes populations along the eastern and western boundaries of the species range show strong support and is disjunct from populations in the central part of the range.

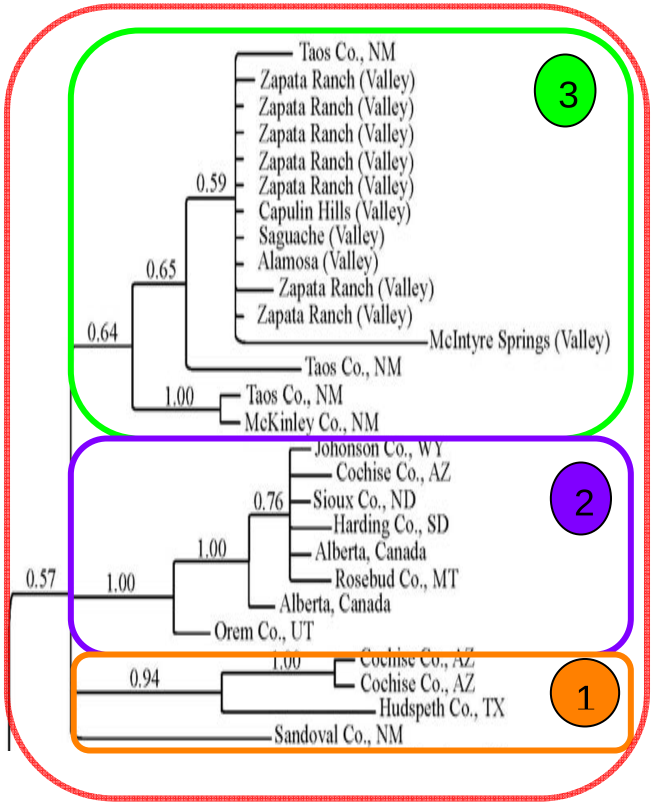




1
2
Taos Co., NM
Zapata Ranch (Valley)
Zapata Ranch (Valley)
Zapata Ranch (Valley)
Zapata Ranch (Valley)
Zapata Ranch (Valley)
Capulin Hills (Valley)
Saguache (Valley)
Alamosa (Valley)
Zapata Ranch (Valley)
Zapata Ranch (Valley)
McIntyre Springs (Valley)
Taos Co., NM
Taos Co., NM
McKinley Co., NM
Johonson Co., WY
Cochise Co., AZ
Sioux Co., ND
Harding Co., SD
Alberta, Canada
Rosebud Co., MT
Alberta, Canada
Orem Co., UT
Cochise Co., AZ
Cochise Co., AZ
Hudspeth Co., TX
Sandoval Co., NM
Walsenburg, CO
Mosca Pass, CO
Forbes Trinchera, CO
Weld Co., CO
Weld Co., CO
Weld Co., CO
Weld Co., CO
Otero Co., NM
Kane Co., UT
Kane Co., UT
Iron Co., UT
Garfield Co., UT
Garfield Co., UT
Morgan Co., UT
Coconino Co., AZ
Coconino Co., AZ
Elko Co., NM
White Pine Co., NM
Tooele Co., UT
Apache Co., AZ
Apache Co., AZ
Cochise Co., AZ
Cochise Co., AZ
Catron Co., NM
Gila Co., AZ
P. ditmarsi
0.59
0.65
0.64
1.00
0.76
1.00
0.57
1.00
1.00
0.94
0.62
0.55
0.61
1.00
1.00
0.91
0.55
1.00
0.99
1.00
1.00
0.51
0.64
1.00

Fig. 3-4. Distribution of the second major *P. hernandesi* clade using the ND4 gene that includes Valley populations. Although there is weak basal support for this second clade, there is strong support for two of the three internal clades that include populations from the most northerly and southern populations, as well as populations from within the Valley.





3
Taos Co., NM
Zapata Ranch (Valley)
Zapata Ranch (Valley)
Zapata Ranch (Valley)
Zapata Ranch (Valley)
Zapata Ranch (Valley)
Capulin Hills (Valley)
Saguache (Valley)
Alamosa (Valley)
Zapata Ranch (Valley)
Zapata Ranch (Valley)
McIntyre Springs (Valley)
Taos Co., NM
Taos Co., NM
McKinley Co., NM
0.59
0.65
0.64
1.00
2
Johonson Co., WY
Cochise Co., AZ
Sioux Co., ND
Harding Co., SD
Alberta, Canada
Rosebud Co., MT
Alberta, Canada
Orem Co., UT
0.76
1.00
1.00
0.57
1
Cochise Co., AZ
Cochise Co., AZ
Hudspeth Co., TX
Sandoval Co., NM
1.00
0.94

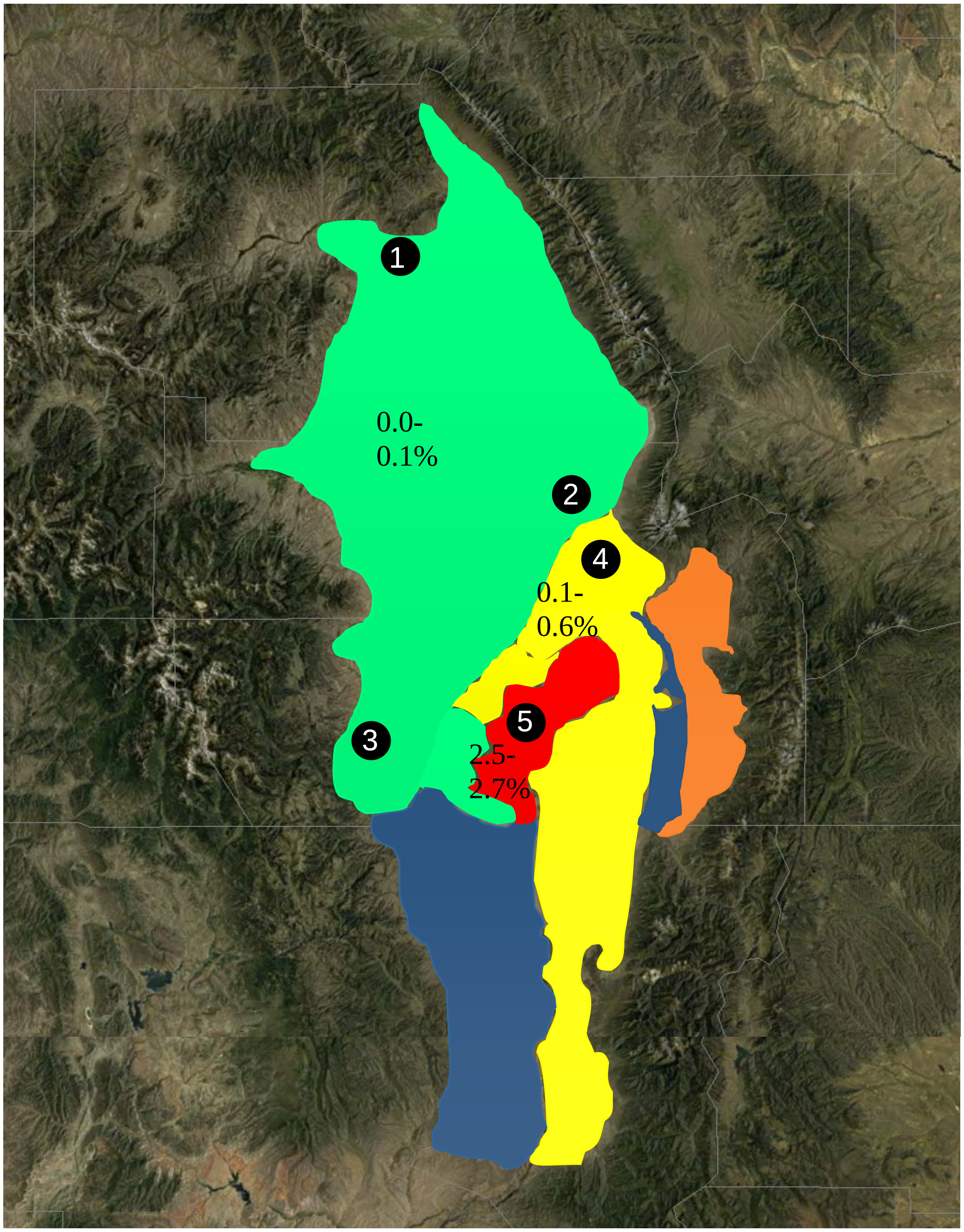


Fig. 3-5. Distribution of lizard haplotypes within the 5 physiographic subdivisions within the Valley based on the ND4 gene. Lizards within the Alamosa formation (green) are most similar to the lizard population in the Costilla Plains formation (yellow), while lizards in the San Luis Hills formation (red) show high divergence from any other lizard population sampled. Lizard populations from the Culebra Reentrant (orange) and Taos Plateau (blue) were not sampled. Circled numbers indicate populations sampled: 1 = Saguache, 2 = Zapata Ranch, 3 = Capulin Hills, 4 = south Zapata Ranch, 5 = McIntyre Springs.

hernandesi in the Alamosa formation show minimal genetic divergence from each other. This divergence is even more evident when compared to *P. hernandesi* within the Costilla plains or San Luis Hills formations. The *P. hernandesi* individual sampled within the San Luis Hills formation shows a relatively high divergence from any other lizard population within the Valley (2.5-2.7%), while the Costilla plains lizard is minimally divergent from *P. hernandesi* populations within the Alamosa formation (0.1-0.6%).

Phrynosoma hernandesi within the Alamosa formation are more similar to lizards in northern New Mexico than the lizard within the San Luis Hills formation (McIntyre Springs); lizards at Zapata Ranch, Saguache, and Capulin Hills are 2.46-2.70% divergent from lizards at McIntyre Springs and 0.5-3.80% divergent from populations in Taos, Sandoval, and McKinley Counties, New Mexico while the McIntyre Springs lizard is 2.90-6.35% divergent from the New Mexico lizards (Table 3-4).

Valley *P. hernandesi* are 0.53-8.50% divergent in the number of base pair substitutions compared to lizards outside the Valley (Fig. 3-6, Table 3-4). The highest divergence rates among Valley populations occur in Colorado populations adjacent to the Valley including Mosca Pass (8.50%), Forbes Trinchera (8.37%), Walsenburg (8.26%), Pinion Canyon (7.67%), and Weld Co (8.13%). In contrast, Valley populations are most similar to lizards just beyond the southern end of the Valley in Taos (0.53-2.7% sequence divergence) and McKinley Counties, New Mexico (0.53-3.71% sequence divergence) followed by populations from Grand County, Utah (2.18-3.61% sequence divergence) and throughout the northern extent

Table 3-4.Genetic distances (uncorrected "p") for *P. hernandesi* throughout its range compared to San Luis Valley populations.

		1	2	3	4	5	6	7
1	San Luis Valley	-						
2	Mosca Pass	7.08-8.50%	-					
3	Forbes Trinchera	6.94-8.37%	0.12%	-				
4	Walsenburg	7.60-8.26%	0.26%	0.13%	-			
5	Pinion Canyon	6.58-7.67%	1.22%	1.10%	1.33%	-		
6	Arizona (Apache Co.)	3.73-5.20%	6.74-11.14%	6.43-6.69%	6.42-6.68%	7.18-7.49%	-	
7	Arizona (Coconino Co.)	5.44-7.36%	4.695.27%	4.38-4.96%	4.95%	4.33-4.91%	5.81-6.67%	-
8	Arizona (Cochise Co.)	2.89-5.20%	6.99-9.09%	6.68-8.77%	4.60-6.66%	7.16-9.26%	4.06-5.80%	6.38-7.25%
9	Arizona (Gila Co.)	2.65-4.83%	6.40%	6.09%	6.07%	7.14%	1.74-2.03%	5.22-5.8%
10	Arizona (Mohave County)	5.01-6.93%	4.38%	4.08%	4.08%	4.02%	5.51-5.80%	0.30-0.90%
11	Colorado (Weld Co.)	6.83-8.13%	0.30-0.45%	0.00-0.31%	0.00-0.45%	1.04-1.10%	6.38-6.73%	4.35-5.00%
12	Montana (Ro Co.)	3.02-4.17%	7.25%	6.95%	6.94%	7.41%	4.35-4.64%	6.09-6.67%
13	Nevada (Elko Co.)	5.71-8.40%	6.14%	5.83%	5.83%	6.33%	5.51-5.80%	3.12-3.77%
14	Nevada (White Pine Co.)	6.23-8.24%	4.81%	4.68%	4.93%	5.17%	5.48-5.78%	3.18-3.75%
15	New Mexico (Taos Co.)	0.53-2.7%	6.96-7.27%	6.66-7.24%	6.64-7.21%	6.57-7.43%	3.77-4.06%	5.22-6.97%
16	New Mexico (Sandoval Co.)	3.88-4.48%	6.66%	6.35%	6.33%	6.82%	4.06-4.35%	6.09-6.67%
17	New Mexico (Otero Co.)	5.93-7.23%	2.32%	2.02%	2.02%	2.54%	5.51-5.80%	3.48-4.06%
18	New Mexico (Catron Co.)	2.71-4.70%	6.97%	6.67%	6.66%	7.13%	2.03-2.32%	5.22-5.8%
19	New Mexico (McKinley Co.)	1.43-3.71%	6.98%	7.25%	7.22%	7.17%	3.77-4.06%	5.81-8.43%
20	North Dakota (Sioux Co.)	2.72-3.87%	6.96%	6.66%	6.66%	7.12%	4.06-4.35%	5.80-6.38%
21	South Dakota (Harding Co.)	2.95-4.17%	7.22%	6.93%	6.92%	7.37%	4.35-4.64%	6.09-6.67%
22	Texas (Hudspeth Co.)	4.30-5.78%	7.58%	7.27%	7.25%	7.74%	5.22-5.51%	7.54-8.12%
23	Utah (Kane Co.)	5.45-6.93%	4.64-5.23%	4.33-4.92%	4.32-4.91%	4.27-4.86%	4.06-6.67%	2.32-2.90%
24	Utah (Garfield Co.)	5.15-7.53%	4.94%	4.63%	4.62%	4.56%	6.09-6.38%	2.03-2.61%
25	Utah (Grand Co.)	2.18-3.61%	6.37%	6.06%	6.05%	6.53%	3.48-3.77%	5.22-5.80%
26	Utah (Toole Co.)	5.68-6.81%	6.11	5.81%	5.80%	5.74%	6.09-6.38%	3.19-3.77%
27	Utah (Iron Co.)	5.15-7.20%	4.94	4.63%	4.62%	4.57%	6.09-6.38%	2.03-2.61%
28	Wyoming (Johnson Co.)	3.02-3.87	6.96	6.66%	6.66%	7.12%	4.06-4.35%	5.80-6.38%
29	Canada (Alberta)	2.72-3.87%	6.66-6.96%	6.36-6.66%	6.35-6.66%	6.82-7.82%	3.77-4.35%	5.51-6.38%

Table 3-4 continued.

	8	9	10	11	12	13	14	15	16
1									
2									
3									
4									
5									
6									
7									
8	-								
9	3.77-5.22%	-							
10	6.09-6.96%	4.93%	-						
11	6.67-8.87%	5.86-6.09%	4.06-4.11%	-					
12	4.06-4.93%	4.06%	5.80%	6.96-7.04%	-				
13	6.67-6.96%	5.80%	3.48%	5.80-5.87%	6.09%	-			
14	6.61-6.96%	5.13%	3.43%	4.84-5.77%	6.00%	6.23%	-		
15	4.06-5.51%	3.48-4.06%	4.93-6.09%	6.67-7.30%	3.48-4.06%	5.51-6.67%	5.38-6.58%	-	
16	4.93-5.22%	3.77%	6.38%	6.39%	4.06%	5.80%	5.69%	3.12-3.77%	-
17	6.38-7.83%	5.22%	3.12%	2.02%	6.96%	4.93%	4.90%	5.80-6.96%	6.67%
18	2.90-4.35%	1.74%	4.93%	6.67-6.73%	3.19%	4.93%	4.85%	3.19-3.77%	3.48%
19	3.49-4.94%	3.49%	4.93%	7.28%	3.49%	5.51%	5.39%	0-2.33%	3.19%
20	3.77-4.64%	3.77%	5.51	6.67-6.74%	0.30%	5.80%	5.72%	3.19-3.77%	3.77%
21	4.06-4.93%	4.06%	5.80%	6.97%	0.60%	6.09%	5.97%	3.48-4.06%	3.48%
22	2.9-6.09%	5.80%	7.25%	7.25-7.33%	4.64%	7.83%	7.79%	4.06-4.64%	4.93%
23	5.80-7.83%	5.80%	2.61%	4.32-4.93%	6.96%	3.19-3.77%	3.14-3.73%	5.80-6.38%	6.68%
24	6.09-7.54%	5.51%	2.32%	0.00-4.64%	6.67%	2.90%	2.85%	5.51-6.67%	6.38%
25	3.77-4.35%	3.19%	4.93%	6.09%	1.45%	5.22%	5.11%	2.61-3.19%	3.19%
26	6.67-7.54%	5.80%	3.48%	5.87-5.98%	6.09%	2.9'0%	2.85%	5.51-6.67%	6.38%
27	5.51-7.54%	5.51%	2.32%	4.64%	4.67%	2.90%	2.85%	5.51-6.09%	6.38%
28	3.77-4.67%	3.77%	5.51%	6.67-6.74%	0.30%	5.80%	5.72%	2.32-3.19%	3.77%
29	3.77-4.93%	3.48-3.77%	5.22-5.51%	6.38-6.67%	0.45%	5.51-5.80%	5.41-5.72%	2.90-6.38%	3.48-3.77%

Table 3-4 continued.

	17	18	19	20	21	22	23	24	25	26
1										
2										
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8										
9										
10										
11										
12										
13										
14										
15										
16										
17	-									
18	5.80%	-								
19	6.39%	3.19-4.65%	-							
20	6.67%	2.90%	3.19%	-						
21	6.96%	3.19	3.48%	0.30%	-					
22	6.96%	4.93	4.65%	4.35%	4.64%	-				
23	3.48%	5.80-6.67%	5.81%	6.67%	6.96%	6.96%	-			
24	3.12%	5.51%	5.52%	6.67%	6.67%	7.25%	0.90-1.45%	-		
25	6.09%	2.90%	2.61%	1.16%	1.45%	4.35%	6.09%	5.80%	-	
26	4.35%	4.93%	5.51%	5.80%	6.09%	7.83%	2.61-3.19%	6.09%	5.80%	-
27	3.19%	5.51%	5.51%	6.38%	6.38%	6.67%	0.30-0.90%	0.60%	5.80%	2.32%
28	6.67%	2.90%	3.19%	0.00%	0.30%	4.35%	6.67%	6.38%	1.16%	5.80%
29	6.38-6.67%	2.90-3.19%	2.90-3.19%	0.00-0.30%	0.30-0.60%	4.35-4.64%	5.80-6.67%	6.09-6.38%	0.90-1.16	5.51-5.80%

Table 3-4 continued.

	27	28
1		
2		
3		
4		
5		
6		
7		
8		
9		
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11		
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15		
16		
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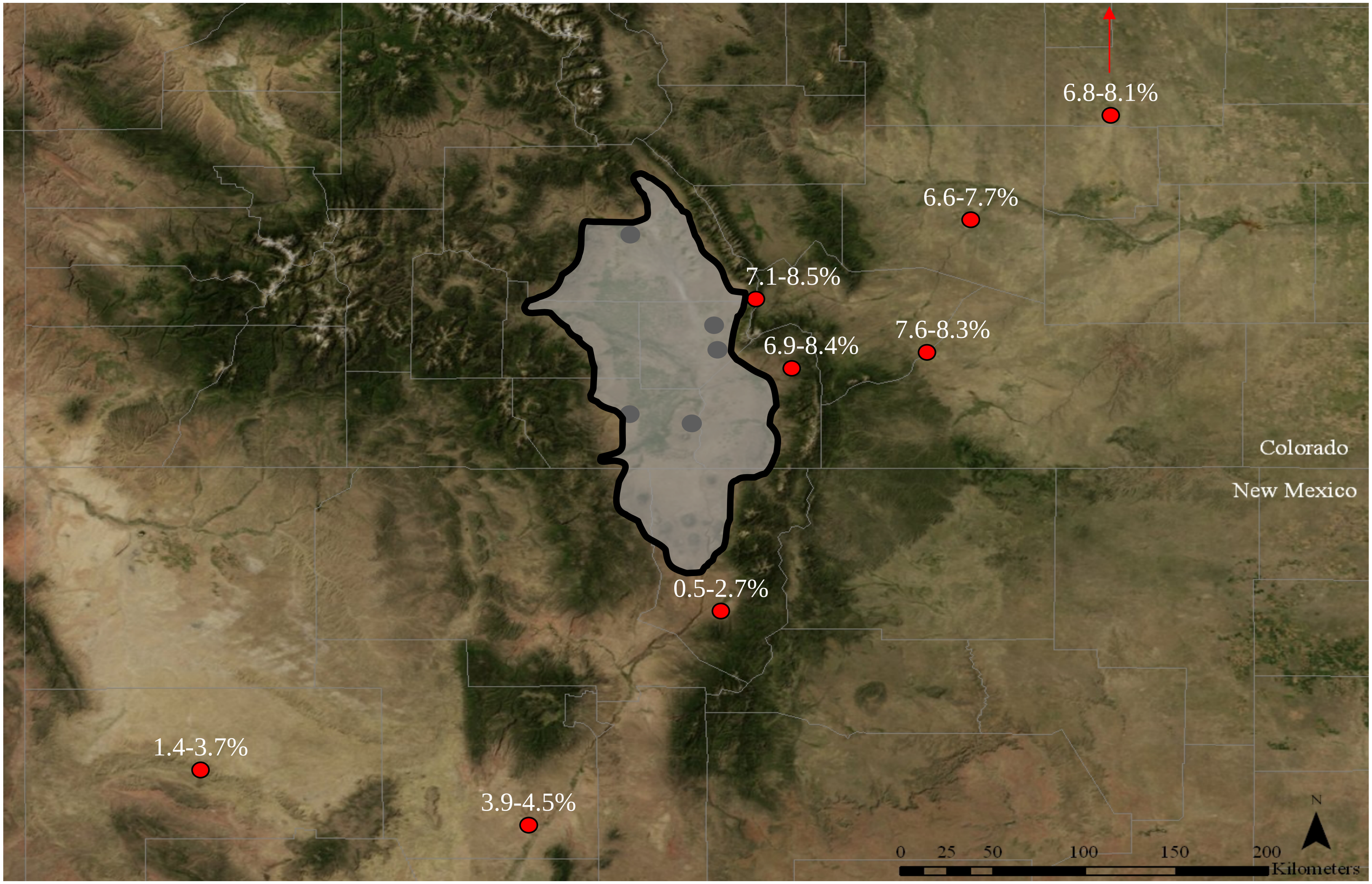
18		
19		
20		
21		
22		
23		
24		
25		
26		
27	-	
28	6.38%	-
29	6.09-6.38%	0.00-0.30%

Fig. 3-6. Sequence divergence values for Valley *P. hernandesi* populations compared to populations surrounding the Valley using the ND4 gene. Highest divergence (7.1-8.5%) occurs with the georgraphically closest population to Valley populations at Mosca Pass, Colorado, while Valley populations are most similar (0.5-2.7% divergence) to *P. hernandesi* populations in Taos, New Mexico.


6.8-8.1%
6.6-7.7%
7.1-8.5%
7.6-8.3%
6.9-8.4%
Colorado
New Mexico
0.5-2.7%
1.4-3.7%
3.9-4.5%
N
0
25
50
100
150
200
Kilometers

of its range including Canada (Alberta), Wyoming (Johnson County), North Dakota (Sioux County), and South Dakota (Harding County) (2.72-4.17% collective sequence divergences). *Phrynosoma hernandesi* from throughout northern New Mexico do not show dwarfism; females from this locality measured 65.1-80.0 cm SVL and males measured 55.4-68.6 mm SVL (see Zamudio, 1996; Degenhardt et al., 1996).

Anaxyrus cognatus

Due to few samples included in the analysis, an unrooted tree was constructed (Fig. 3-7). The two *A. cognatus* within the Valley share one haplotype and form a well-supported node (1.0 probability). The Valley populations of *A. cognatus* are most similar to the population in south-central Kansas (Harper County; 0.93 probability; Fig. 3-8), followed by southern California (San Bernardino County) and northwest Texas (Parmer County; 0.93 probability). *Anaxyrus cognatus* shows relatively low sequence divergence rates across the localities; Kansas and California populations show the greatest sequence divergence (2.71%) while Valley populations are only 0.92-1.19% divergent from any other population (Table 3-5).

DISCUSSION

The spatial structuring of the genetic variation among Valley populations of *P. hernandesi* in relation to the geology of the Valley is suggestive of their colonization history, while *A. cognatus* colonization history remains inconclusive. There is genetic diversity in the Valley, particularly within the San Luis hills formation that

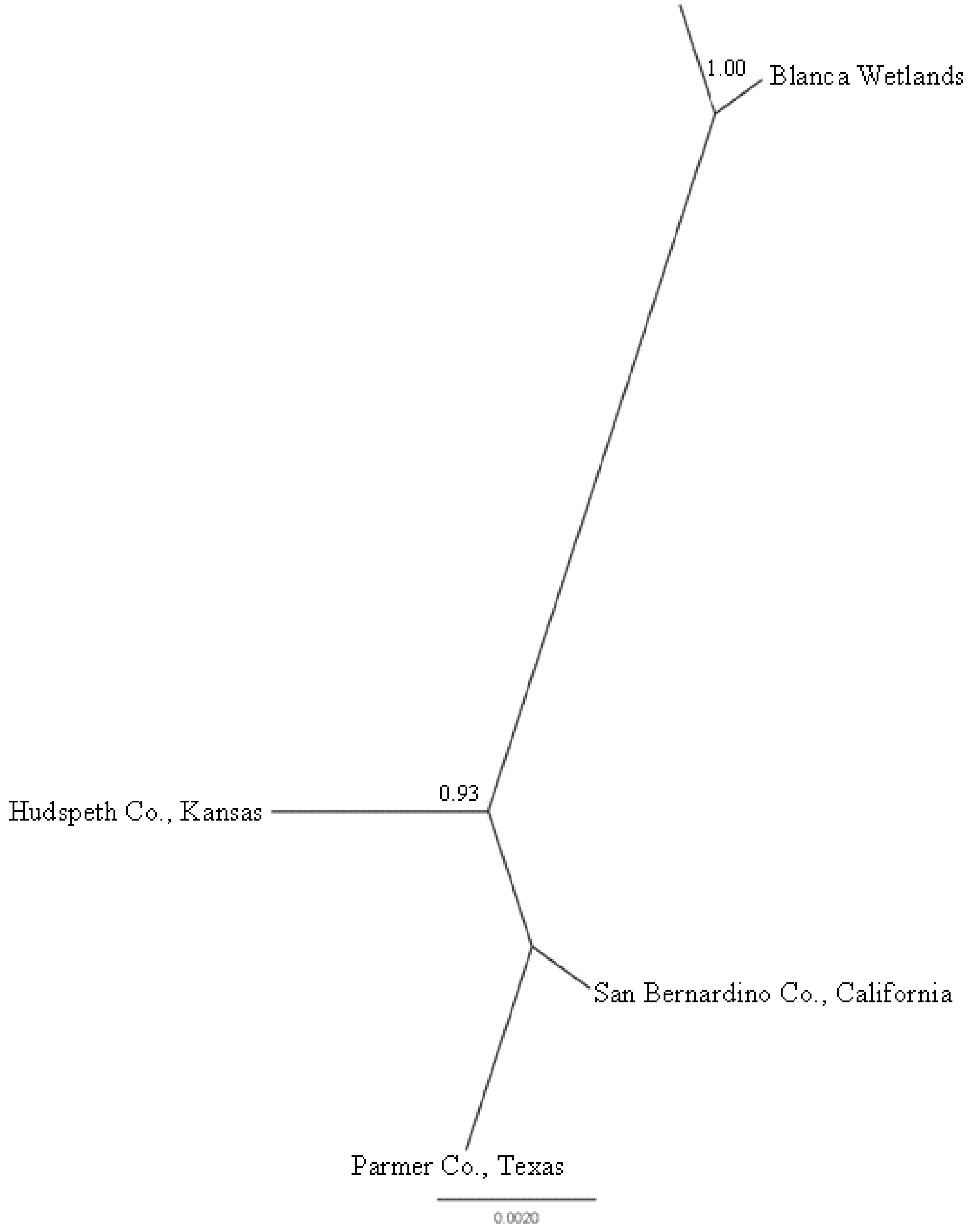


Fig. 3-7. Maximum likelihood tree using Bayesian inference for *A. cognatus* using the 16S/tRNA gene including posterior probabilities. Due to relatively few samples throughout its range, assessing the phylogenetic history of *A. cognatus* within the Valley is difficult.

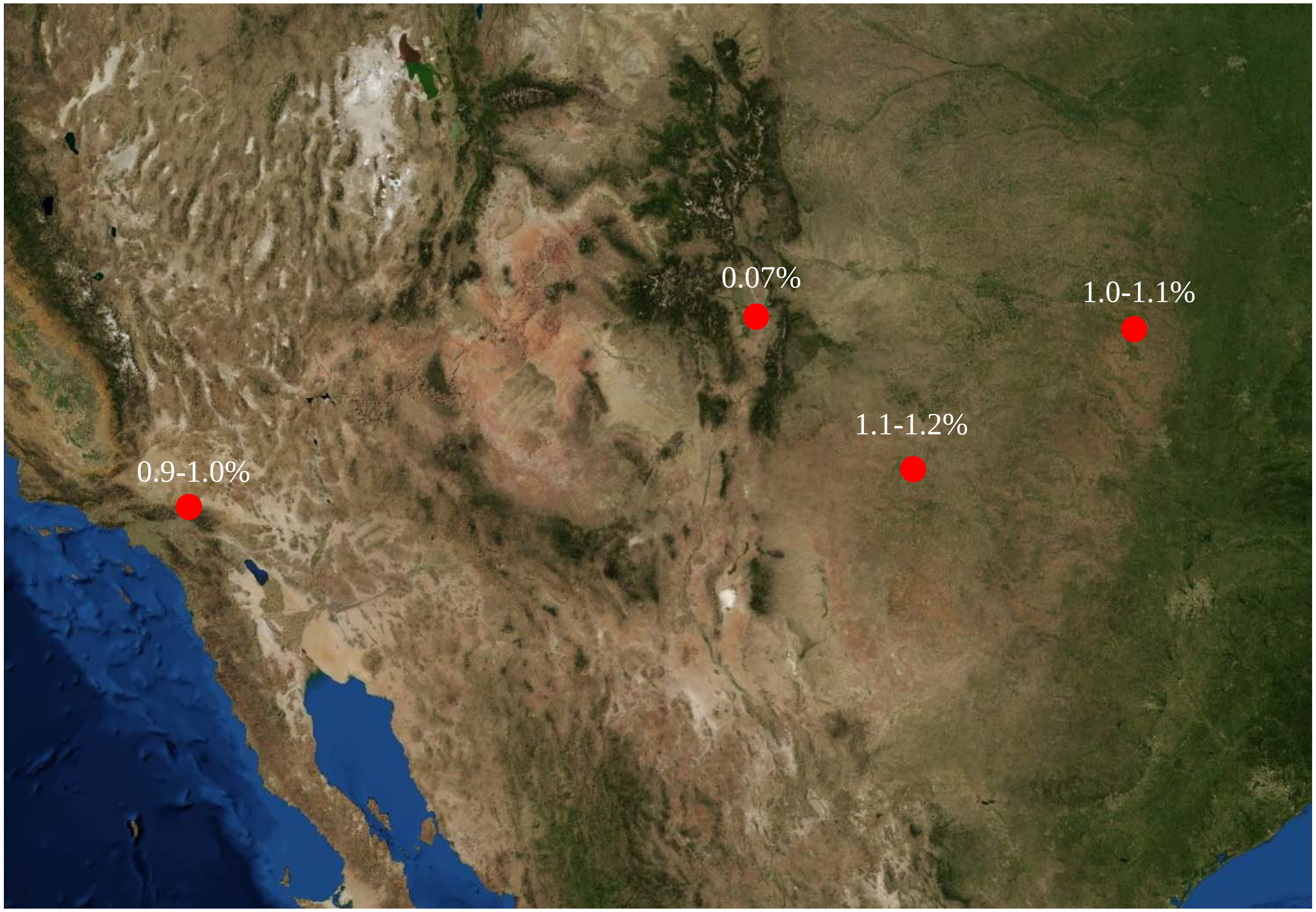


Fig. 3-8. Genetic variation among Valley populations of *A. cognatus* compared to populations throughout its range based on the 16S/tRNA gene. There is a low amount of genetic variation throughout the range of this species.

Table 3-5. Genetic distances (uncorrected “p”) for *A. cognatus*.

	Locality	1	2	3	4	5
1	California	-				
2	Texas	1.81%	-			
3	Kansas	2.71%	0.45%	-		
4	Valley (San Luis Lake)	1.01%	1.19%	1.10%	-	
5	Valley (Blanca Wetlands)	0.92%	1.11%	1.01%	0.07%	-

consists of rocky, sparsely vegetated terrain unlike the Alamosa formation that contains stabilized and active sand dunes. The south Zapata Ranch *P.hernandesi* has a low divergence rate from the other *P.hernandesi* populations, and most likely demonstrates natural population variation since south Zapata *P.hernandesi* are less than 4mi distance from Zapata Ranch lizards within contiguous stabilized sand dune habitat.

Given the distribution of haplotypes and divergence among populations, there are two most likely hypotheses for the colonization history of *P.hernandesi* within the Valley: colonization occurred during a single migration event and populations have since diverged or colonization occurred during multiple migration events and populations have not converged (Avise, 2000). Climate conditions during the last 0.80 MYA offer insight into these two hypotheses.

Based on fossil record evidence, the Valley experienced 3 major shifts in climate since the late Miocene where the earliest fossil records of *P. hernandesi* and *A. cognatus* are known (Rogers et al., 1985). From about 0.82-0.81 MYA, the climate was warm/hot and dry, the Valley floor consisted of sagebrush grassland, and ground water levels were high. Freezing duration was similar to current freezing duration (90-200 days), winters were relatively warm (normal minimum and mean daily temperatures in winter were probably greater than -5-0°C), and annual temperatures were 3-8°C warmer than today. This climate pattern is favorable for *P. hernandesi* and *A. cognatus* and likely facilitated their distribution within the Valley. A cooler, wetter period occurred from 0.82 and 0.74 MYA where the Valley floor was montane forest habitat. During this time, species indicative of warmer periods are absent from the fossil record while aquatic species were present, indicating more deep, permanent aquatic habitats. This cooler, wetter period indicates a glacial period. During the most recent climate shift, 0.74 MYA to current, fossil records indicate drier, terrestrial conditions.

It is possible that *P. hernandesi* and *A. cognatus* have colonized the Valley at least twice in the past 0.8 MYA. *Phrynosoma hernandesi* and *A. cognatus* fossils are not well-known from the cooler, wetter time periods although data for only two excavation sites are reported within the Valley (Rogers et al., 1985; Rogers, 1987; Rogers et al., 2000). It is likely that *P. hernandesi* and *A. cognatus* persisted within the Valley during the cool, wet period by retreating to smaller areas of suitable habitat, since *P. hernandesi* occupies montane habitats throughout its range and *A. cognatus* can tolerate cooler temperatures; however, it is also possible that original populations of both species within the Valley became extinct or nearly extinct during the cool, wet period and re-colonized once the climate became warmer and drier. *Phrynosoma hernandesi* populations could have persisted within the San Luis hills formation during the cool, wet period while populations from northern New Mexico migrated upwards to the Alamosa formation during the most recent warm, dry period.

Genetic information for *A. cognatus* is insufficient to determine whether it persisted throughout the wet, cool period. This hypothesis, in support of multiple migration events, is evidenced by the high divergence rate among *P. hernandesi* at the San Luis hills formation, and low divergence of Alamosa formation populations compared to northern New Mexico populations.

It is also possible that *P. hernandesi* migrated once into the Valley and has since diverged (due to the low divergence rates and sample sizes for *A. cognatus*, determining whether it experienced a single migration event is not possible). The San Luis Hills function as a topological barrier to the sand-filled basin in the northern portion of the Valley (Alamosa basin), consisting of a contiguous landform up to 300 m elevation (Upson, 1939). The division between the Alamosa basin and the San Luis Hills is distinct; terrain shifts from primarily stabilized sand dunes with many desert-evolved plant species to volcanic rocks with sparse upland vegetation. It is unlikely that *P. hernandesi* traverse such distinct habitat types, given their preference for various microhabitat features (e.g. Burrow et al., 2001; Lahti et al., 2010). Interestingly, the McIntyre springs sample shows a consistent amount of genetic variation compared to Valley populations within the Alamosa formation (0.22% maximum difference) yet compared to *P.hernandesi* adjacent to the southern end of the Valley, the McIntyre springs sample shows twice the divergence rate than *P.hernandesi* within the Alamosa formation. This discrepancy suggests that *P.hernandesi* at McIntyre Springs, and possibly throughout the San Luis hills landform, are the result of an independent colonization event or divergence. Without further analysis of more individuals and genetic data, the colonization history of *P. hernandesi* and *A. cognatus* within the Valley remains unresolved.

Genetic variation across *A. cognatus* populations is minimal, though its distribution within the Valley is localized due to its amphibious life/natural-history requirements such as reproductive activity (standing water for egg laying and tadpole development) development. It would not be surprising that all *A. cognatus* within the Valley share minimally divergent mtDNA haplotypes, since no population is no more than 30 km from the next. I observed *A. cognatus* toadlets and adult *A. cognatus* migrating from Blanca Wetlands to San Luis Lakes (a distance of 16 km) and other ephemeral pools throughout Medano-Zapata Ranch in July during the monsoon rains. No genetic samples were obtained from toads at Mishak Lakes, which is 30 km northwest of San Luis Lake though given the adjoining habitat (greasewood salt flats and sand dunes with ephemeral wetlands) is suitable, it is likely that *A. cognatus* also migrate between Mishak Lakes and San Luis Lake regularly.

Minimal genetics work for *A. cognatus* exists, and from current information, there seems to be minimal variation in the mitochondrial or nuclear DNA (Masta et al., 2002; Pauly et al., 2004). I was unsuccessful at collecting samples from *A. cognatus* populations adjacent to the Valley, so limited knowledge exists about the evolutionary history of Valley populations. Based on the three previously sequenced *A. cognatus* throughout its greater species range, Valley populations of toads are most similar to populations in south central Kansas. Interestingly, there are fewer base pair substitutions among California, or Texas populations of toads compared to Valley populations. As with *Phrynosoma*, any genetic distinction among Valley and non-Valley toads is most likely an artifact of isolation.

The nearest reported locality for *A. cognatus* at the southern boundary of the Valley is approximately 100 air miles southeast of the Valley near Colfax, New Mexico (Degenhardt et al., 1996). It is possible that *A. cognatus* migrate up the Rio Grande River into the Valley where the headwaters form, although the nearest reported locality for *A. cognatus* is over 140 river mi downstream from the Colorado border (Degenhardt et al., 1996). Thus, *A. cognatus* would likely have a more difficult time migrating across this distance, especially since there suitable habitat is lacking between these localities such as wetlands, grasslands, or desert scrub (Lanoo, 2005). Migration distances reported for *A. cognatus* in Minnesota ranges from 100-1,300 m depending on whether toads are traversing from wetlands to feeding sites or overwintering sites (Ewert, 1969). Similarly, *A. cognatus* is not able to traverse mountain ranges, as their maximum elevation range is typically <1,900 m and its maximum known elevation at 2,300 m is within the San Luis Valley (Lanoo, 2005).

Orogenous uplift of the mountain ranges forming the Valley's boundary isolated populations within the Valley ~30-27 MYA. This geographic isolation is evidenced by *P. hernandesi* at Zapata and Capulin Hills having sequence divergence rates of nearly 10% from Mosca Pass, which is 16 km and 96 km from either Valley population, respectively. Interestingly, Mosca Pass and Zapata Ranch are geographically the closest populations and suitable habitat exists between these two localities, though isolation is evident. Instead, Valley populations of *P. hernandesi* are most genetically similar to populations below the southern portion of the Valley in New Mexico, indicating that migration occurred from the southern end of the Valley.

Although estimates of colonization history or dwarfism are not determined, it is possible that Valley *P. hernandesi* have been isolated since the Valley began forming. Similarly, *P. hernandesi* observed in the Hansen Bluff excavation (~0.8 MYA) were identical in size to current dwarfed populations (Rogers et al., 1985). It is also possible that dwarfed *A. cognatus* were present in the Hansen Bluff formation; fossil records indicate the presence of ilia from small adults (<2.5 cm SVL) as well as a normal distribution of frontal-parietal fossils from small and large adults (up to 5-6 cm SVL). Rogers (1987) suggests this discrepancy in body size is a function of predators consuming larger toads. Similarly, there is at least one other prehistoric record of dwarfism reported within the Valley; dwarfed *L. catesbeianus* (bullfrogs) 4-5 cm SVL (20-56% smaller than *L. catesbeianus*) were observed up to 0.9 MYA in the Hansen Bluff formation (Rogers et al., 1985; Stebbins, 2003) though it remains unclear whether *L. catesbeianus* populations, either prehistoric or modern, show genetic variation from populations outside the Valley; recent *L. catesbeianus* invasions are known and these populations are not dwarfed (Hahn, 1968; Hammerson, 1999).

Although there is a unique genetic sub-structuring of *P. hernandesi* and possibly *A. cognatus* populations within the Valley, genetics data from this study alone are insufficient to provide any definitive conclusions of the taxonomic status of either species. Discordance among datasets still remains the prevailing issue with horned lizard phylogenetic reconstruction. Among the 17 currently recognized horned lizard species (Leaché and McGuire, 2006; Mulcahy et al., 2006; Leaché et al., 2009), *P. hernandesi*, like *P. cornutum*, has undergone multiple revisions of its taxonomic status, largely because it is such a widespread species that shows great morphological, life/natural-history variation throughout its range (Smith, 1946; Reeve, 1952; Montanucci, 1987; Powell and Russell, 1991; Zamudio, 1996; Zamudio et al., 1997). Previously recognized subspecies designations among *P. hernandesi* were derived from unique morphological and life-history states throughout its species range although in areas of overlap, characters became undifferentiated among so-called species (e.g. Smith, 1946; Reeve, 1952; Nussbaum et al., 1983; Zamudio, 1996; Stebbins, 2003). This poor resolution brought into question the validity of each subspecies and instead suggested that the subspecies simply represented ecomorphs (e.g. Smith, 1946).

Zamudio et al. (1997) provide the most recent analysis of *P. hernandesi* throughout most of its range in the United States using primarily genetic data, in combination with life-history and morphological data. Based on the same mitochondrial gene used in this study (ND4), Zamudio et al. (1997) removed any distinction among 4 of the 6 subspecies (*ornatissimum*, *brevirostre, ornatum,* and *hernandesi*) and elevated *P.h. douglasii* (formerly subspecies *P. douglasi douglasi*) to species status because of incongruence between subspecific range boundaries and previous morphological data. The remaining subspecies, *P.h. brachcercum,* was retained because genetic data for this subspecies was lacking.

Compared to Zamudio et al.'s (1997) range map for these subspecies, my findings support and clarify a distinct boundary between two subspecies (*P.d.*

ornatissimum and *P.d. brevirostre*) that was previously ambiguous. My findings also show great variation from the previously recognized boundaries for all subspecies from Reeve (1952) (Fig. 3-3). Although my dataset included the analysis of only one mitochondrial gene, it is likely that *P. hernandesi* represents multiple species throughout its range. The high divergence among geographically local populations (7.99-9.33% for San Luis Valley vs. Mosca Pass) underlies the importance of revisiting this species' taxonomy. A comprehensive assessment of the genetics and morphology of *P. hernandesi* throughout its range needs to be conducted, as there is definite morphological and life-history variation structuring throughout its expansive range that extends from Canada to central Mexico.

Based on limited ND4 results from this study, it appears that there are at least some unique qualities about Valley populations of *P. hernandesi* and that recent introgression is minimized or absent within the Valley. Avise (2000) describes four categories of phylogenetic patterns that imply different historical relationships within lineages. Valley populations of *P. hernandesi*, and likely *A. cognatus*, are classified within category 3 that includes taxa with low haplotype divergence but high geographic localization. What is not known is whether this isolation has been long-standing on an evolutionary time scale. Establishing accurate molecular clocks for the *P. hernandesi* lineage is difficult to assess because of variations in life-histories (i.e. clutch size, fecundity), body size, and ectothermy (as a function of metabolic activity) which are known to influence estimates (Bromham, 2002). However, both species appear to be undergoing unique evolutionary histories.

The taxonomic history of *A. cognatus* appears to be much more simplified than *Phrynosoma* but is similar in that *A. cognatus* was previously identified by morphological characteristics (Goebel et al., 2009), which are currently supported by genetic data (Masta et al., 2002; Pauly et al., 2004). There has been no sub-specific designation for this fairly ubiquitous species and morphological and genetic variation throughout its expansive range that parallels the distribution of *P. hernandesi* is minimal (Gonzalez et al., 2004; Lanoo, 2005; Chan, 2007); the range is from south-central Canada, throughout the Great Plains into the desert southwest, and central Mexico (Lanoo, 2005). *Anaxyrus cognatus* shows variation in body size though it occurs across elevational and/or latitudinal gradients typical to many species (i.e. Bergman's Rule) though they do not show the regional variation in color or pattern observed in horned lizards (Lanoo, 2005).

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CHAPTER 4

LIFE- AND NATURAL-HISTORY VARIATION AND DIET ANALYSIS OF SAN LUIS VALLEY POPULATIONS OF *PHRYNOSOMA HERNANDESI*

INTRODUCTION

The two integral components of an organism's morphology are genetics and environment. Together, they dictate the form and function of an organism, which enables species to persist in novel or shifting environments via adaptation. The San Luis Valley is unique because it is geographically isolated and is a high-elevation alpine valley that supports an arid desert ecosystem. Populations of *P. hernandesi* within the Valley are morphologically unique (Chapter 2) and genetically distinct (Chapter 3). The extent to which the environmental conditions within the Valley are responsible for the variations seen within Valley populations (either morphological or genetic) is not known; however, what can be determined are the associated changes in life/natural-histories of the dwarfed Valley *P. hernandesi*.

An organism's environment is inherently complex and constitutes the entirety of variables directly and indirectly affecting the organism (Riclefs and Miles, 1994). An organism is largely a product of its environment because these abiotic and biotic factors influence morphology and therefore ultimately influence evolution and adaptation. Although not constant across microhabitats, various abiotic and biotic factors influence morphology, behavior, and potentially genetic mutations such that species are adapted to perform more optimally in their own microhabitats than other microhabitats (Garland and Losos, 1994).

There are many implications of body size for life and natural history variables. For instance, diet can contribute to dwarfism when resources are limited or when competition is high (Wilkelski and Thom, 2000). Conversely, diet can also facilitate gigantism under the right conditions (Schwaner and Sarre, 1988). A shift in body size correlates to a shift in natural and life history variables such as reproductive capacity, predator evasion, and competition (Irschick et al., 2000; Bergmann et al., 2008). Additionally, body size commonly correlates with latitude and elevation, including Bergmann's Rule and Allen's Rule, as discussed in Chapter 2. Or, for many species, exploitation of new or novel habitats can promote selection on various traits including morphology, behavior, and performance (Bergmann et al., 2009), which can all directly correlate to body size; in *Anolis* lizards, perch attributes correlate directly with the evolution of limb proportions which in turn affects locomotor performance and display behavior (Losos, 1990a; Losos, 1990b; Losos et al., 2006).

Although the Valley does not exceed latitudinal or elevational ranges where *P. hernandesi* are known to occur, the Valley still offers a novel habitat for this species. Although in a high-elevation alpine valley, the San Luis Valley is dominated by high-elevation desert. The climate, consisting of monsoon summers and below-freezing temperatures during winter, is dissimilar from surrounding landscape that includes grasslands to the east and pine forests to the west. The Valley is far from an optimal climate for horned lizards, let alone many other reptile and amphibian species (Hahn, 1968; Hammerson, 1981). Thus, the reduced body size and genetic uniqueness among the isolated population of *P. hernandesi* within the Valley is likely coupled with a shift in life/natural-history variables.

Life/natural-history information is of importance because this unique population of *P. hernandesi* remains undetermined, including its taxonomic status. Although genetic data suggest that these populations have historically unique genotypes that span the formation of the Valley, it is ideal to integrate biological data prior to delineating its taxonomic history; dwarfism alone is not necessarily a convincing case to delineate a species or subspecies, while genetics alone does not necessarily provide any information on the realized or functional differences among or within populations.

In this chapter, I explore life/natural-history variables of *P. hernandesi* within the San Luis Valley in comparison to populations surrounding the Valley and throughout the greater species' range. I present distribution and abundance data, comprehensive diet analyses among years, localities, and age/sex groups, and finally population characteristics for a local population at Zapata Ranch.

METHODS

Distribution

Historic and known localities within and surrounding the San Luis Valley were opportunistically searched for the presence of *P. hernandesi*. *Phrynosoma hernandesi* were considered absent from a locality if none were observed within 4 hours of searching during optimal conditions (i.e. peak activity hours). Locality and elevation were recorded for each individual encountered using a Magellan Meridian GPS (Thales Navigation Inc., Santa Clara, CA). Localities where individuals were observed or searched for were mapped using ArcView GIS (vers. 9.2, ESRI, Redlands, CA).

Diet

Lizards were held in captivity up to 2 days for scat collection. Scat was air-dried, weighed to the nearest 0.001g. Scat length and width were measured using Digital calipers (0.001 mm accuracy) and a dissecting scope with an occipital micrometer (= 0.01 mm).

Insect head capsules were identified to order and tallied using a dissecting scope (Borror and DeLong, 1964; Fisher and Cover, 2007; Lahti and Beck, 2007). In addition to head capsules, the presence of Coleopteran insects was quantified using wing pair counts if a.) no head capsules were present, b.) the number of wing pairs exceeded the number of head capsules, or c.) wing pairs belonged to a different species than the head capsules. Pebbles greater than 1mm diameter and pieces of vegetation (i.e. leaves, twigs) were tallied.

To determine whether *P. hernandesi* showed ontogenetic and annual variation in the size of prey items consumed, I measured 10 randomly selected ant heads from each individual. Additionally, all ant heads from Zapata lizards collected in 2009 were identified to genus to provide a more comprehensive analysis for this population. Ants were identified to genus using previously identified ants from Lahti and Beck (2007), specimens collected associated field localities, The Ants of New Mexico (Hymenoptera: Formicidae) (Mackay and Mackay, 2002), and with the assistance of Dr. James Pitts (USU Department of Biology).

Ant head lengths and widths were measured to the nearest 0.1 mm using an ocular micrometer. Ant head length was measured from the forehead apex to the tip of the clypeus and head width was measured from the widest distance between the eyes. Head size measurement was measured as a function of head length* head width.

Analysis

Multiple fecal pellets collected from individual *P. hernandesi* were averaged and analyzed as one pellet. Linear regressions were used to determine relationships between scat morphology and lizard size and mass. An ANCOVA was used to determine whether scat mass varies across age and sex classes (male, female, pregnant female, neonate) (SAS Institute Inc., Cary, NC). Multi-response permutation procedure (MRPP) and Indicator Species Analysis (ISA) was used to determine variation in diet a.) among age and sex classes, b.) annually, and c.) across localities (PC-ORD4; McCune and Mefford, 1999). An MRPP analysis is a non-parametric analysis to test differences between multiple a priori groups (i.e. sex/age classes, year, locality) and an ISA analysis determines which a priori groups associate with the sample units (i.e. type of prey item) using indicator values. A Monte Carlo test using 1,000 random iterations was used to determine the significance of the indicator values, which range from 0 (no indicator value) to 100 (perfect indicator value).

I conducted a 1-way ANOVA using Tukey's HSD post-hoc test to determine differences in head capsule size among *P. hernandesi* a.) age and sex classes (male, female, pregnant female, neonate), b.) location (outside the Valley, inside the Valley), and c.) years (2007-2009). Proportion of each species consumed by each group (male, female, neonate) was calculated. I conducted a 1-way ANOVA using Tukeys HSD to test whether ants vary significantly in head size across genera. Mean head size for each ant species was calculated by averaging all heads measured for each respective ant species. Head size here is used as a proxy for body size, since ants have head sizes consistently proportional to body size as previously reported in the literature (e.g. Lahti and Beck, 2007).

I used ANCOVA and Tukey's HSD tests to determine whether the ontogenetic and annual variation in prey items consumed was significant. A scatterplot and Pearson's correlation coefficient was used to show the relationship between body size and average size of prey items (ant head length*width).

Population Demographics

Annual surveys were conducted on a population of *P. hernandesi* within the Valley at Zapata Ranch, since it is most abundant in stabilized sand dune habitat, where the Ranch is located. Zapata Ranch is a conservation property owned by The Nature Conservancy and managed by conservation ranchers. I chose a population located within the stabilized sand dune habitat at Zapata Ranch near State Well 052 (SW052). The Zapata *P. hernandesi* population at SW052 is a healthy population in a relatively undisturbed habitat. The habitat is lightly grazed to mimic grazing historically done when bison were still present. Monthly annual climate data were downloaded from the Western Regional Climate Center from a weather station located approximately 13.7 km north from the Zapata Ranch lizard population within sand dune habitat at Great Sand Dunes National Park. Monthly and annual climate averages from 2006 to 2009 are presented and shown in comparison to historical climate averages (calculated from 1951-2009). Climate data include average daily maximum temperature (˚C), average daily minimum temperature (˚C), precipitation (cm), and snowfall (cm).

Intense population surveys were conducted 13 August 2007, 17 July 2008, and 4 August 2009 with the help of Southwest Conservation Corps crew members and additional volunteers. We haphazardly searched for *P. hernandesi* within the ~75 ha plot by walking throughout the terrain within the study area, including stirring up individuals under bushes and flipping old cow patties and other vegetative debris. Since tissue samples were collected for all individuals encountered, recaptures are also identified by tail or toe clip scars. Dorsal photographs were taken for future identification of individual *P. hernandesi* including recapture information. Since only one female was a confirmed recapture, recapture rate was not calculated.

Age was determined using natural breaks in size-class data as well as timing of birth activities (i.e. lizards within neonate size class but observed in May during other surveys throughout the Valley were counted as juveniles since birth events do not occur until mid- to late-summer). *Phrynosoma hernandesi* were categorized into these age/sex classes: adult male, adult female, adult pregnant female, juvenile, neonate. Pregnant females were determined based on palpation of the body cavity and showed disproportionate increases in mass (relative to SVL) compared to non-pregnant females.

Analyses

I used a MANCOVA using RegWQ to determine whether *P. hernandesi* groups (adult male, adult female) showed annual variation in morphology including tail length, femur length, head length, and shield width. I used an ANCOVA using RegWQ to determine if groups (adult male, adult female) vary significantly in body size and whether lizards within both groups show annual variation in body size, using mass as the covariate. Pregnant females and neonates were not analyzed since the timing of sweeps is likely to confound the size variation, since both lizard groups show great variation in size throughout the activity season.

RESULTS

Distribution

A total of 182 *P. hernandesi* were observed from 11 localities within and surrounding the Valley (Fig. 4-1 and Table 4-1). Within the Valley, 161 individuals were observed at five localities and outside the Valley, 21 lizards were observed at six localities. Within the Valley, *P. hernandesi* were found at historically-documented localities (Hahn, 1968; Hammerson, 1981; Tim Armstrong, pers. comm.). Densities varied across the Valley but lizards were consistently most abundant in stabilized sand dune habitats near the east-central portion of the Valley (Alamosa county) (Fig. 4-2). Outside the Valley, *P. hernandesi* were not always encountered at historically-documented localities (based on museum records) although searches for lizards were not always exhaustive.

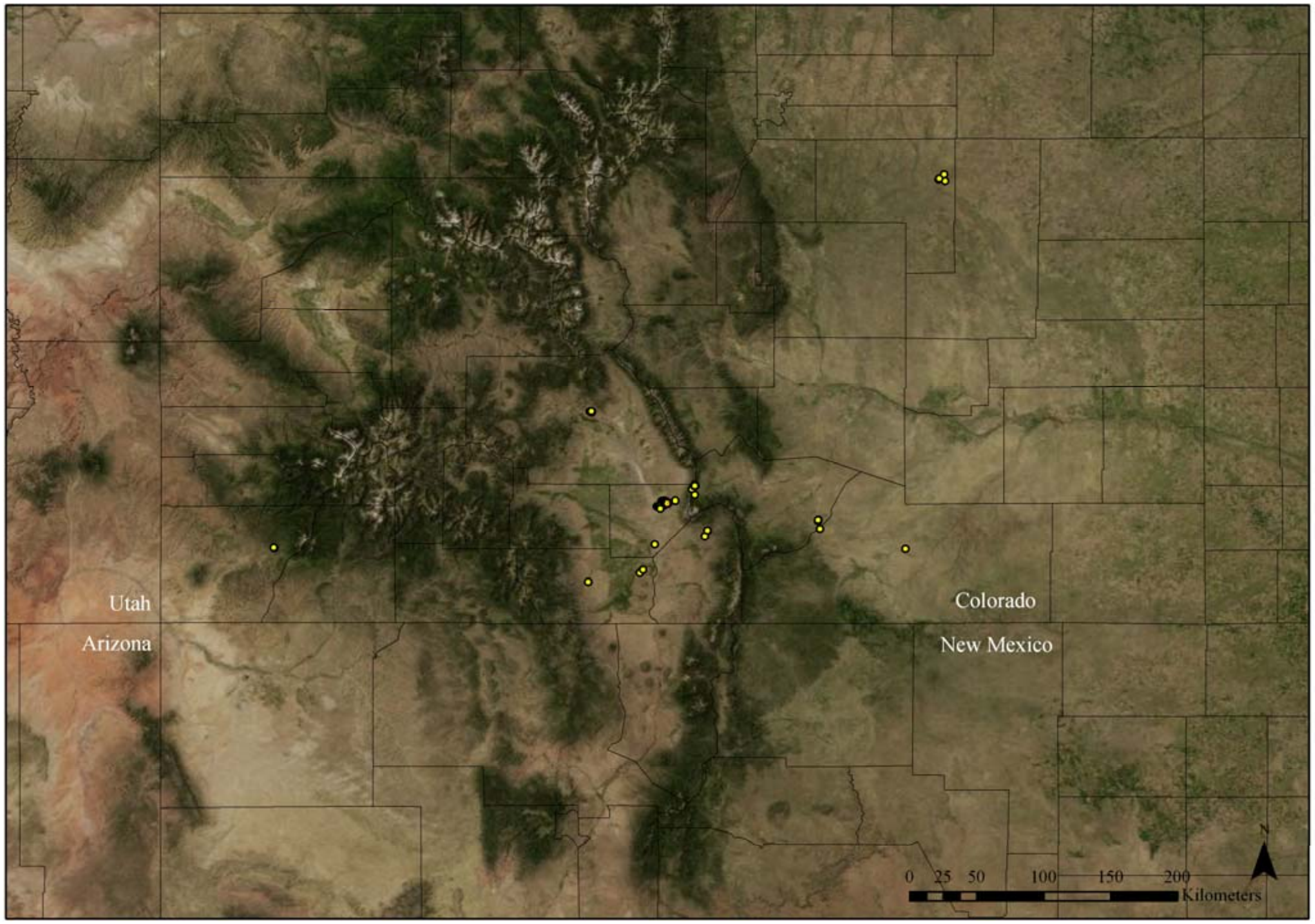

Fig. 4-1. Distribution of *P. hernandesi* encountered during the survey period from 2007-2009.

Table 4-1. Numbers and age/sex classes of *P. hernandesi* encountered at each locality within (bold) and surrounding the Valley.

County	Locality	Total	Adult Male	Adult Female	Juvenile	Neonate
Alamosa	**Medano-Zapata Ranch**	**145**	**74**	**35**	**2**	**34**
Alamosa	**Alamosa National Wildlife Refuge**	**1**	**1**	**0**	**0**	**0**
Conejos/Rio Grande	**Capulin BLM Hills**	**5**	**0**	**3**	**2**	**0**
Conejos/Rio Grande	**McIntyre Springs**	**2**	**1**	**0**	**0**	**1**
Saguache	**Saguache BLM Hills**	**8**	**4**	**1**	**0**	**3**
Costilla	Forbes-Trinchera	3	1	0	2	0
Huerfano	Mosca Pass	11	2	9	0	0
Huerfano	Walsenburg	4	2	2	0	0
Las Animas	Pinon Canyon Maneuver Site	1	1	0	0	0
Montezuma	Jackson Gulch	1	0	1	0	0
Montezuma	Mancos	1	0	1	0	0
	Totals	182	86	52	6	38

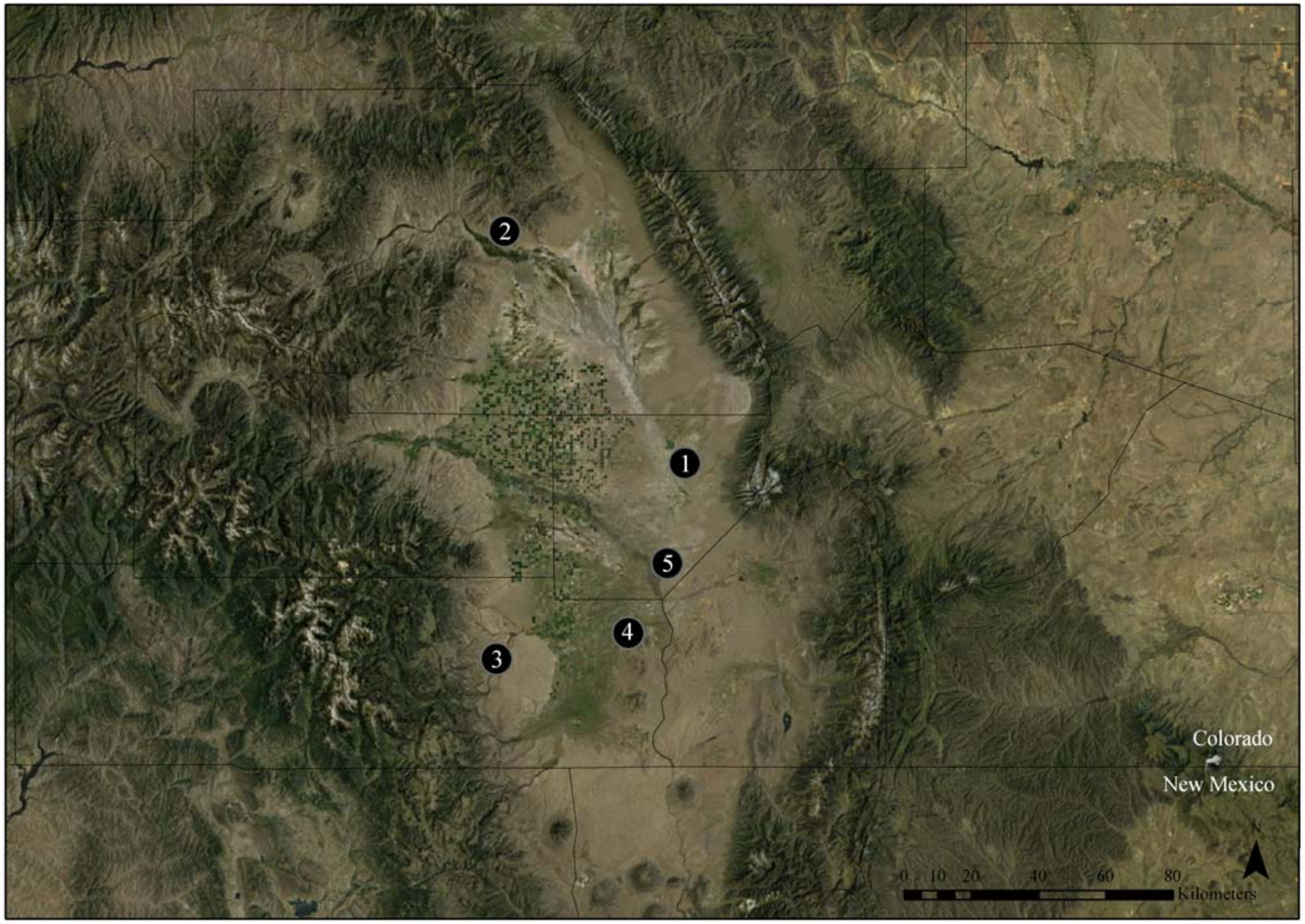

Fig. 4-2. Distribution of *P. hernandesi* encountered within the Valley.

Phrynosoma hernandesi showed localized variation in dorsal coloration and pattern within and outside the Valley that matched the habitat (Fig. 4-3). West of the Valley in southwest Colorado (Mancos, CO), *P. hernandesi* showed a high degree of red, orange, and yellow coloration with broad patches of uninterrupted colors and robust scalation. Mosca Pass, located at the east boundary of the Valley in Great Sand Dunes National Park, supports a population of *P. hernandesi* with similar bright and bold coloration and scalation as lizards west of the Valley, though some individuals deviate from this form. Within the Valley, *P. hernandesi* throughout the Medano-Zapata Ranch sand dunes showed a high amount of pattern with small dots of coloration, primarily white, yellow, and brown. Most lizards east of the Valley in grasslands were the least descript, having coloration and pattern with mostly hues of browns and intermediate pattern, which is more typical of *P. hernandesi* throughout its greater species range.

Diet

A total of 162 scat were collected from 123 individuals, including 126 scat from 104 individuals within the Valley (Saguache and Zapata Ranch) and 36 scat from 19 individuals outside the Valley (Walsenburg, Mosca Pass, Limon, Forbes-Trinchera). A total of 39 scat is from 29 males, 55 scat is from 38 females, 9 scat is from 4 yearlings, and 59 scat is from 54 neonates.

Scat length correlates significantly to scat mass ($t_{104} = 15.37$, $N = 106$, $P < 0.001$; Fig. 4-4). *Phrynosoma hernandesi* has significantly different scat masses relative to body size (ANOVA: $F_{3,130} = 2.96$, $P = 0.038$; Tukey HSD not significant

Fig. 4-3. *Phrynosoma hernandesi* within the Valley that show variation in dorsal color and pattern relative to the habitat substrate. a.) adult female from Zapata Ranch where the substrate is sand and b.) adult female from Capulin Hills where the substrate is volcanic gravel and rocks.

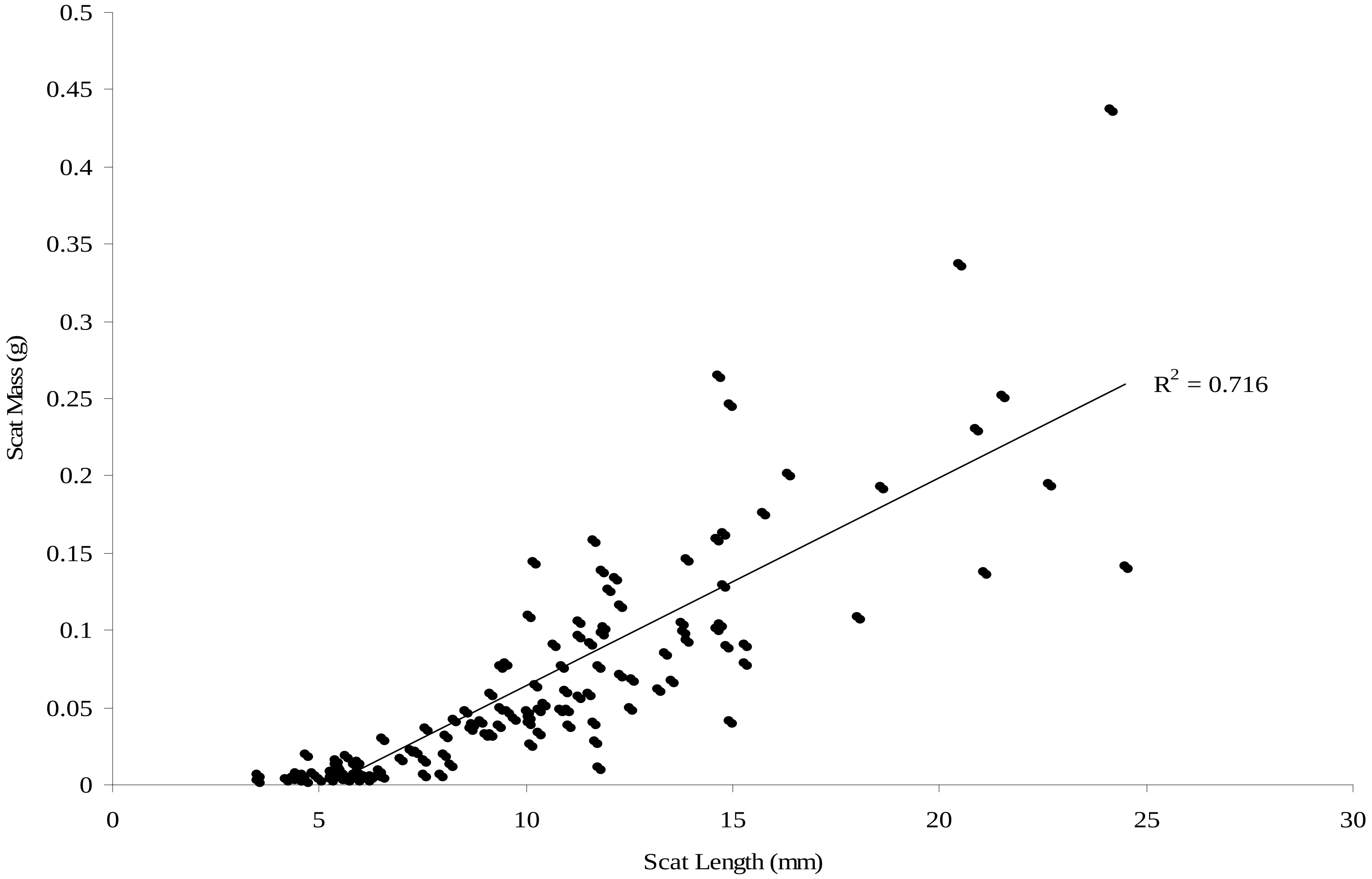

Fig. 4-4. Scat length plotted against scat mass for all *P. hernandesi* scat.

for any pairings) and body mass (ANOVA $F_{3,130}$ = 4.27, P = 0.008; Tukey HSD $p < 0.05$ for all pairings). Pregnant females have the highest scat mass relative to body size (N = 10, 0.00285g/SVL), followed by females (N = 16, 0.00264g/SVL), males (N = 22, 0.00168g/SVL), and neonates (N = 24, 0.00096g/SVL) (Fig. 4-5). However, compared to body mass, females have the highest scat mass (0.01298g/g), followed by males (0.01151g/g), pregnant females (0.01298g/g), and neonates (0.00466g/g) (Fig. 4-6).

In total 6,224 prey items were found in all 173 scat; 4,441 prey items were from Valley *P. hernandesi* and 1,783 prey items were from outside *P. hernandesi*. Overall, *Phrynosoma hernandesi* consumed the highest proportion of ants, which consisted of between 58-94% ants during any year. Valley populations show ontogenetic variation in diet (Table 4-2). Neonates consume mostly ants (92%) and nearly double the intake of Coleopterans as adults (3.7% as neonates and 7.1-8.4% as adults) while consuming over 10% fewer ants (83-84%). Coleopterans were consumed in highest proportions by pregnant females (14.3%) and in the lowest proportions by neonates (3.7%). Interestingly, pregnant females consumed 2-4 times greater proportion of Coleopterans than non-pregnant females (8.4%), but showed negligible differences in proportion of other prey items consumed.

Although *P. hernandesi* consumes mostly ants and beetles, a diversity of other prey items exists in its diet. In the Valley, neonates consumed the highest proportions of Dipteran (0.25%) insects and females consumed the highest proportion of hemipterans (0.72%). Juveniles consumed the highest proportion of pebbles (11.3%)

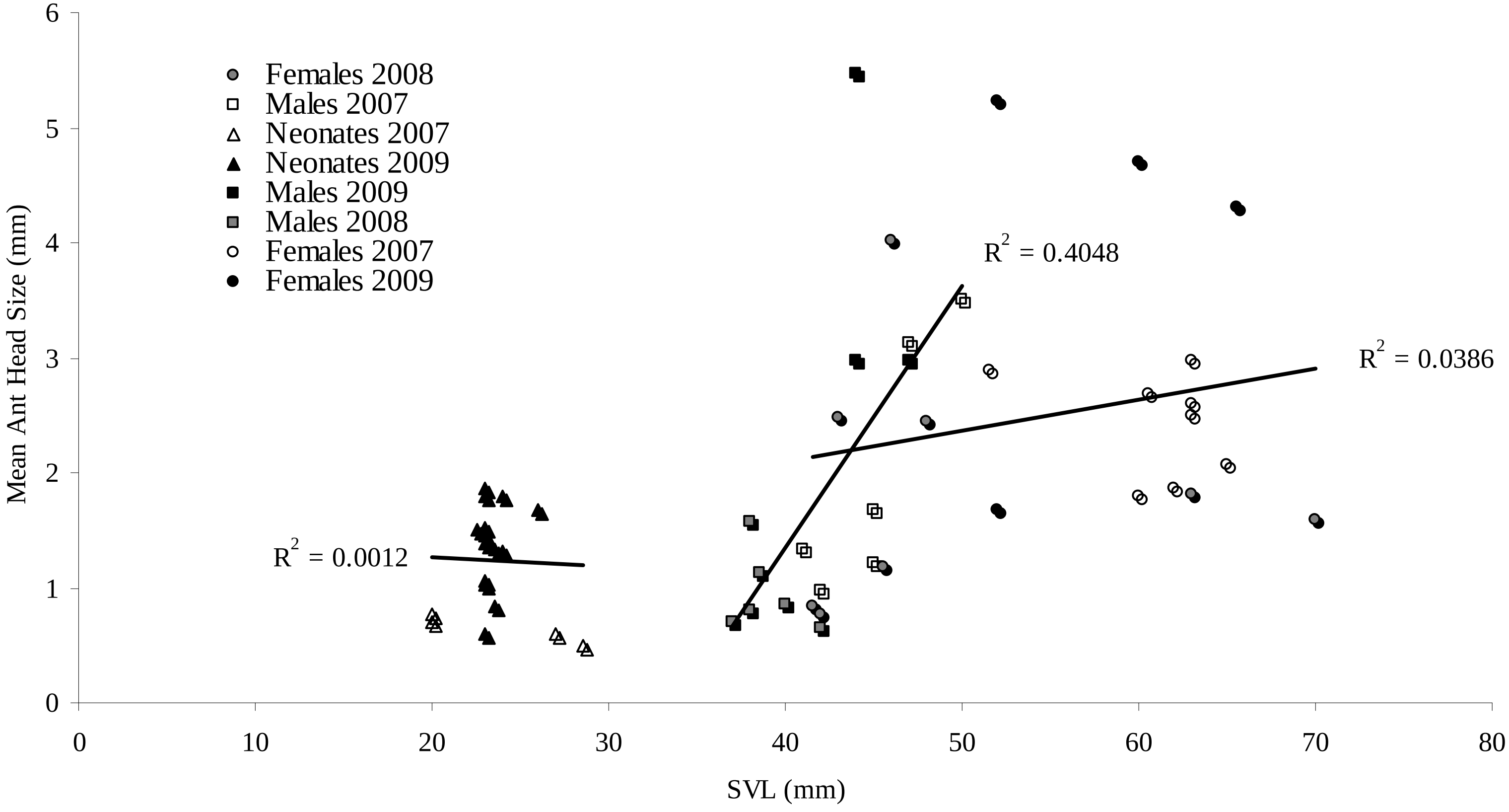

Fig. 4-5. Annual variation of ant sizes consumed by *P. hernandesi*. *Phrynosoma hernandesi* shows variation in the sizes of ants consumed which correspond to the donimant ant genera consumed annually.

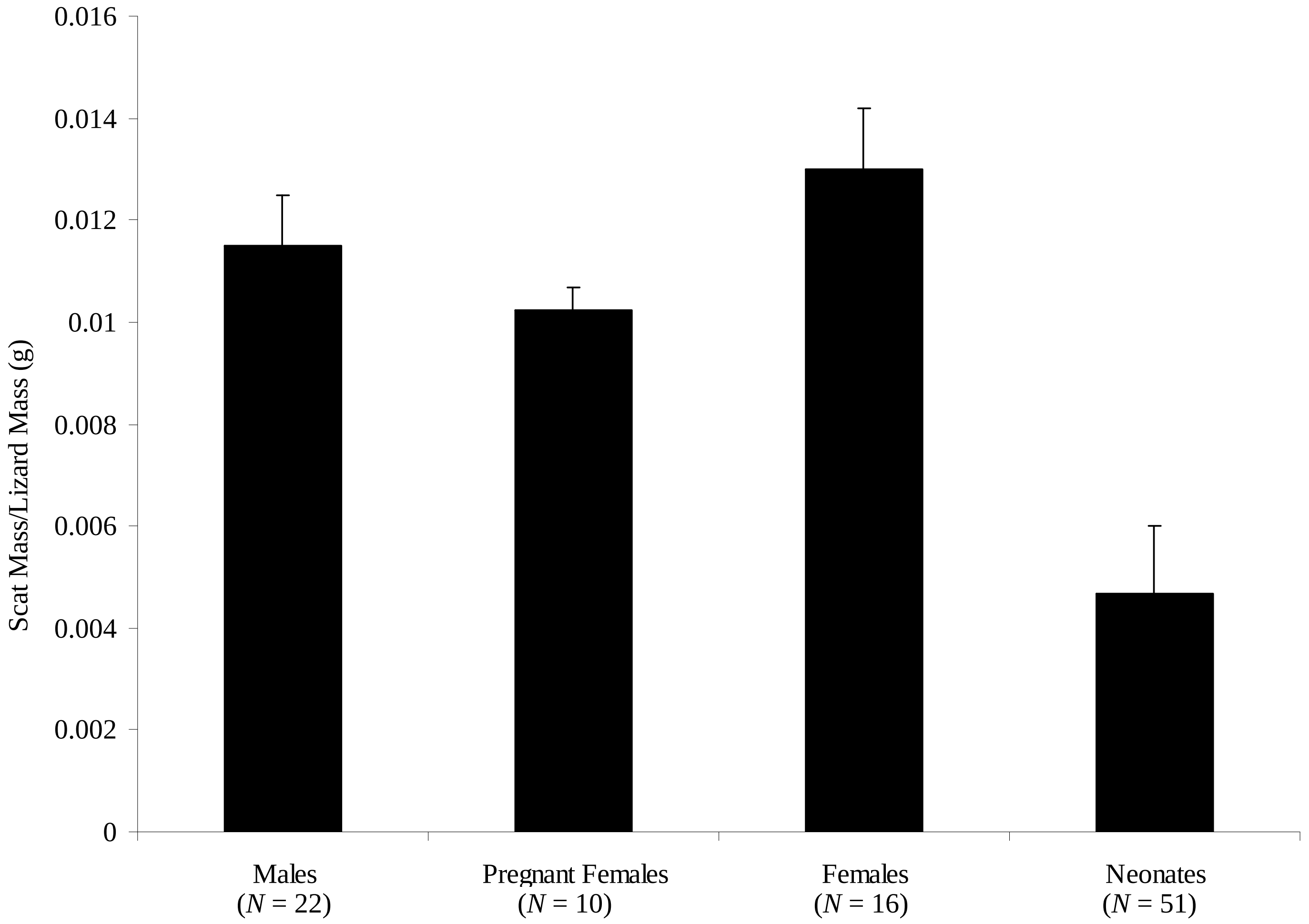

Fig. 4-6. Scat mass relative to *P. hernandesi* mass at Zapata Ranch, including standard error bars.

Table 4-2. Number and proportion of prey items consumed by *P. hernandesi* within the Valley from 2007-2009. A total of 126 scat from 104 lizards is reported. Values in parentheses for each lizard groups indicates the number of lizards and number of scat analyzed. Multiple scat from one individual were averaged and counted as one scat prior to analysis.

			Formicidae		Coleoptera		Diptera		Hemiptera		Hymenoptera		Isoptera		Pebbles		Organic Debris	
Year	Locality	Lizard Group	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
2007-2009	Zapata	Male (*N* = 22, 32)	1462	*83.4%*	125	*0.7%*	3	*0.2%*	6	*0.3%*	1	*0.1%*	0	*0.0%*	1	*8.8%*	1	*0.2%*
		Female (*N* = 15, 19)	1395	*83.8%*	140	*8.4%*	1	*0.1%*	12	*0.7%*	0	*0.0%*	0	*0.0%*	2	*5.8%*	4	*1.2%*
		Pregnant Female (*N* = 13, 16)	538	*81.6%*	94	*14.3%*	0	*0.0%*	4	*0.6%*	0	*0.0%*	0	*0.0%*	0	*2.3%*	0	*1.2%*
		Neonate (*N* = 54, 59)	743	*91.7%*	30	*3.7%*	2	*0.2%*	4	*0.5%*	0	*0.0%*	1	*0.1%*	22	*3.5%*	2	*0.2%*
2007	Zapata	Male (*N* = 12, 17)	572	*86.8%*	65	*9.9%*	1	*0.2%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	20	*3.0%*	1	*0.2%*
		Female (*N* = 4, 5)	577	*89.5%*	44	*6.8%*	1	*0.2%*	5	*0.8%*	0	*0.0%*	0	*0.0%*	15	*2.3%*	3	*0.5%*
		Pregnant Female (*N* = 7, 9)	426	*91.2%*	29	*6.2%*	0	*0.0%*	3	*0.6%*	0	*0.0%*	0	*0.0%*	6	*1.3%*	3	*0.6%*
		Neonate (*N* = 8, 12)	164	*90.1%*	11	*6.0%*	0	*0.0%*	1	*0.5%*	0	*0.0%*	0	*0.0%*	6	*3.3%*	0	*0.0%*
2008	Zapata	Male (*N* = 7, 11)	823	*80.9%*	52	*5.1%*	2	*0.2%*	6	*0.6%*	0	*0.0%*	0	*0.0%*	133	*13.1%*	1	*0.1%*
		Female (*N* = 7, 10)	672	*78.2%*	87	*10.1%*	0	*0.0%*	7	*0.8%*	0	*0.0%*	0	*0.0%*	80	*9.3%*	13	*1.5%*
		Pregnant Female (*N* = 6, 7)	112	*58.3%*	65	*33.9%*	0	*0.0%*	1	*0.5%*	0	*0.0%*	0	*0.0%*	9	*4.7%*	5	*2.6%*
		Neonate (*N* = 3, 4)	0	*0.0%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	0	*0.0%*
2009	Zapata	Male (*N* = 3, 4)	67	*85.9%*	8	*10.3%*	0	*0.0%*	0	*0.0%*	1	*0.1%*	0	*0.0%*	154	*1.3%*	3	*1.3%*
		Female (*N* = 4, 4)	146	*90.7%*	9	*5.6%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	97	*1.2%*	20	*2.5%*
		Pregnant Female (*N* = 0, 0)	0	*0.0%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	0	*0.0%*	15	*0.0%*	8	*0.0%*
		Neonate (*N* = 43, 43)	579	*92.2%*	19	*3.0%*	2	*0.3%*	3	*0.5%*	0	*0.0%*	1	*0.2%*	28	*3.5%*	2	*0.3%*
2007	Saguache	Male (*N* = 3, 6)	320	93.2%	13	3.8%	0	*0.0%*	0	0.0%	0	*0.0%*	0	*0.0%*	11	3.2%	0	*0.0%*
		Female (*N* = 1, 2)	66	90.4%	3	4.1%	0	*0.0%*	1	1.4%	0	*0.0%*	0	*0.0%*	3	4.1%	0	*0.0%*
		Pregnant Female (*N* = 0, 0)	0	0.0%	0	0.0%	0	*0.0%*	0	0.0%	0	*0.0%*	0	*0.0%*	0	0.0%	0	*0.0%*
		Neonate *(N* = 2, 5)	98	98.9%	1	1.0%	0	*0.0%*	0	0.0%	0	*0.0%*	0	*0.0%*	0	0.0%	0	*0.0%*

while pregnant females and neonates consumed the least amount (2.3 and 3.5%, respectively). Although males and females consumed lower portions of pebbles (5.8% and 8.8%, respectively), the pebbles they did consume were often over 3 times larger in diameter than those consumed by juveniles (personal observation). Organic debris consumption was highest among all females (1.2% each) and absent from juvenile diets. Hymenopteran and Isopteran insects constitute a negligible proportion of their diets; only one each of Hymennoptera and Isoptera insects were consumed by a male and neonate, respectively. Also observed in scat from 4 individuals was shed skin from the mouth region of the head.

Phrynosona hernandesi also showed variability in diet across localities within the Valley (Table 4-2). At Saguache, individuals consumed almost exclusively ants (90.4-99% for all age/sex classes) and consumed in almost equal proportions Coleopterans and pebbles (3.2-4.1% each). Similar to Zapata populations, Saguache individuals consume the highest amount of ants as neonates (99.9%) and increase their intake of Coleopterans as adults (1% as neonates and 3.2-4.1% as adults).

At Zapata, *P. hernandesi* diets showed annual fluctuation across age and sex classes. Females decreased ant consumption by 12% from 2007 to 2008, but increased it by 12% in 2009. Males consumed ants in fairly consistent proportions but consumed the most ants (87%) in 2007. Males, however, did show fluctuation in the proportion of Coleopteran insects consumed; in 2007, 9.9% of male diets consisted of Coleopterans but decreased their intake by half in 2008, and returned to consuming 10% Coleopterans in 2009. Females showed a converse trend where they consumed the most Coleopterans in 2008 (10.2%) and the least in 2007 (6.8%) and

2009 (5.6%). No juvenile scat were found in 2007 and 2009, so annual comparisons cannot be made. No neonate scat were collected in 2008, but neonates were fairly consistent in diet proportions in 2007 and 2009 with the exception of the amount of Coleopterans; neonates consumed half as many Coleopterans in 2009 (3.0%) than 2007 (6.0%). In 2008, males consumed 13% pebbles, as compared to 3.0% and 1.0% in 2007 and 2009, respectively. Female pebble consumption increased 6-fold from 2007 to 2009 while neonate consumption remained constant in 2007 to 2009. Organic debris was consumed in highest proportions in 2009 by males (1.2%) but was constant in 2007 and 2008 (0.1%). Organic debris consumption by females increased gradually from 0.2% in 2007 to 2.5% in 2009, and no organic debris was observed in neonate scat.

Phrynosoma hernandesi diets outside the Valley were variable across age/sex classes and compared to Valley populations (Table 4-3). Populations outside the Valley consumed ants in higher proportions than Valley populations. Males consumed 94% ants, females 82%, and juveniles 93%. No neonate scat was collected. Dipterans were consumed in negligible amounts by all populations (0-0.6%). In contrast to Valley populations, Coleopterans were consumed in highest proportions by males (5.8%) and juveniles (5.0%) and the least by females (2.9%) outside the Valley. Hymenopteran and Isopteran insects were not consumed by individuals outside the Valley. Females consumed the most pebbles (12.6%) while pebbles consisted of <1% of male and juvenile diets. Organic debris was absent from males while females consumed 2% and juveniles consumed negligible amounts (0.2%).

Table 4-3. Number and proportion of prey items consumed by *P. hernandesi* outside the Valley.

			Formicidae		Coleoptera		Diptera		Hemiptera		Hymenoptera		Isoptera		Pebbles		Organic Debris	
Year	Locality	Lizard Group	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
2007-2009	Outside (all sites)	Male (*N* = 5, 7)	289	92.6%	18	5.8%	0	0.0%	2	0.6%	0	0.0%	0	0.0%	3	1.0%	0	0.0%
		Female (*N* = 10, 20)	827	81.7%	29	2.9%	4	0.4%	4	0.4%	0	0.0%	0	0.0%	128	12.7%	20	2.0%
		Juvenile (*N* = 4, 9)	429	93.5%	23	5.0%	1	0.2%	1	0.2%	0	0.0%	0	0.0%	4	0.9%	1	0.2%
		Neonate (*N* = 0)	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2008-2009	Mosca Pass	Male (*N* = 0)	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
		Female (*N* = 6, 13)	546	79.3%	15	2.2%	4	0.6%	1	0.2%	0	0.0%	0	0.0%	109	15.8%	14	2.0%
		Juvenile (*N* = 0)	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
		Neonate (*N* = 0)	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2008	Forbes Trinchera	Male (*N* = 1, 1)	49	77.8%	3	4.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	11	17.5%	0	0.0%
		Female (*N* = 2, 5)	0	81.0%	0	2.7%	0	0.4%	0	0.1%	0	0.0%	0	0.0%	0	13.6%	0	2.1%
		Juvenile (*N* = 2, 5)	176	83.0%	12	5.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	24	11.3%	0	0.0%
		Neonate (*N* = 0)	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2008	Limon	Male (*N* = 2, 3)	32	75.0%	9	2.3%	0	0.0%	1	20.9%	0	0.0%	0	0.0%	1	0.9%	0	0.0%
		Female (*N* = 0)	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
		Juvenile (*N* = 0)	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
		Neonate (*N* = 0)	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
2008	Walsenburg	Male (*N* = 2, 3)	208	95.9%	6	2.8%	0	0.0%	1	0.5%	0	0.0%	0	0.0%	2	0.9%	0	0.0%
		Female (*N* = 2, 2)	78	89.7%	4	4.6%	0	0.0%	3	3.5%	0	0.0%	0	0.0%	2	2.3%	0	0.0%
		Juvenile (*N* = 2, 4)	253	93.4%	11	4.1%	1	0.4%	1	0.3%	0	0.0%	0	0.0%	4	1.5%	1	0.3%
		Neonate (*N* = 0)	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Phrynosoma hernandesi showed diet variation across localities (MRPP: A = 0.0702, t = 6.96, P = 0.0002; Table 4-4). Individuals at Mosca Pass consumed significantly higher proportions of Formicidae (Indicator Value = 52.3), Hymenoptera (Indicator Value = 49.3), pebbles (Indicator Value = 67.3), and debris (Indicator Value = 90.2) compared to populations within the Valley ($P < 0.02$) and outside the Valley at other locations while individuals outside the Valley consumed significantly higher amounts of Hemipterans (ISA: Indicator Value = 30.0).

Phrynosoma hernandesi at Zapata Ranch showed annual fluctuation in diet (MRPP; A = 0.0607, t = 5.07, P = 0.0015). In 2007, *P. hernandesi* consumed significantly more Coleopterans (Indicator Value = 49.8, P = 0.002) and in 2008, *P. hernandesi* consumed significantly more pebbles (Indicator Value = 76.4, P = 0.001). These differences in annual diet were driven by age and sex class groups (MRPP: A = 0.1197, t = 9.999, $P < 0.0001$; Table 4-5). Pregnant females showed no significant variation in diet proportions compared to non-pregnant females ($P > 0.05$ for all prey items) and all females were combined for analysis. Females consumed significantly higher amounts of Formicidae (Indicator Value = 45.4, P = 0.02), Coleopterans (Indicator Value = 44.0, P = 0.011), and debris (Indicator Value = 24.6, P = 0.011) compared to males and neonates while males consumed the highest proportion of pebbles (Indicator Value = 43.0, P = 0.02).

There was no significant difference in the size of ants eaten by females, males, or neonates in the Valley compared to *P. hernandesi* within the same age/sex class outside the Valley ($P > 0.05$; Table 4-6). Pregnant females did not eat ants significantly different in size than non-pregnant females ($P > 0.05$) so all females

Table 4-4. Indicator Species Analysis to test for prey item associations across localities. High indicator values indicate an association and the associated *P*-value shows the significance of that relationship.

Prey Item	Maximum Group	Indicator Value	Mean	SD	*P*- value
Formicidae	Mosca Pass	52.30	40.60	4.49	0.013
Coleoptera	Outside	40.90	34.10	6.89	0.146
Hemiptera	Outside	30.00	14.30	7.77	0.046
Diptera	Mosca Pass	11.60	9.90	5.75	0.267
Isoptera	Valley	1.10	2.70	4.21	1.000
Hymenoptera	Mosca Pass	49.30	7.40	4.73	0.001
Pebbles	Mosca Pass	67.30	31.00	8.92	0.003
Debris	Mosca Pass	90.20	14.70	7.70	0.001

Table 4-5. Indicator Species Analysis testing prey item associations across age/sex classes. High indicator values indicate an association and the associated *P*-value shows the significance of that relationship.

Prey Item	Maximum Group	Indicator Value	Mean	SD	*P*-value
Formicidae	Female	45.40	38.20	3.16	0.020
Coleoptera	Female	44.00	29.90	4.56	0.022
Hemiptera	Female	12.70	9.10	3.67	0.167
Diptera	Male	6.30	6.20	3.12	0.397
Isoptera	Neonate	2.30	3.70	1.44	1.000
Hymenoptera	Male	5.90	3.70	1.44	0.212
Pebbles	Male	43.00	26.30	6.41	0.020
Debris	Female	24.60	10.30	4.37	0.011

Table 4-6. ANOVA using Tukey's HSD test for ant head sizes consumed by *P. hernandesi* across localities and among age/sex groups and years at Zapata Ranch. Valley lizards include individuals from Saguache and Zapata, since there was no difference in the sizes of ant heads consumed by either group ($P > 0.05$).

Lizard Group	Grouping	Variable	*N*	Mean	SE	df	*F*	*P*-value	Tukey's HSD
Males	Locality	Outside^{a}	5	1.44	0.31	1	1.47	0.2507	
		Valley^{b}	8	1.97	0.81				
Females	Locality	Outside^{a}	8	1.97	0.81	1	0.75	0.3938	
		Valley^{b}	22	2.38	0.27				
Zapata	Lizard Groups	Male^{a}	15	1.93	0.36	2	15.46	<0.0001	ac $P < 0.05$, bc $P < 0.01$
		Female^{b}	20	2.52	0.28				
		Neonate^{c}	46	1.23	0.05				
Zapata Males	Year	2007^{a}	6	1.97	0.44	2	9.34	0.0036	ac $P < 0.05$, bc $P < 0.01$
		2008^{b}	6	0.95	0.14				
		2009^{c}	3	3.81	0.83				
Zapata Females	Year	2007^{a}	8	2.42	0.16	2	5.84	0.0117	ac $P < 0.05$, bc $P < 0.01$
		2008^{b}	8	1.89	0.38				
		2009^{c}	4	3.98	0.79				
Zapata Neonates	Year	2007^{a}	4	0.64	0.06	1	15.34	0.0003	
		2009^{c}	41	1.29	0.05				

were combined for analysis. Similarly, *P. hernandesi* at Saguache do not eat ants of significantly different sizes than at Zapata ($P > 0.05$), so these two localities were combined for the locality analysis. *Phrynosoma hernandesi* showed no significant variation in mean size of ants consumed within each age/sex class; however, individuals at Zapata showed annual variation in the size of ants consumed (Fig. 4-7). Ants eaten by males in 2009 were 2-4 times larger in size than ants eaten in 2008 and 2007, respectively (Table 4-6). Females showed the same difference in ant head sizes as males; females in 2009 consumed ants 2 times larger than in 2007 and 4 times larger than in 2008 (Table 4-6). No scat was collected from neonates in 2008, although neonates consumed ants 2 times larger in 2009 than 2008 ($F_{1,43} = 15.34$, $P = 0.0003$) (Table 4-6). However, ants showed significant variation in mean head size from 2007 to 2009 that can partially explain the trend in annual variation of ant head sizes consumed by age/sex groups (Table 4-7). Dorymyrmex, Formica, and Camponotus mean head sizes were significantly smaller in 2007 and 2008 than in 2009 ($P < 0.01$) while Pheidole head sizes did not vary significantly across years ($P > 0.05$).

The 7 ant genera present within scat from Zapata Ranch in 2009 varied significantly in head size ($F_{6,2} = 119.4$, $P < 0.0001$; Fig. 4-8). *Crematogaster* was significantly smaller than 4 other species ($P < 0.05$), *Camponotus* was larger than 3 other species ($P < 0.05$), *Leptothorax* was larger than 1 other species ($P < 0.05$), and *Formica* was larger than all 7 other species ($P < 0.01$). Based on the 10 randomly selected ant heads from each scat, Valley populations show the greatest diversity in

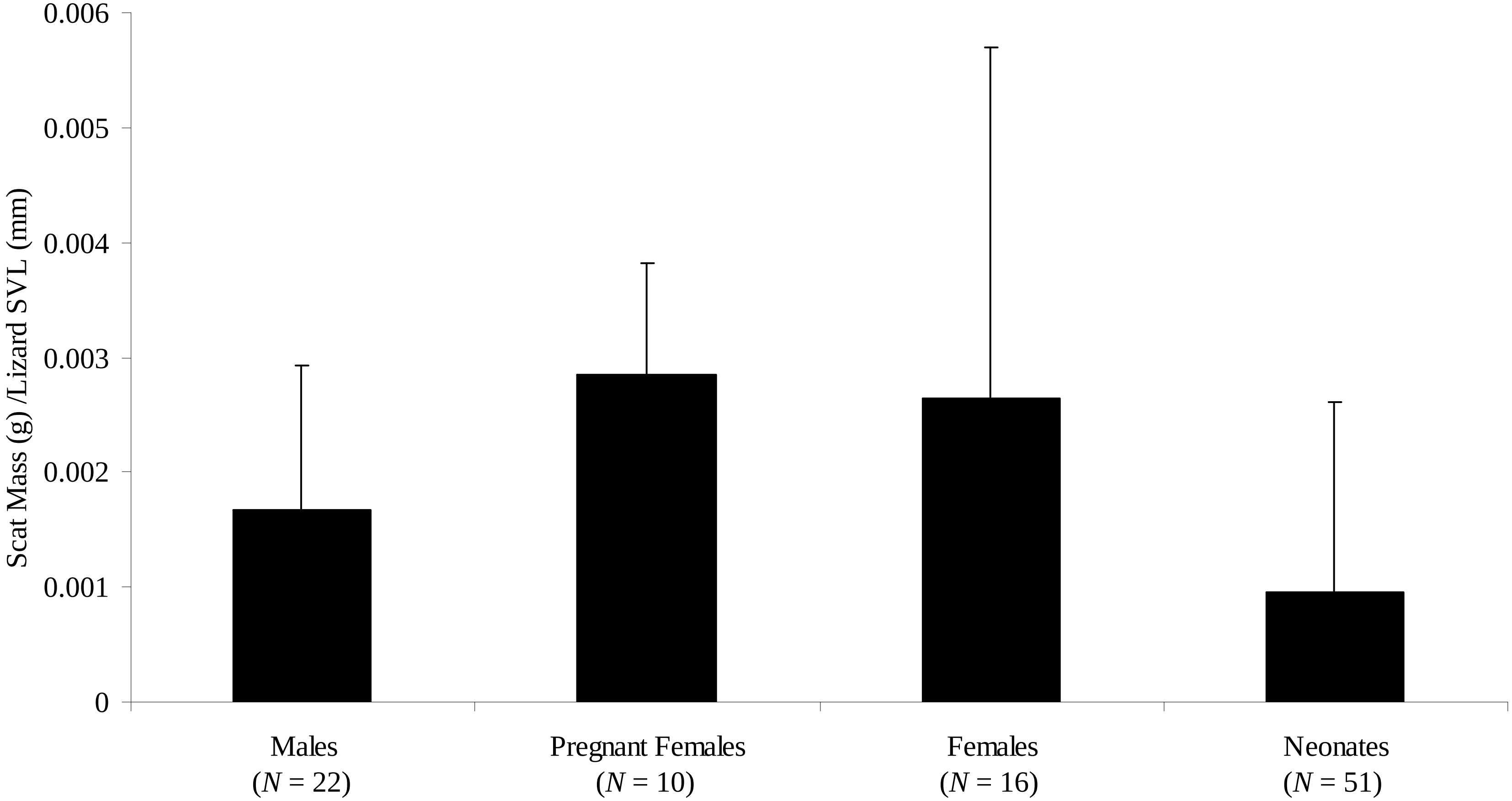

Fig. 4-7. Scat mass relative to *P. hernandesi* size at Zapata Ranch, including standard error bar.

Table 4-7. ANOVA using Tukey's HSD test for annual variation in ant head sizes (length*width, mm) at Zapata Ranch.

Ant Genus	Year	*N*	Mean	SE	df	*F*	*P*-value	Tukey's HSD
Doromyrmex	2007^a	54	0.600	0.012	2	186.46	<0.0001	ac, bc $P < 0.01$
	2008^b	47	0.640	0.010				
	2009^c	160	0.990	0.015				
Formica	2007^a	120	2.440	0.125	2	16.30	<0.0001	ac, bc $P < 0.01$
	2008^b	71	2.810	0.289				
	2009^c	82	3.910	0.198				
Camponotus	2007^a	62	0.960	0.046	2	105.33	<0.0001	ac, bc $P < 0.01$
	2008^b	26	0.850	0.041				
	2009^c	157	1.860	0.044				
Pheidole	2007^a	6	1.890	0.546	2	0.07	0.507	n.s.
	2008^b	6	2.660	0.249				
	2009^c	3	2.220	0.796				

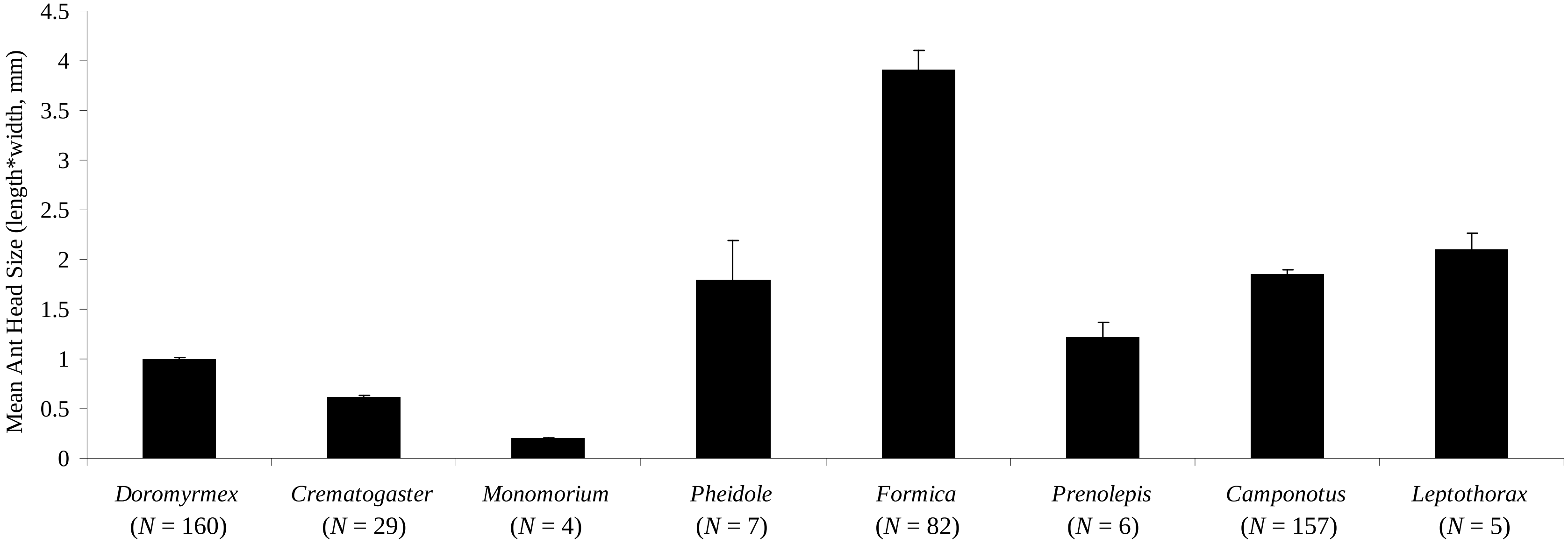

Fig. 4-8. Mean head sizes for ant genera from *P. hernandesi* scat collected at Zapata Ranch in 2009. Adults mostly consume larger genera (*Formica*, *Camponotus*) while neonates consume smaller genera (*Dorymyrmex*) or smaller individuals from larger genera (*Camponotus*)

the number of ant species consumed than lizards outside the Valley (Table 4-8). Males mostly consume *Formica* species (41.6%), but also *Doromyrmex* (31.5%) and *Camponotus* (24.7%). Females mostly consume *Formica* (52.5%) and *Camponotus* (40%) while pregnant females consume primarily *Formica* (80%). Neonates consume mostly *Camponotus* (40.5%) and *Doromyrmex* (43.2%).

At Zapata Ranch, females mostly consumed *Formica* species across all years, but ate only half as many *Formica* in 2008 (50%) than 2007 (100%) or 2009 (96.7%). Males in 2007 and 2008 consumed mostly *Formica* (62.7% and 63.6%, respectively) but in 2008 they consumed mostly *Doromyrmex* (47/1%) and *Camponotus* (31.4%) species; *Formica* was consumed only one-third the amount as in 2007 and 2009. Neonates in 2007 consumed in equal proportions *Doromyrmex* and *Camponotus* (47.1%) and nearly equal proportions of the same genera in 2009 (44.5% and 37.9%, respectively). No neonate scat was obtained in 2008. Individuals at Saguache consumed mostly *Camponotus* and *Doromyrmex* species. Outside the Valley, the proportion of ants in adult male and female diets was both dominated by *Camponotus*.

Population Demographics

A total of 103 hours was spent searching for *P. hernandesi* at SW052 from 2007-2009 (19.5, 34, and 49.5 person hours on those respective years). In total, 122 individuals were captured at Zapata during the three annual sweeps; 19 individuals were captured in 2007, 31 in 2008, and 72 in 2009 (Fig. 4-9). Population densities varied from 0.69-1.03 lizards/ha. Only one *P. hernandesi* was recaptured; a female at

Table 4-8. Proportion of ant genera (reported as percentage) consumed by *P. hernandesi* across localities and years based on 10 randomly-selected ant heads from each scat. Multiple scat from one lizard were grouped prior to analysis to avoid repeated measures.

				Ant Genus							
Locality	Year	Lizard Group	# Prey Items	*Dorymyrmex*	*Crematogaster*	*Monomorium*	*Pheidole*	*Formica*	*Prenolepis*	*Camponotus*	*Leptothorax*
Zapata	2007	Female (*N* = 2)	20	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%
		Male (*N* = 6)	59	27.1%	0.0%	0.0%	3.4%	62.7%	0.0%	6.8%	0.0%
		Neonate (*N* = 3)	17	47.1%	0.0%	0.0%	5.9%	0.0%	0.0%	47.1%	0.0%
		Prego (*N* = 6)	60	3.3%	0.0%	0.0%	0.0%	80.0%	0.0%	16.7%	0.0%
Zapata	2008	Female (*N* = 8)	80	17.5%	0.0%	1.3%	1.3%	50.0%	10.0%	20.0%	0.0%
		Male (*N* = 7)	70	47.1%	0.0%	0.0%	1.4%	20.0%	0.0%	31.4%	0.0%
Zapata	2009	Female (*N* = 3)	30	3.3%	0.0%	0.0%	0.0%	96.7%	0.0%	0.0%	0.0%
		Male (*N* = 3)	22	4.5%	0.0%	0.0%	0.0%	63.6%	4.5%	27.3%	0.0%
		Neonate (*N* = 47)	348	44.5%	8.3%	0.0%	0.9%	6.9%	1.4%	37.9%	0.0%
Saguache	2007	Female (*N* = 2)	20	10.0%	0.0%	0.0%	5.0%	5.0%	0.0%	80.0%	0.0%
		Male (*N* = 3)	30	40.0%	0.0%	0.0%	0.0%	0.0%	0.0%	60.0%	0.0%
		Neonate (*N* = 2)	20	40.0%	0.0%	0.0%	5.0%	20.0%	0.0%	35.0%	0.0%
Outside	2008	Female* (*N* = 4)	39	0.0%	0.0%	0.0%		28.2%	0.0%	56.4%	12.8%
		Female (*N* = 5)	50	4.0%	0.0%	0.0%	8.0%	6.0%	0.0%	82.0%	0.0%
		Male (*N* = 4)	40	10.0%	0.0%	0.0%	2.5%	12.5%	0.0%	75.0%	0.0%

* = Mosca Pass

Fig. 4-9. Proportion of *P. hernandesi* age and sex classes observed at Zapata Ranch from 2007-2009. No subadults were observed during the surveys any year, indicating that lizards mature within their first full season of activity.

SW052 at Zapata Ranch was initially captured in 2007 and recaptured in 2008 during the sweep. On average, *P. hernandesi* were found at rates of 0.71 to 1.45 lizards/hour. The highest density of *P. hernandesi* occurred in 2009, while 2007 showed the lowest density. Overall, 59% of the individuals observed were adults while 41% were neonates. Of the total adults observed, over half (64%) were females. No yearlings were observed during any year during the sweeps. The annual proportion of females to males remained a constant 1:2 ratio annually. The proportion of neonates fluctuated greatly in 2009; in 2007 and 2008, 21-22% of individuals encountered were neonates while in 2009, 54% were neonates.

Compared to historic climate data for the area, 2006-2009 was higher than average for both minimum and maximum daily temperatures while there was great variability in the timing and amount of rainfall and snowfall across years (Figs. 4-10 to 4-13). Temperatures in 2006 showed the greatest variation compared to 2007-2009 temperature data; Spring (April-June) minimum and maximum temperatures were relatively high while fall (September-October) temperatures were relatively lower. Total annual precipitation was 116% and 147% above historical average (28.42 cm) in 2006 and 2007, 100% of average in 2008, and 9% below average in 2009. There is great variation in the timing of rainfall across years although most rainfall occurs during summer. In 2006, 75% of the total rainfall occurred from July-October. In 2007, 50% of rainfall occurred from July-September while the next largest amount, 25%, occurred from April-May. In 2008, 50% of rainfall occurred from July-August while in 2009, 50% occurred from April-June. The highest variation in snowfall occurs during April, when snowfall begins to cease. Over half (53%) the snowfall in

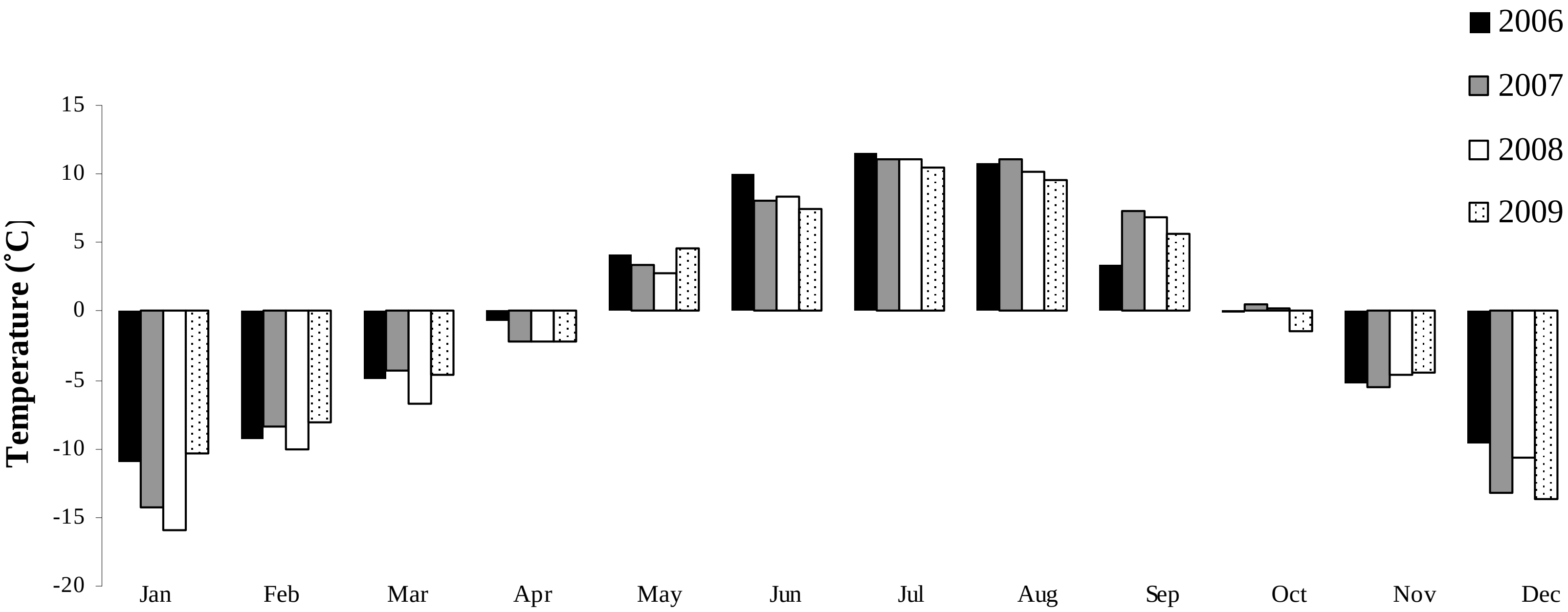

Fig. 4-10. Average minimum daily temperatures at Zapata Ranch.

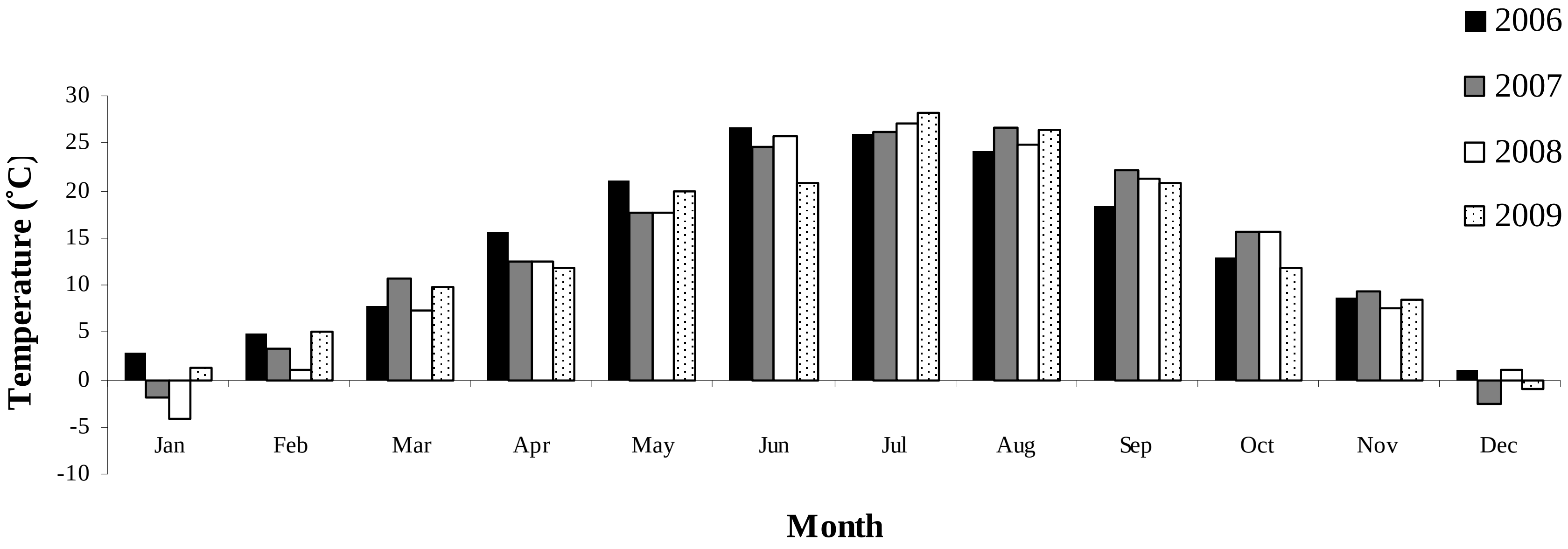

Fig. 4-11. Average maximum daily temperatures at Zapata Ranch.

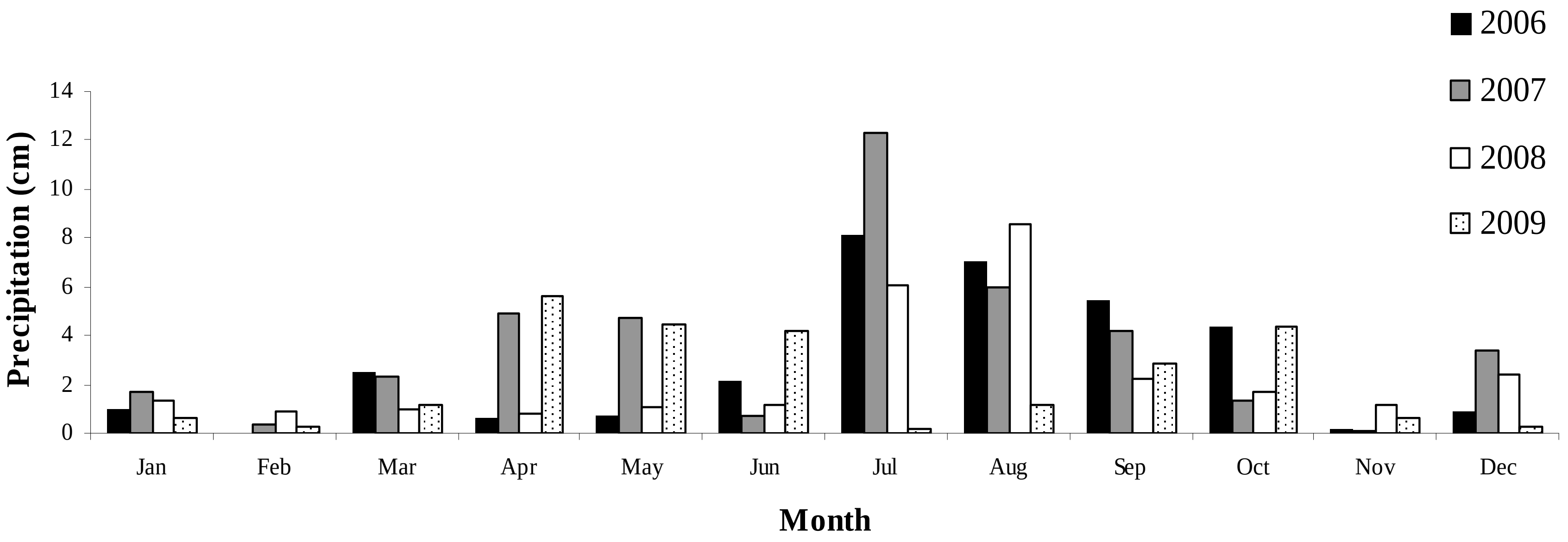

Fig. 4-12. Average precipitation at Zapata Ranch.

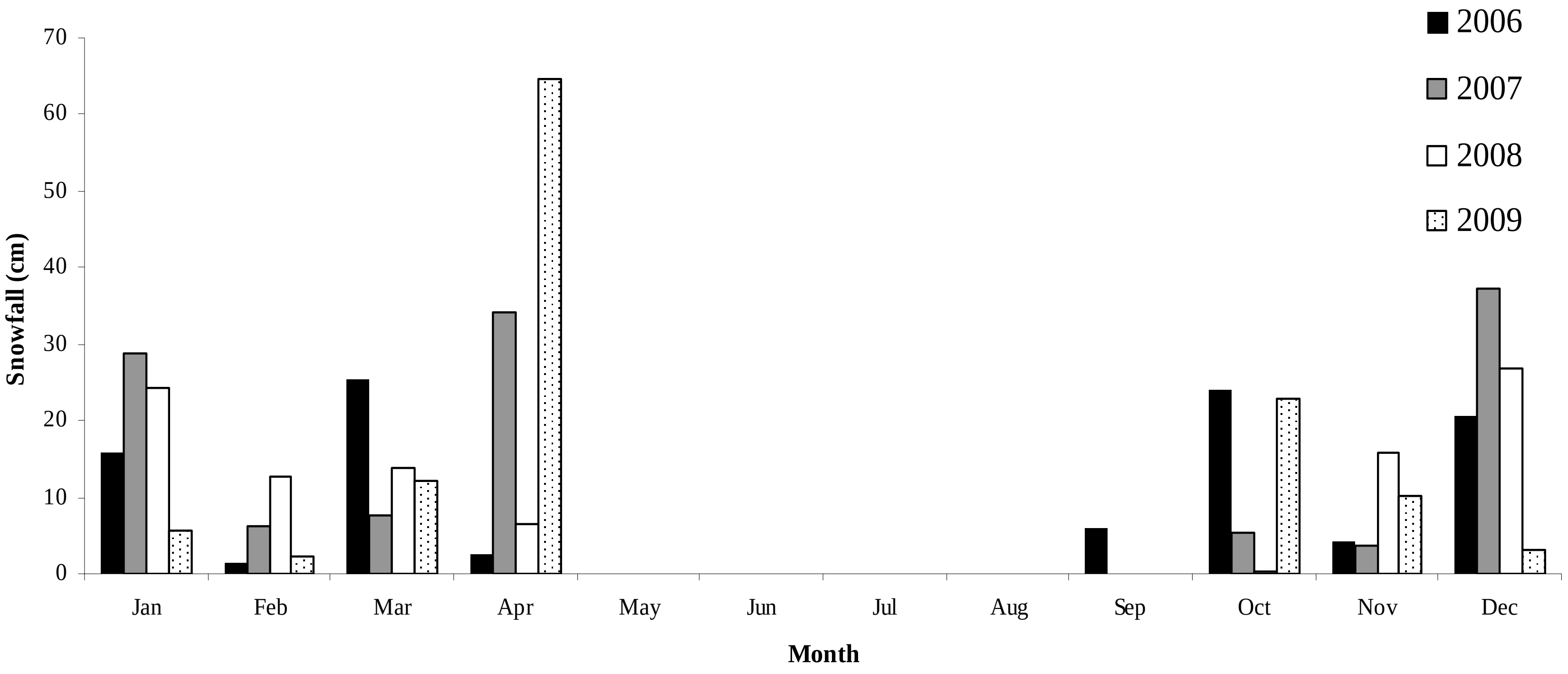

Fig. 4-13. Average snowfall at Zapata Ranch

2009 arrived in April while only 2.5% occurred in 2006. On average, April receives approximately 14% of total snowfall.

During the 3 years Zapata *P. hernandesi* were measured, both male and female adults showed significant variation in morphologies (Tables 4-9 and 4-10). Females showed annual variation in tail length, femur length, head length, and shield width across all years ($P < 0.001$) and although significant, males showed less variation than females ($P < 0.001$); only variation in tail length, femur length, and head length was seen in 2008 males compared to 2007 and 2009 males while no annual variation in shield width occurred across years.

Phrynosoma hernandesi also varied significantly in size during the 3 years (ANCOVA: Males: $F_{2,24} = 7.73$, $P = 0.003$; Females $F_{2,44} = 6.79$, $P = 0.003$; Neonates $F_{2,48} = 19.74$, $P < 0.0001$; Fig. 4-14). Pregnant females vary mass/svl proportions from 0.17 to 0.37 ($N = 17$, $\bar{x} = 0.26$). Non-pregnant females, or females with minimal fetal development, range from 0.11 to 0.20 (n = 29, mean = 0.14). Males range from 0.09 to 0.18 ($N = 26$, $\bar{x} = 0.13$) and neonates are 0.02 to 0.05 ($N = 49$, $\bar{x} = 0.03$). Although no yearlings were observed during the sweeps, a total of 7 yearlings were observed at other localities at Zapata Ranch earlier in the season (June and July 2007) and these are what the neonate values are based on. In 2008, males and females were each roughly 4mm the smaller than any other year. Males in 2007 were similarly sized to males in 2009 (45 and 46.5mm, respectively) but were of greater mass (2007: average = 6.39 g, SE = 0.6599, $N = 6$; 2009 average = 5.90 g, SE = 0.3206, $N = 11$; Fig. 4-15). Non-pregnant females in 2007 and 2008 were of similar body size distributions overall and females in 2009 were longer but

Table 4-9. MANCOVA using RegWQ analysis to test for morphological variation of adult male *P. hernandesi* at Zapata Ranch from 2007-2009.

Morphological Feature	Grouping Mean 2007 (*N* = 9)	Grouping Mean 2008 (*N* = 14)	Grouping Mean 2009 (*N* = 22)	Critical Range	Significance*
Tail Length	25.420	21.560	24.090	1.950	07 + 09 vs 08
Femur Length	8.950	7.830	9.260	0.450	07 + 09 vs 08
Head Length	11.200	11.130	10.130	0.370	07 + 09 vs 08
Head Shield Width	8.260	7.900	8.290	0.350	n.s.

* $P < 0.05$

Table 4-10. MANCOVA using RegWQ analysis to test for morphological variation of adult female *P. hernandesi* at Zapata Ranch from 2007-2009.

Morphological Feature	Grouping Mean 2007 (*N* = 6)	Grouping Mean 2008 (*N* = 9)	Grouping Mean 2009 (*N* = 11)	Critical Range	Significance*
Tail Length	24.72	20.61	22.93	1.480	07 vs 08 vs 09
Femur Length	11.44	8.90	10.32	0.450	07 vs 08 vs 09
Head Length	13.45	11.04	12.57	0.310	07 vs 08 vs 09
Head Shield Width	10.06	8.39	9.11	0.230	07 vs 08 vs 09

* $P < 0.05$

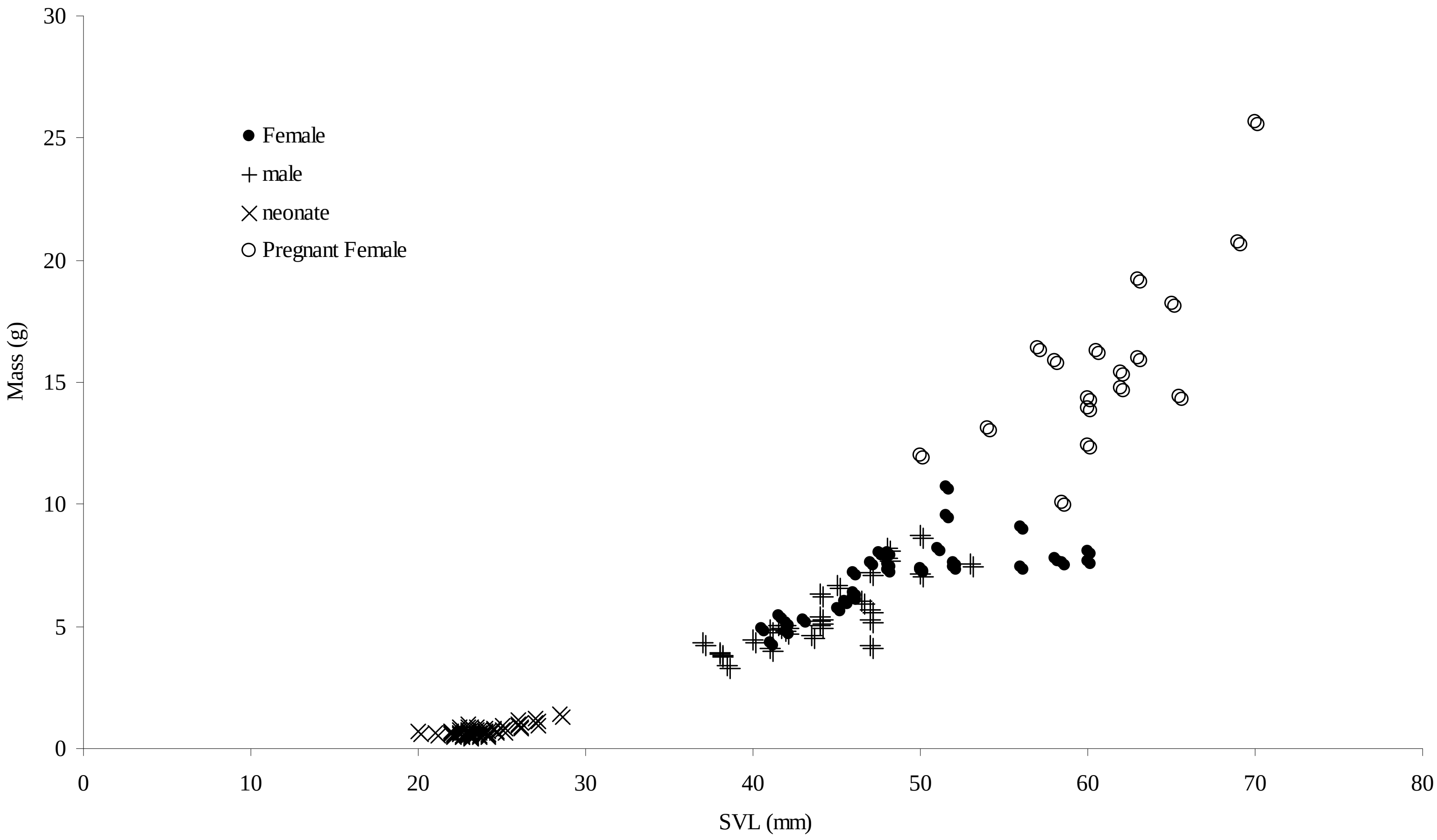

Fig. 4-14. *Phrynosoma hernandesi* size and mass distributions from 2007-2009 at Zapata Ranch.

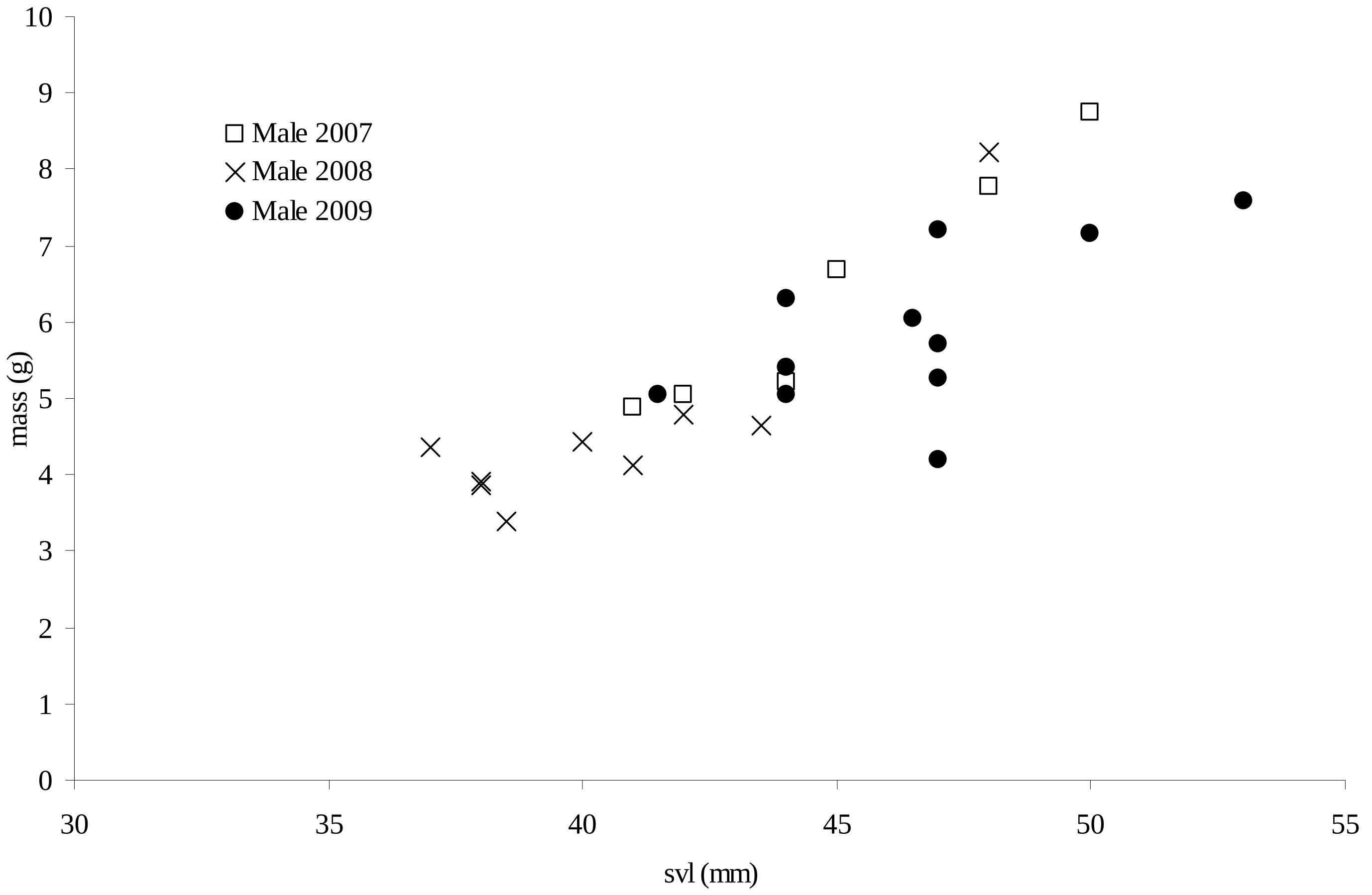

Fig. 4-15. Annual variation in male *P. hernandesi* size and mass at Zapata Ranch. Males were 4 mm smaller on average in 2008 than 2007 or 2009.

disproportionately smaller in mass (Fig. 4-16). Non-pregnant masses: $\bar{x}$ 2007 = 10.14 g, SE = 0.585, N = 2; $\bar{x}$ 2008 = 5.88, SE = 0.411, N = 11; $\bar{x}$ 2009 = 7.57 g, SE = 0.108, N = 16). Pregnant females in 2009 were smaller in size and mass than either 2007 or particularly 2008 pregnant females, which were highest in mass and body size than any year (Fig. 4-16). However, comparing pregnant females is conditional, since in 2007, the population was surveyed in mid-August, in 2008 lizards were surveyed in mid-July, and in 2009, lizards were surveyed in early August. Neonates were largest in 2007 in both mass and size compared to 2008 and 2009 (Fig. 4-17).

Other interesting findings occurred during this study. First, two individuals were observed squirting blood while being captured (Fig. 4-18). In 2007, an adult male from Saguache squirted blood after being chased and captured by a volunteer (9 August 2007). The second individual, an adult female, observed squirting blood was also while being pursued by a volunteer and occurred on 4 September 2009 at Zapata Ranch. Second, females are capable of developing at least 10 fetuses (personal observation of a sacrificed female). Based on examination of the fetuses and capture date (29 June 2007), it is likely that this female would have given birth within 2 weeks; fetuses were fully developed but weighed under 0.5 g each and neonates appeared as early as mid-July at this site. Additionally, one female birthed 7 neonates on 17 July 2008 while in captivity (Table 4-11). All neonates were between 0.31 and 0.35 mass/SVL. The female was 0.16 mass/SVL immediately following birth, and was at least 0.25 mass/SVL while pregnant. Thus, over one third her body mass was from the fetuses (9.07 g post-birth, 5.14 g combined fetus mass). Third, *P. hernandesi* at Zapata were found using cow patties both individually and in groups

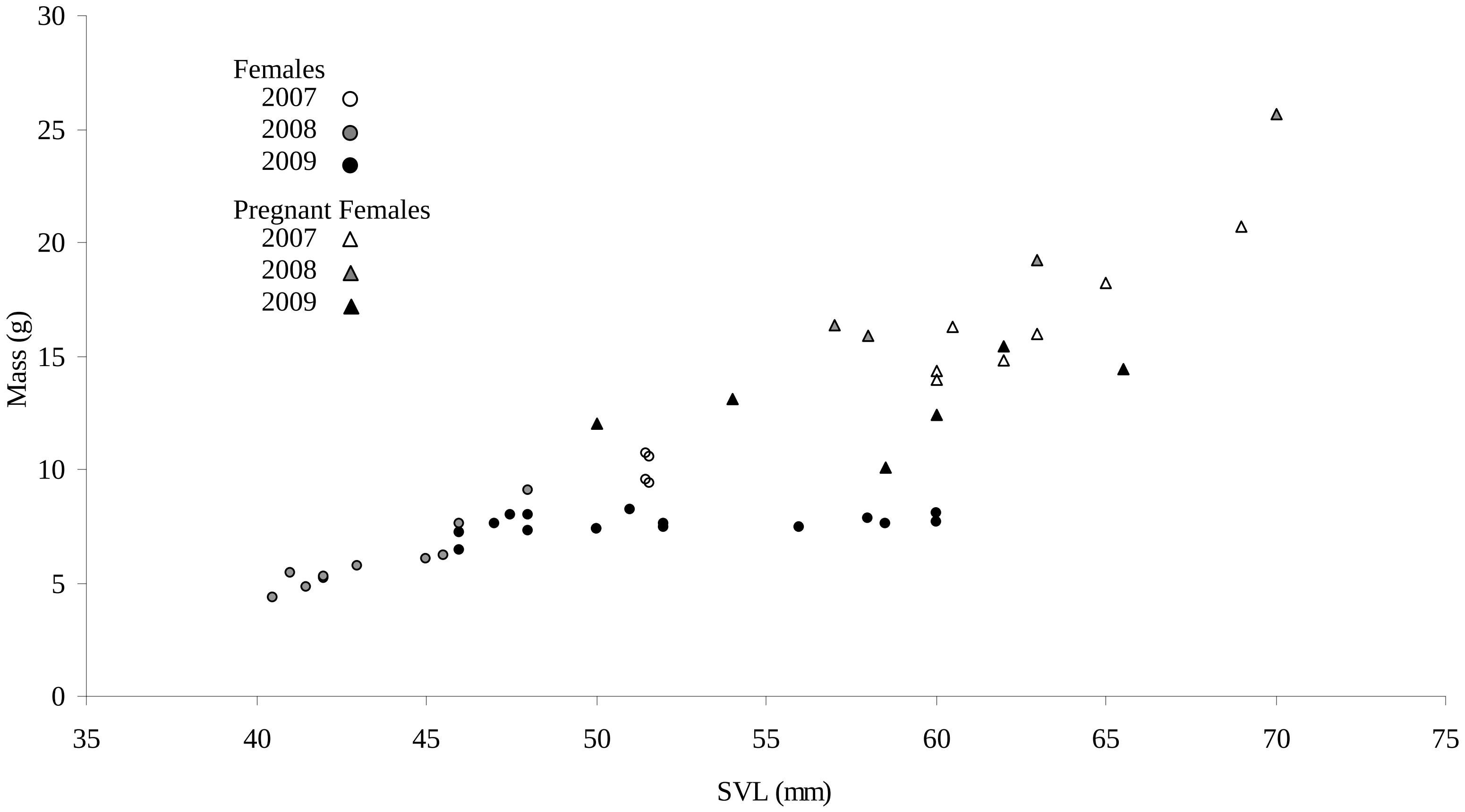

Fig. 4-16. Annual variation in female *P. hernandesi* size and mass at Zapata Ranch. Feales were 4 mm smaller on average in 2008 than 2007 or 2009.

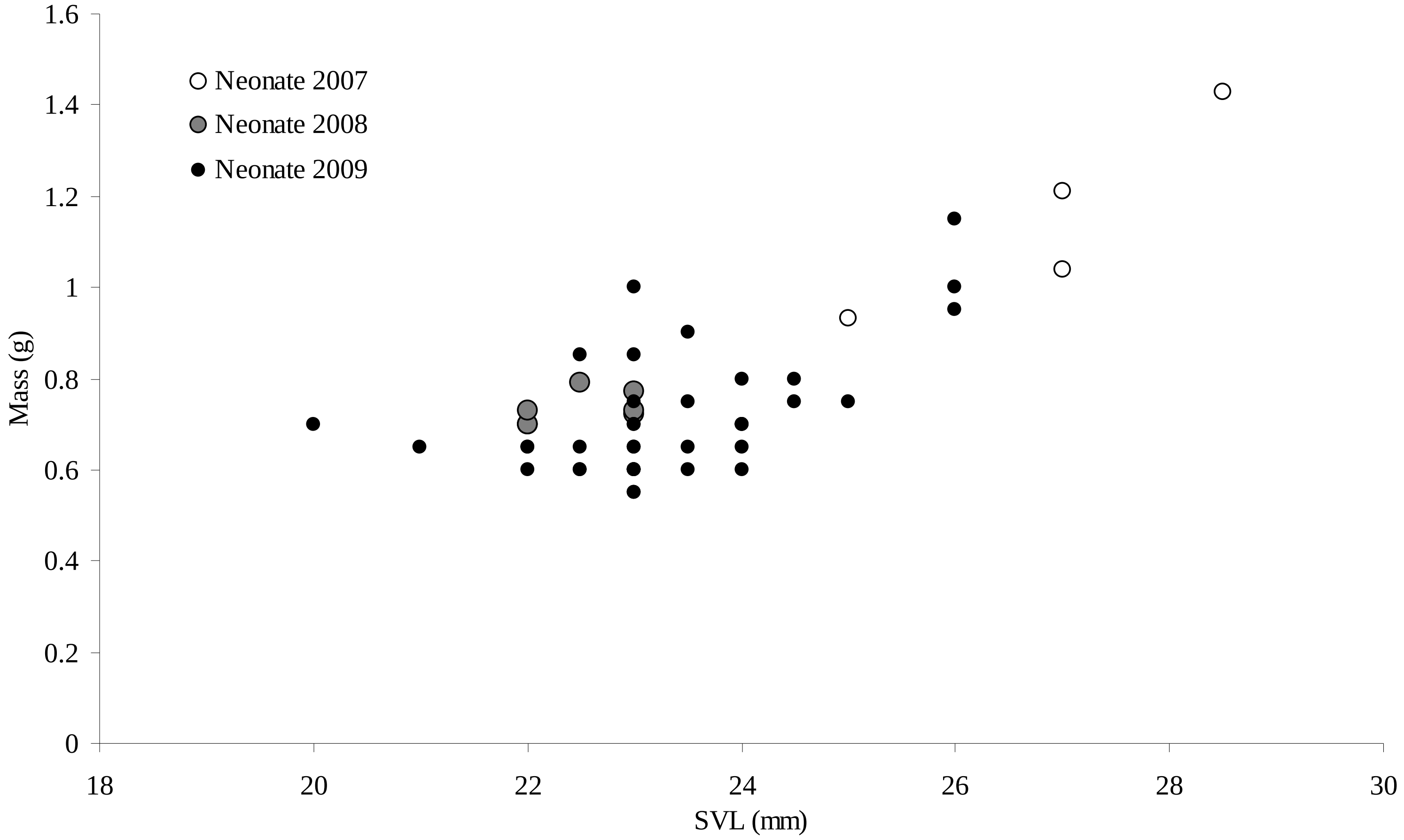

Fig. 4-17. Annual variation in neonate *P. hernandesi* size and mass at Zapata Ranch. Annual surveys were conducted 2-4 weeks later in the season, so annual variation in neonate size is not known; however, neonates show rapid size increase within ~4 weeks of birth (2007 compared to 2008 or 2009).

Fig. 4-18. Two *P. hernandesi* were observed immediately after squirting blood. a.) an adult male from Saguache in 2007 squirted blood after being pursued by a volunteer and b.) an adult female from Zapata in 2009 squirted blood after being picked up by a volunteer.

Table 4-11. Size and Weight of neonates birthed by female 2008-97 on 17 July 2008 while in captivity.

Neonate #	SVL (mm)	Tail Length (mm)	Mass (g)
1	2.20	0.95	0.70
2	2.30	1.00	0.77
3	2.30	0.90	0.72
4	2.30	0.90	0.73
5	2.20	0.80	0.73
6	2.20	0.80	0.70
7	2.25	0.85	0.79

(Fig. 4-19 and 4-20). In one instance, three neonates were encountered under a flipped patty and a single neonate was also observed under another patty. Another instance occurred when an adult female was captured within a couple feet of a patty that contained four neonates, presumably belonging to the female. A third instance occurred where three male subadults were observed under one patty. In these three

Fig. 4-19. An adult female *P. hernandesi* observed under a cow patty.

Fig. 4-20. An adult and neonate *P. hernandesi* observed under a cow patty.

incidents, all *P. hernandesi* were observed under cow patties in the morning prior to emerging from sleeping. Finally, one female was discovered partially submerged into the sand while sleeping under a cow patty, and was still nearly buried in sand at 7:17am (Figs. 4-21 and 4-22). The lizard was fully emerged after about 5 min.

DISCUSSION

Phrynosoma hernandesi were found at the same historic localities reported by Hahn (1968) and Hammerson (1981), though no formal surveys were conducted, and so it is not known whether densities have persisted across localities. The most abundant population occurred at Zapata Ranch in stabilized sand dune habitat, where multiple captures per hour is standard. Conversely, over 30 hours were spent searching for lizards at McIntyre springs and only two individuals were observed, each within 3m of each other on the same day: a neonate and an adult male. *Phrynosoma hernandesi* have been reported historically (Hahn, 1968; Hammerson, 1981) and recently (Tim Armstrong, pers. comm.) at this site. Horned lizards are considered sensitive to habitat alteration, such that their abundances correlate with local conditions such as climate, resource availability, and disturbances (Rissing, 1981; Munger, 1984; Suarez et al., 2000; McIntyre, 2003). A relatively large portion of the Valley remains undisturbed or undeveloped and there are at least 6 distinct habitats within the undisturbed areas (based on vegetation and soil associations). *Phrynosoma hernandesi* persists and appears to thrive most readily in the stabilized sand dune habitats, where conservation threat is highest because of this habitat's rich

Fig. 4-21. An adult female *P. hernandesi* shimmied under the sand while sleeping.

Fig. 4-22. An adult female *P. hernandesi* emerging from her sleep site.

diversity. The stabilized sand dune habitat is not typical to high-elevation alpine valleys and functions more as a hospitable desert, where the substrate heats and cools rapidly, insect diversity is high, precipitation is low, and vegetation is relatively dense. Desert habitat is what *Phrynosoma* are adapted to, which would seemingly correspond to their disproportionately higher abundance within sand dune habitat in the Valley (Sherbrooke, 2003). *Phrynosoma hernandesi* is known to occur at elevations nearly 3,200 m throughout its range (Sherbrooke, 2003), but only up to 2,500 m in northwest Colorado and 3,400 m in southwest Colorado (Hammerson, 1981) in forest habitat.

Phrynosoma hernandesi likely responds to within-year variation in climate. In 2008 when *P. hernandesi* densities were least abundant, snowfall was 121% average but 28% occurred in late spring (April) and precipitation was 100% average but mostly occurred in mid-summer. In contrast, *P. hernandesi* was most abundant in 2009 when rainfall and precipitation were at historical average but the timing of snowfall and precipitation was offset from previous years; only 6% of snowfall occurred in April but most precipitation occurred in late spring. Individuals begin emerging in late spring to early may when temperatures are no longer freezing. Few snowstorms allows *P. hernandesi* to emerge sooner, and abundant rainfall increases overall habitat productivity that directly correlates with *P. hernandesi* activity, including reproductive efforts. Annual variation in temperatures showed no discernable pattern.

Horned lizards are considered highly cryptic and are known to match the substrate coloration within their habitats (Norris and Lowe, 1964; Stebbins, 2003).

Bundy and Neese (1958) analyzed hue across a population of *P. modestum* (round-tail horned lizard) from habitat with locally diverse substrate coloration. The authors found that local variation in dorsal coloration and pattern is most prevalent among adults. Dorsal coloration and pattern also exists among *P. hernandesi* within the Valley. It is not known whether individuals adopt this variation before or after parturition, or whether the variation is regulated by genetics, environment, or a combination of genetic and environmental interactions. As observed in this study, Bundy and Neese (1958) reported a lack of coloration that correlated with the local substrate among hatchlings and juveniles.

Although horned lizards are considered ant specialists, variation in their diets is not uncommon; species within the short-horned clade (*P. hernandesi, P. douglasii, P. orbiculare, P. ditmarsi*) tend to consume fewer ants while species in the long-horned clade have diets more characteristic of true ant specialists, consuming almost exclusively all ants (Pianka and Parker, 1975; Powell and Russell, 1984; Lahti and Beck, 2007). The variation in diet among clades correlates with cranial morphology where lizards within the short-horn clade tend to have reduced features associated with feeding (Montanucci, 1989; Meyers et al., 2006). Reduced cranial morphologies are considered adaptive for these semi-generalist since they consume higher proportions of larger and harder insects, primarily Coleopterans.

Throughout its range, *P. hernandesi* shows variation in diet, consuming anywhere from 49-99% ants (Pianka and Parker, 1975). Although the variations in diets among populations within and outside the Valley are both within ranges expected for this species, the observed variations in diet should not be interpreted definitively; differences may be an artifact of low sample sizes for lizards outside the Valley or simply a difference in the prey items available. Regardless, it can be stated with certainty that like *P. douglasii*, *P. hernandesi*, is not truly a dietary ant specialist (Lahti and Beck, 2007). Myrmecophagy is possibly relaxed due to species within the short-horned clade radiating to cooler, higher elevation climates where insect diversity shifts (Zamudio, 1996; Meyers et al., 2006).

Ontogenetic variation in diet is known to occur in insectivorous lizards, including horned lizards, and has many potential causes (Pough, 1973; Lahti and Beck, 2007). Potential causes include differences in gape size and bite force capacity associated with lizard body size (Herrel and O'Reilly., 2006), and a preference for prey items in proportion to lizard size (Whitford and Bryant, 1979; Rissing, 1981; Suarez and Case, 2002). Other factors associated with ontogentic variation in lizard diets include temporal and spatial distribution of prey items (Pianka and Parker, 1975; Rissing, 1981) and optimal foraging strategies among age classes (Schoener, 1971).

Species within the short-horn clade show similar patterns in ontogenetic variation in diet; both *P. douglasii* and *P. hernandesi* neonates consume primarily ants and transition to eating more beetles as adults (Lahti and Beck, 2007). Other shared ontogenetic shifts among *P. douglasii* and *P. hernandesi* include juveniles consuming the most pebbles and neonates consuming the fewest, as well as adults consuming the largest pebbles. Within the genus *Phrynosoma*, there is a relatively consistent proportion of non-food items consumed across all age classes (Pianka and Parker, 1975; Lahti and Beck, 2007), and is likely attributed to incidental consumption or misidentification (Weese 1917, 1919), since horned lizards discern prey and potential prey items based on movement (Milne and Milne, 1950; Lahti and Beck, 2007; pers. obsv.); however, it is also possible that lizards consume pebbles to aid in digestion of a diet with high levels of chitin, as seen with gastroliths in dinosaurs and other vertebrates (e.g. Baker 1956; Darby and Ojakangas, 1980; Christiansen, 1996).

Data on diet of pregnant females is nearly lacking and the degree to which pregnant females consume prey during gestation remains largely unknown. Zamudio (1996) showed that pregnant females reduced their food intake throughout the entirety of embryonic development, although pregnant females in this study consumed 26-43% fewer prey items than non-pregnant females. In 2008, for instance, pregnant females consumed 26% fewer prey items, though consumed 600% more Coleopterans than non-pregnant female; however, the findings from this study must be carefully interpreted, as the diet data are calculated from head capsule tallies and overlook potentially critical variables associated with prey items such as nutritional content or soft-bodied prey items. The actual caloric gain from many small ants might not be dissimilar from the caloric gain from beetles. In support of Zamudio's (1996) findings, it is expected that females decrease their prey intake as the fetuses develop, largely because there is little room left in the body cavity; organs are compacted into the upper region of the body cavity and the stomach becomes compressed (Goodman, 2009).

Annual variation in diet does occur, though factors underlying the cause, such as insect availability and resource competition, were not accounted for in this study. *Phrynosoma hernandesi* at Zapata Ranch in 2008 showed high variability for most variables measured, including diet, morphology, and density. Scat in 2008 contained fewer prey items among all groups, suggesting a potential resource limitation. Also in 2008, males consumed 13% fewer ants and instead increased consumption of beetles and pebbles, while females showed the converse trend with ants and beetles.

Scat mass showed a positive correlation with both lizard body size and mass. Neonates expected to have the highest scat mass relative to both body mass and SVL, since the main objective to a neonate is to survive its first overwintering, a period that lasts approximately 6 months in the Valley, and larger individuals are though to have a higher lifetime reproductive success (Anderson, 1994). On the contrary, rapid growth of neonates may lead to a reduced survivorship, possibly due to a trade-off in immune system function (Olsson and Shine, 2002). Pregnant females, also unexpectedly, have the second highest scat mass ratio comparisons; Zamudio's (1996) findings support the expectation that scat mass ratios would be lower since overall consumption is decreased during pregnancy and consuming becomes difficult for females if their digestive tracts experience crowding from the fetuses.

Ants consumed by individuals within the Valley are not significantly different in size from ants consumed by populations outside the Valley. This lack of difference suggests that either ants are a.) similarly-sized inside and outside the Valley or b.) lizards prefer ants of a particular size, regardless of the lizard's size. The prior explanation is most likely, since intraspecific variation in body size of ants does show variation across latitudes though all populations included in this study occur along the same latitude (Cushman et al., 1993). Though, ant head sizes do show temporal variation at Zapata Ranch. In 2009, three of the four ant genera were significantly larger than in 2007 or 2008. Annual variation among ant species is not uncommon, and reflects fluctuation in resources that correspond to an increase in successful harvesting of food and defense against colony predators (Rissing, 1987). Variation in ant sizes could also correlate to the proportion of soldiers and workers, which is known to shift annually due to social and ecological factors such as density-based competition and plant productivity (Elmes, 1987a; Elmes, 1987b; Holldobler and Wilson, 1990).

Phrynosoma hernandesi body size shows a positive correlation with ant size consumed; neonates are smallest and eat the smaller ant species while adults are largest and eat the larger ant species. As a result of having a specialized diet consisting of ants, horned lizards have evolved a reduced mandible length and thickness, dentition, head height, and area of jaw adductor muscle insertion (Meyers et al., 2006). Horned lizards swallow prey whole, such that lizards must sufficiently immobilize and ingest the prey item mechanically, a process that is facilitated by mucus sacs and a greater bite force in larger lizards (Schwenk and Sherbrooke, 2003; Meyers et al., 2006; Sherbrooke and Schwenk, 2008). Lizards that capture prey and attempt to consume it beyond their capacity to mechanically ingest the item must either expel the item or risk death (Sherbrooke, 2002). Thus, the ontogenetic shift in ant consumption might correlate more greatly with mechanical limitations correlated with body size than prey item preference or intraspecific competition, an explanation that could be supported by adults shifting to a higher proportion of beetles as adults.

Phrynosoma hernandesi at Zapata Ranch show annual fluctuations in population demographics, a characteristic common to species with shorter life-spans and high reproductive output (Molles, 2004). In 2009, precipitation was above average, resulting in dense vegetation and higher numbers of insects throughout the landscape (Western Regional Climate Center; pers. obsv.) and the high rate of neonates suggests that reproductive output increased as a result.

No juveniles were observed at Zapata Ranch during any of the sweeps and there is a distinct break in the body size data between neonates and adults. Hammerson (1999) noted that *P. hernandesi* in Colorado do not become sexually mature until their second full year (Hammerson, 1999). Most likely, yearlings have already attained or nearly attained the sizes of mature adults though they are not reproductively active until their second full season.

Valley individuals are morphologically distinct from outside lizards (Chapter 2), but their morphologies also show annual fluctuations within a local population. Plasticity is the most likely explanation for the small-scale in morphological shifts, and often correlated with food and water resource availability. Marine iguanas (*Amblyrhynchus cristatus*) shrink up to 6.8 cm (20% of their body sizes) during El Niño events when algal food resources are limited (Wilkelski and Thom, 2000). Similarly, flat-tailed horned lizards (*Phrynosoma mcallii*) and the Sonoran horned lizard (*Phrynosoma goodei*) show a 4-fold decrease in time to sexual maturity during years with abundant rainfall; juveniles attain sizes of sexually mature adults in 1.5-2 years during below-average rainfall while juveniles during years with above-average rainfall reach adult size within 6 months (Young, 2010). Both *P. mcallii* and *P. goodei* shrink during low rainfall years, which correlates with limited prey availability (Young, 2010). As with diet, *P. hernandesi* in 2008 showed a drastic shift in morphology.

In 2009, a majority of precipitation occurred in April, when lizards are emerging from hibernation and beginning to mate. The early and abundant rainfall in April likely contributed to the high number of neonates, since the majority of rainfall in 2007 and 2008 occurred in mid-summer and late summer, respectively, after mating activities have occurred. It is unknown whether limited precipitation and possibly limited resources affected lizard morphology. Though, head morphology should not vary greatly in comparison to other body measurements, such as body and limb length, since the head is constructed primarily of bone, which does not shift rapidly or much in size (Montanucci, 1987). The annual variation in morphology is a curious finding and should be further investigated.

Phrynosoma hernandesi is viviparous and Valley females must provide space in their body cavities for developing fetuses. Thus, the cost of dwarfism creates two possible scenarios: 1.) smaller offspring and 2.) fewer offspring. Dwarfed females have adopted the latter scenario, where fewer offspring are produced. *Phrynosoma hernandesi* body size has been shown to correlate positively with reproductive output (Zamudio, 1996); in more northerly latitudes or higher elevations where *P. hernandesi* tend to be smaller, females produce fewer offspring (6-11 in Alberta, Canada; Powell and Russell, 1991) while in lower elevations and latitudes where lizards tend to be larger, females produce significantly more offspring (16-17 in Arizona and 14-18 in Colorado; Goldberg, 1971; Pianka and Parker, 1975;

Hammerson, 1999). In the southwest US and Mexico, females attain their maximum body sizes and are reported to have as many as 48 offspring (Howard, 1974).

Annual variation in neonate size at Zapata should be considered carefully; the 8.5 mm gap in body size and 0.73 mm gap in mass among neonates across annual sampling periods varying up to 4 weeks highlights the rapid growth in neonates during their first few months prior to hibernation. Tanner (1918) observed a similar growth pattern in *P. hernandesi* neonates from Sanpete County, Utah. Eight 15-hr-old neonates weighed an average of 0.74 g each, while 12 neonates from Beaver County approximately 4 weeks in age weighed an average of 2.1 g.

Although not all species of horned lizards are known to blood-squirt, there is variation in the willingness and degree of this behavior among species known to squirt blood (Sherbrooke and Middendorf, 2001). Lizards within the short-horn clade rarely display blood-squirting behavior, although they often arch their backs and tuck their heads in a posture that is precursory to blood-squirting (pers. obsv.; Zamudio, 1996; Sherbrooke and Middendorf, 2001; St. John, 2002). Reports of blood-squirting within *P. hernandesi* are reported throughout its range (Stebbins, 2003; St. John, 2003; Sherbrooke and Middendorf, 2001) and is further reported here among populations within the San Luis Valley.

The use of novel habitat by organisms is more commonly observed, especially in more recent years since the encroachment of humans. In addition to long-term disturbances such as loss of native vegetation species (i.e. Rittenhouse et al., 2008; Watson et al., 2010), organic and inorganic debris is a source of disturbance often unaccounted for. To my knowledge, the use of cow patties by horned lizards as refugia is not reported in the literature. Cow patties are structurally beneficial, as they preserve ground moisture and can provide refugia from temperature extremes and predators (pers. obsv.).

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CHAPTER 5

CONCLUSIONS

The goal of this study was to characterize dwarfed populations of *P. hernandesi* and *A. cognatus* within the San Luis Valley, Colorado using a combination of morphological, genetic, and natural/ life-history data. In Chapter 2, I confirmed the previously-reported dwarfism and elaborated on the extent and patterns of this dwarfism that included an increase in SSD among dwarfed populations of both species. Because body forms of males and females are different and selection acts on components of body size (as opposed to overall body size), forces driving selection and fitness are experienced differently for males than females (Stuart-Fox, 2009). This varying selection leads to different morphological states for males versus females (i.e. the small-male-advantage hypothesis; Zamudio, 1998) and contributes to underlying causes of SSD (Fairbairn, 1997). Typically, larger body sizes promote the evolution of SSD as a direct response to sexual selection (Zamudio, 1998). Valley populations of *P. hernandesi* and *A. cognatus* contradict this trend; dwarfism is associated with an increase in SSD. In short-horned lizards, large body size is not likely associated with a strong selective advantage (Zamudio, 1998) and is evidenced by males typically maturing a year earlier than females. Thus, potential absence of selective advantage for larger body size in Valley populations of *P. hernandesi* supports the observed dwarfism and could also explain the same patterns observed in Valley populations of *A cognatus*. However, the proximal causes of this dwarfism remain unknown.

There are three main influences on body size: physiological, environmental, and genetic (Schmidt-Nielsen, 1984). Often, body size reduction is a response to release from an ecological or environmental constraint (i.e. predators, habitat) or a change in resource availability (i.e. food, climate). Because none of the other 12 reptile and amphibian species in the Valley are dwarfed (Hahn, 1968) and they are all subject to the same environmental conditions, it is unlikely that environmental variables are the primary cause of this dwarfism. It is possible that genetic variability resulting from isolation within the Valley is involved since populations of both species are considered disjunct. And, both species have been dwarfed within the Valley for at least 0.7 MYA.

In Chapter 3, I addressed whether Valley lizards and toads have unique genetic histories, whether Valley populations are isolated, and investigated their colonization histories in theValley. The unique morphology of *P. hernandesi* and *A. cognatus* within the Valley corroborates their genetic structures; the divergence rates in Valley populations of *P. hernandesi* from surrounding populations suggests that lizards have historically occupied the Valley and are undergoing a distinct evolutionary trajectory. *Phrynosoma hernandesi* and *A. cognatus* are known from within the Valley up to approximately 0.8 MYA and are thought to be prehistorically dwarfed based on fossil records (Rogers et al., 1985; Rogers, 1987; Rogers et al., 2000). Based on knowledge of the historic climate and limited genetic data, it is not known whether populations have colonized the Valley once or during two events. Further genetic research is necessary to more accurately assess the historic occurrence of both species in the Valley.

Although subspecies of *P. hernandesi* are not currently recognized, the distinction among subspecies is evident, particularly along the eastern border of the

Valley from the Sangre de Cristo mountain range. Although the amount of genetic data analyzed in this study is not comprehensive, a pattern among groups of lizards is evident. The geographic pattern does not parallel subspecific boundary distinctions as previously suggested by Reeve (1952) and is instead most similar to patterns revealed in the first genetic analysis of *P. hernandesi* (Zamudio, 1997). Further genetic and morphological analysis should be conducted to determine whether there is concordance among genetic and morphological data and whether subspecific, or new specific, designations are recommended. Although the genetic findings for *A. cognatus* are minimal, evidence suggests there is minimal variation in its mitochondrial DNA across its range. As with *P. hernandesi*, more genetic data are needed, including other mtDNA as well as nuclear markers, to determine a more accurate assessment of *A. cognatus*.

It is not known whether the variation in morphology or genetics has altered the life/natural-history of *P. hernandesi*. A comprehensive assessment of differences in the life/natural-history of a species is critical to understanding the effects of morphological and/or genetic variation. Body size is known to have various effects on a species as a result of size-constrained functions, including physiological limitations (number and size of offspring, capillary action) and mechanical limitations (prey item size, movement rate) (Calder, 1984; Schmidt-Nielsen, 1984). For instance, island populations of tiger snakes obtain body sizes nearly twice as large as their mainland counterparts also show an increase in fecundity, producing nearly twice the amount of eggs as mainland populations (Schwaner and Sarre, 1988). Similarly, they consume new and larger prey items. Thus, body size alteration among isolated populations can affect life/natural-history of a species. Over time, if these differences are substantial enough, isolated populations are subject to speciation.

In Chapter 4, I determined whether the differences seen in the morphology and genetics of *P. hernandesi* are evident in its life/natural-histories. As predicted, body size reduction on Valley populations of *P. hernandesi* shows variability in the effects of their life/natural-histories. Valley populations are most prevalent in stabilized sand dune habitat, which is not characteristic habitat for this cold-climate species that occurs primarily in short-grass prairies and mountain hillsides and valleys with pinion-juniper, pine, and juniper vegetation (Sherbrooke, 2003; Stebbins, 2003). Diet of dwarfed *P. hernandesi* does not vary substantially from populations throughout its species range and horned lizards show highly localized variation in the diversity and amount of various prey items consumed. Prey availability across sites was not assessed, so it is not certain whether *P. hernandesi* discriminate in favor of certain prey items or whether they are more similar to generalists. Although many previous studies have shown that horned lizards consume primarily ants, observations on their foraging behaviors suggest that horned lizards indiscriminately uptake prey items, such as rolling shot-leads and pebbles, based on movement and do not reject the item until it has been assessed orally (Milne and Milne, 1950; Lahti and Beck, 2008). Interestingly, the effects of body size on dwarfed females has caused a reduction in the number of offspring produced per reproductive event, which has implications for population dynamics, such as recovery rate from disturbance; however, Valley populations of *P. hernandesi* appear to persist at localized densities normally observed for this species.

The findings from this study demonstrate the effects of isolation on the evolution of species both genetically and morphologically. The effects of body size shift and isolation within the Valley has also led to a shift in the life/natural-histories of *P. hernandesi*. Although Valley populations of lizards are not listed for conservation, additional evidence from morphological and molecular data suggest that Valley populations represent a unique taxa. Further genetic data will help resolve the ambiguities from this study and provide more insight to the amount of divergence and colonization histories of Valley populations of *P. hernandesi* and *A. cognatus* as well as accurately concluding their taxonomic and conservation status.

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APPENDIX

Table A-1. Measurement abbreviations and definitions for *A. cognatus* museum specimens.

Measurement	Code	Definition
Snout-vent length	SVL	tip of snout to anus
Head width	HW	jawline to jawline (end of jaw and widest angle)
Head length	HL	angle of jaw to front middle of jaw (where lip divets up)
Orbit diameter	OD	inside of socket at the bone horizontal from the snout
Eye-nostril distance	END	anterior angle of eye to posterior edge of snout
Inter-nasal distance	ID	anterior edge of snout to anterior edge of snout
Tympanum height	TYH	vertical height of tympanum
Tympanum width	TYW	horizontal width of tympanum
Femur length	FL	kneecap to kneecap (180° orientation perpendicular to the body) divided by two
Tibia length	TIL	top of knee to bottom of heel
Foot length	FTL	back of heel to end of longest toe (4th toe) at last scale (protrusion of nail)
Humerus length	HUL	top of shoulder to end of elbow
Radius length	RL	end of elbow to base of palmside (hand flexed 90° angle to the radius)
Hand length	HAL	base of palm to end of longest toe (4th toe) at last scale (protrusion of nail)
Parotoid length	PAL	along the longest length of the parotoid
Parotoid width	PAW	at the midline of the parotoid length (not the widest width)
Tubercule length	TUB	base of tubercule where it protrudes from the foot

Table A-2. Measurement abbreviations and definitions for *P. hernandesi* museum specimens.

Measurement	Code	Definition
Snout-vent length	SVL	tip of snout to midline of cloaca
Tail length	TL	midline of cloaca to tip of tail
Head width	HW	jawline to jawline (end of jaw to widest angle)
Head length	HL	tip of snout to posterior edge of the parietal
Head shield width	HSW	width of the shield at its widest part
Eye-nostril distance	END	anterior angle of eye to posterior edge of snout
Inter-nasal distance	ID	anterior edge of snout to anterior edge of snout
Tympanum height	TYH	vertical height of tympanum
Femur length	FL	kneecap to kneecap (180° orientation perpendicular to the body) divided by two
Tibia length	TIL	top of knee to bottom of heel
Foot length	FTL	back of heel to end of longest toe (4th toe) at last scale (protrusion of nail)
Longest hindfoot toe	LHT	base of longest toe (4th toe) to the end of the toe at last scale (protrusion of nail)
Humerus length	HUL	top of shoulder to end of elbow
Radius length	RL	end of elbow to base of palmside (hand flexed to 90° angle to the radius)
Hand length	HAL	base of palm to end of longest toe (4th toe) at last scale (protrusion of nail)
Occipital spine medial length	OSML	base of spine (protrusion of spine from scales) to tip of spine
Occipital spine medial width	OSMW	width of the spine at the base (protrusion of spine from scales) at the widest part
Occipital spine distal length	OSDL	base of spine (protrusion of spine from scales) to tip of spine
Occipital spine distal width	OSDW	width of the spine at the base (protrusion of spine from scales) at the widest part

Table A-3. Measurement abbreviations and definitions for *A. cognatus* live specimens.

Measurement	Code	Definition
Snout-vent length	SVL	tip of snout to anus
Femur ength	FL	kneecap to kneecap (180° orientation perpendicular to the body) divided by two
Orbit diameter	OD	inside of socket at the bone horizontal from the snout
Head width	HW	jawline to jawline (end of jaw and widest angle)
Parotoid length	PAL	along the longest length of the parotoid
Parotoid width	PAW	at the midline of the parotoid length (not the widest width)

Table A-4. Measurement abbreviations and definitions for *P. hernandesi* live specimens.

Measurement	Code	Definition
Snout-vent length	SVL	tip of snout to midline of cloaca
Tail length	TL	midline of cloaca to tip of tail
Femur length	FL	kneecap to kneecap (180° orientation perpendicular to the body) divided by two
Head length	HL	tip of snout to posterior edge of the parietal
Head shield width	HSW	width of the shield at its widest part

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Education

Ph.D., Biology, August 2006- December 2010

Utah State University, Department of Biology, Logan, UT

- The status of dwarfed populations of short-horned lizards (*Phrynosoma hernandesi*) and Great Plains toads (*Anaxyrus cognatus*) in the San Luis Valley, Colorado

M.S., Biology, September 2003- June 2005

Central Washington University, Department of Biology, Ellensburg, WA

- Ecology of the pygmy short-horned lizard (*Phrynosoma douglasii*) in Washington

B.A., Ecology, September 1999- May 2003

Whittier College, Whittier Scholars Program, Whittier, CA

- Nest site selection by the Dusky salamander (*Desmognathus fuscus*) over multiple spatial scales

B.A., Sociology, September 1999- May 2003

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Teaching Experience

Graduate Instructor, 2008

Biology Department, Utah State University

Courses: Animal Physiology Laboratory (BIOL5610)

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Biology Department, Central Washington University

Courses: General Biology for non-majors (BIOL101), General Biology for majors (BIOL110), Plants in the Modern World (BIOL200), Plant Ecology (BIOL111), General Ecology (BIOL360), Desert Ecology (BIOL377)

Research/Professional Experience

Biologist, 2009-Current

U.S. Geological Survey, San Diego, CA (Dr. Robert Fisher)

Field Ecology and Herpetology Guest Instructor, 2007, 2008, 2009
Southwest Conservation Corps, Salida, CO (Brinkley Messick)

Journal of Herpetology Reviewer, 2009
American Society of Ichthyologists and Herpetologists

Steering Committee Member, 2008-2009
Allies on Campus, Utah State University (Maure Smith)

Mentor and Seminar Training Facilitator, 2007-2009
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Officer and Member, 2006-2009
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Biologist/Restoration Ecologist, 2005-2006
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Herpetology Department, S.D. Natural History Museum, San Diego, CA (Dr. Brad Hollingsworth)

Washington Herp Atlas Reviewer, 2006
Washington Natural Heritage Program, Washington Department of Fish and Wildlife, U.S. Bureau of Land Management, U.S. Forest Service, WA (Dr. Lisa Hallock)

NatureMapping Mentor, Horned Lizard Radio Telemetry, 2004
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Biology Department, Whittier College, Whittier, CA (Dr. Stephen Goldberg)

Genetics Lab Volunteer, 2000
Biology Department, San Diego State University, San Diego, CA (Dr. Tod Reeder)

Research Grants

Diversity Scholarship, GLBTA Services (USU), 2008-2009
Horned Lizard Conservation Society, 2008
Women and Gender Research Institute (USU), 2008
Travel Award, Graduate Studies Department (USU), 2008
USU Graduate Studies Summer Research Award (USU), 2008
Research Grant, Department of Biology (USU), 2007, 2008
Travel Grant, Department of Biology (USU), 2007, 2008
James and Patty MacMahon Grant, Ecology Center (USU), 2006
Summer Research Grant, Department of Graduate Studies (CWU), 2005
Student Research Award, Biology Department (CWU), 2003, 2004
Travel Grant, Biology Department (CWU), 2003, 2004
Thesis Research Grant, Biology Department (CWU), 2003, 2004

Presentations

Joint Meeting of Ichthyologists and Herpetologists, 2009
Morphological variation in dwarfed populations of short-horned lizards (*Phrynosoma hernandesi*) and Great Plains Toads (*Anaxyrus cognatus*) in the San Luis Valley, Colorado.

Joint Meeting of Ichthyologists and Herpetologists, 2005
Seasonal Variation in Habitat Use and Movement by the Pygmy Short horned lizard (*Phrynosoma douglasii*) over Multiple Seasons.

Society for Northwestern Vertebrate Biology Conference, 2005
Seasonal Variation in Habitat Use and Movement by the Pygmy Short horned lizard (*Phrynosoma douglasii*) over Multiple Seasons.

Pacific Ecology and Evolution Conference, 2005
Seasonal Variation in Habitat Use and Movement by the Pygmy Short horned lizard (*Phrynosoma douglasii*) over Multiple Seasons.

Pacific Ecology and Evolution Conference, 2005
Diet of the Northern Pacific Rattlesnake (*Crotalus viridis oreganus*) in the Yakima River Canyon of central Washington state. (Co-Author with Dr. Robert E. Weaver)

Biology of the Rattlesnakes Symposium, 2005
Diet of the Northern Pacific Rattlesnake (*Crotalus viridis oreganus*) in the Yakima River Canyon of central Washington State. (Co-Author with Dr. Robert E. Weaver)

Pacific Ecology Conference, 2004
Seasonal Variation in Habitat Use and Movement by the Pygmy Short horned lizard (*Phrynosoma douglasii*) over Multiple Seasons.

Whittier Scholars Program Honors Thesis Seminars, 2003
Nest Site Selection by the Northern Dusky Salamander (*Desmognathus fuscus*) over Multiple Spatial Scales.

National Conference for Undergraduate Research, 2002
Nest Site Selection by the Northern Dusky Salamander (*Desmognathus fuscus*) over Multiple Spatial Scales.

Southern California Conference for Undergraduate Research, 2002
Nest Site Selection by the Northern Dusky Salamander (*Desmognathus fuscus*) over Multiple Spatial Scales.

Ecological Society of America, 2003
El Niño Southern Oscillation (ENSO) Event Effects on Southern California Riparian Plant Communities.

Southern California Conference for Undergraduate Research, 2001
The Effects of Prescribed Burns on Coastal Sage Scrub Communities.

Publications

Lahti, M.E. and D.D. Beck. Diel movement, activity patterns, and sleep-site selection by the pygmy short-horned lizard (*Phrynosoma douglasii*). *In preparation*.

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