

ECOMORPHOLOGY OF THE MEXICAN FENCE LIZARDS OF THE
Sceloporus formosus GROUP (SQUAMATA: PHRYNOSOMATIDAE)

by

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ABSTRACT

ECOMORPHOLOGY OF THE MEXICAN FENCE LIZARDS OF THE *Sceloporus formosus* GROUP (SQUAMATA: PHRYNOSOMATIDAE)

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A central belief of ecomorphological studies is the link between morphology and performance, but, surprisingly few analyses demonstrate this relationship. Fence lizards of the genus *Sceloporus* offer a useful system for addressing correlations between morphology and performance because they possess keratinized and keeled scales, a distinctive morphological character that might influence water balance. A common observation across different taxa of reptiles is that many organisms in dry habitats have few scales on their bodies, compared to organisms inhabiting moist environments. This pattern is also present in fence lizards. I investigated if the number of dorsal and lateral scales varies across three environmental variables (elevation, temperature and precipitation) for three species of *Sceloporus* (*S. adleri*, *S. scitulus* and *S. druckercolini*). Among and within species, I found that lizards with the fewest and most heavily keeled scales occur at low elevations and dry environments and lizards with the most and

smoothest scales occur at high elevations and moist environments. A compelling hypothesis to explain this pattern is that fewer and larger scales are generally keeled and overlapping reducing skin exposure and hence the amount of evaporative water loss. On the other hand, smaller, more granular scales increase the exposed skin surface area and increase rates of dehydration. To test this hypothesis, the skin resistance to water loss (R_s) was measured on the sides of lizards and the number of lateral scales was counted for this specific area. The relationship between water loss and scalation was as expected, observing the lowest values of R_s with the highest number of lateral scales for all species together and within species. In addition, I tested if water loss was correlated to any of the three climate variables. I found the lowest values of R_s in lizards occurring at highlands, where temperatures tend to be low and water is usually more readily available. As a consequence of the variation in the lizard's morphology (scalation) with their habitat, I proposed a third hypothesis relating the morphology with the performance of the lizards. I predicted that a higher number of scales will be positively correlated with sprint speed. Out of the three species, only *S. scitulus* increased its performance with a higher scalation. Our results suggest that reptilian changes in scale numbers are correlated with ecological variables such as elevation, temperature and precipitation, and that the number of scales in a population and/or species may be highly plastic in order to maintain a proper osmotic balance. In turn, this important ecomorphological relationship provides also an opportunity to explore trade-offs between the already compromised morphological variation and running performance, as suggested for *S. scitulus*.

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CHAPTER 1

INTRODUCTION

1.1 Ecomorphology

The concept of ecomorphology is based on the premise that the association between morphology and ecology represents the expression of the phenotype – environment interaction (Ricklefs and Travis, 1980). Indeed, environment can shape the morphology of individuals through several processes related to physical factors (like climate) and habitat structure, which relates, for example, to locomotory structures. Likewise, morphology represents aspects of the relationship between the organism and its environment. Ecologists have employed a variety of data and analytical techniques in studying these relationships, and assessing the effects of adaptive plasticity on ecomorphological relationships is one of those techniques (Sinervo and Huey, 1990).

The correspondence between ecology and morphology brings some important advantages. First, morphological characters like scalation are clearly defined as homologous structures that can be measured; these characters are specific to the organism, and are expected to reflect biomechanical principles. Second, morphological characters have high repeatability of measurement, they are measured independently of the environmental background, and they facilitate broad comparison. Conversely, the link between ecology and environment also has some limitations. Morphology provides little information about the breadth of niche utilization by a single individual or even a

population, or about overlap of niche exploitation between individuals or populations. In addition, morphology is usually less responsive than physiology and behavior to short term temporal change in the environment during the life span of a species. Also, even though morphology is measured independently of the habitat background, it is highly taxon-dependent (Ricklefs and Miles, 1994).

Ecomorphological analyses generally seek to link the structure and function of organisms with relevant features of the environment (Losos and Miles, 2004) through three different goals 1) make ecological inferences from morphological pattern; 2) measurement of the ecology-morphology correlation to validate such ecological inferences; and 3) elucidation of the functional relationship between morphology and ecology as it is mediated by the behavior and performance (ethotype) of the organism (Ricklefs and Miles, 1994). In this study, I will be addressing the first two goals.

1.2 The *Sceloporus formosus* group

Phrynosomatids are a remarkably diverse group of lizards that have frequently served as subjects of research in ecology and life history theory. Phrynosomatids, previously identified as Sceloporines, are represented by ten genera, and more than 120 species. *Sceloporus* is the largest genus of reptiles endemic to North America with approximately 80 species currently recognized (Sites et al., 1992). *Sceloporus* occur from southern Canada to Panama, but are most diverse in the southwestern USA and Mexico. In most of the areas where they occur, *Sceloporus* are among the most abundant, conspicuous and diverse vertebrates present. Because of this, *Sceloporus* have

been the subject of intense and diverse biological research, including studies of behavioral ecology, hybrid zone dynamics, host-parasite interactions, life history evolution, cytogenetics, reproductive biology, biogeography, physiological ecology, and allometric engineering (Sites et al., 1992).

Within the species groups of the genus *Sceloporus* recognized by several authors (Smith 1939; Larsen and Tanner, 1974, 1975; Sites et al., 1992; Wiens and Reeder, 1997; among others), the *formosus* group (sensu lato) is outstanding by including morphologically conservative species, procryptic species (avoid depredation by stealth and mimicry), chromospecies (colorful species) and homochromatic species (similar coloration). In the most recent systematic revision of the genus, Wiens and Reeder (1997) recognized the monophyly of the *S. formosus* group.

Members of the *S. formosus* group inhabit the mountains of southern Mexico and Central America (Fig. 1.1). The *S. formosus* group, as defined by Hall (1973, 1977), included twelve species, *S. acanthinus* Bocourt (1873), *S. adleri* Smith and Savitzky (1974), *S. cryptus* Smith and Lynch (1967), *S. formosus* Wiegmann (1834), *S. lundelli* Bocourt (1873), *S. malachiticus* Cope (1864), *S. salvinii* Günter (1890), *S. smaragdinus* Bocourt (1873), *S. stejnegeri* Smith (1942), *S. subpictus* Lynch and Smith (1965), *S. taeniocnemis* Cope (1885), and *S. tanneri* Smith and Larsen (1975). Today, 16 species are considered within the *S. formosus* group, to the ones mentioned previously, it has been added *S. hartwegi* (Stuart, 1971), *S. internasalis* (Smith and Bumzahem, 1955), *S. scitulus* (Smith, 1942), and the most recently described *S. druckercolini* (Perez-Ramos & de la Riva 2008).

Sceloporus adleri is a small species that inhabits the mountain slopes of the Sierra Madre del Sur of Guerrero west of Chilpancingo (Mexico), at elevations between 2,415 and 2,935 m and probably higher. *Sceloporus scitulus* (Smith, 1942), inhabits the same mountain range and the Sierra Madre del Sur of Guerrero west of Chilpancingo at elevations between 1700-2160 m, and the Sierra Madre del Sur of Guerrero east of Chilpancingo (Sierra de Malinaltepec) between about 1800 and 2400 m. Both species differ from each other in many aspects of coloration, size, and in certain scale counts. Geographical distributions of these two species likely overlap for at least portions of their range.

Sceloporus druckercolini (Perez-Ramos and de La Riva, 2008), also known as mountain lizard, is the most recent species added to the *S. formosus* group. The differentiation of *S. druckercolini* is based on morphological characters, coloration, ecological and geographical distribution that, for the moment, allow the recognition of this species of *Sceloporus* in the region of the Sierra Madre del Sur in the state of Guerrero (Mexico). *S. druckercolini* ranges from the hillsides of the Sierra de Atoyac to the summits of the Sierras Campo Morado and Igualatlaco (from 887 to 2600 m of elevation). The environment of the type locality, Filo de Caballo, is a mountainous area mainly in the hillsides and summits, of dense temperate and semi warm forests covered with conifers, oaks, pines-oaks, and pines, and of cloud forest patches.

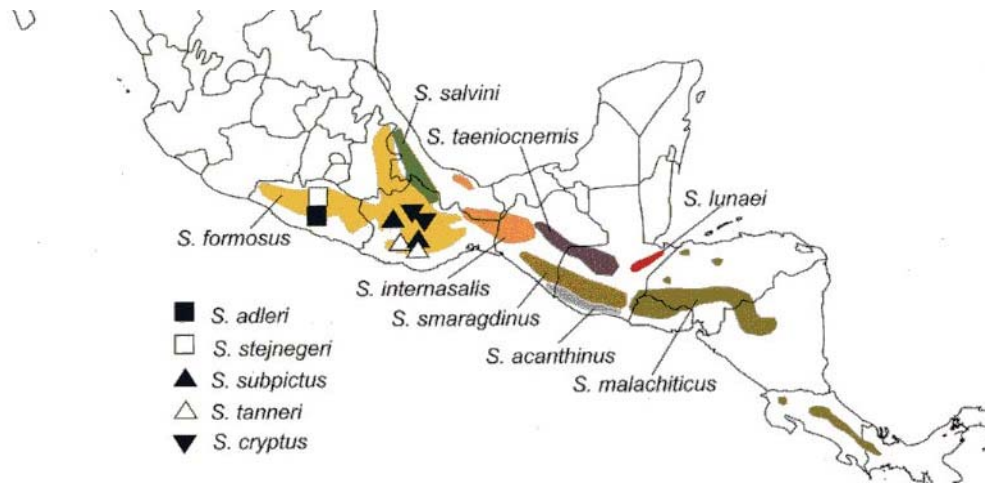


Figure 1.1 Geographical distribution of some of the species of the *Sceloporus formosus* group (adapted from Köhler and Heimes, 2002).

1.2.1 Study Site

During June 2007, a total of 18 localities were sampled and 122 individuals were collected among three species of the *Sceloporus formosus* group (*S. druckercolini*, *S. adleri* and *S. scitulus*) in the state of Guerrero, Mexico (Fig. 1.2).

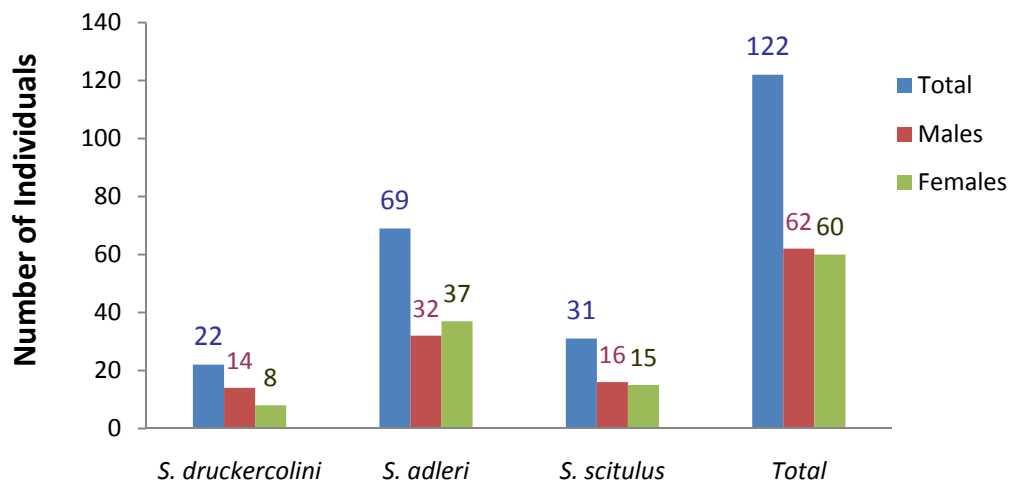


Figure 1.2 Sample sizes for *S. druckercolini*, *S. adleri* and *S. scitulus*.

All collecting sites were located in the Sierra Madre del Sur west of Chilpancingo, at elevations from 1,004 to 3,232 m (Fig. 1.3). Different elevations recorded in the same site were grouped as one locality when the altitudinal difference was less than 45 m (Table 1.1). Elevation, GPS coordinates and body temperature were recorded every time a lizard was captured.

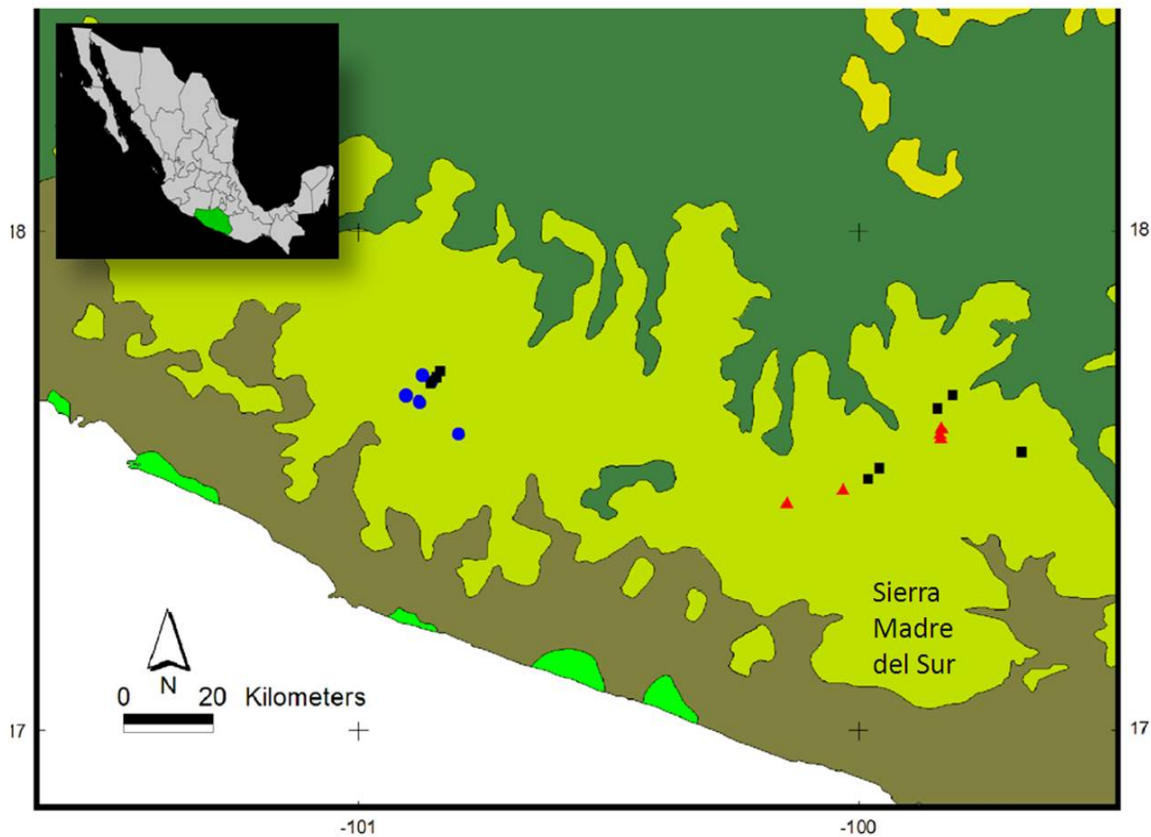


Figure 1.3 Map of the state of Guerrero, Mexico. Collecting sites are showing for each species: *Sceloporus scitulus* (black squares), *S. adleri* (red triangles) and *S. druckercolini* (blue circles). Shades of green represents terrestrial ecoregions (following Olson et al., 2001), collecting sites are found in the Sierra Madre del Sur ecoregion, characterized by a pine-oak forest. Map was generated using ArcView.

Table 1.1. Number of specimens of three species of the *Sceloporus formosus* group collected per locality.

Locality	Site	Elevation (m)	<i>S. druckercolini</i>	<i>S. adleri</i>	<i>S. scitulus</i>
1	Asoleadero	2669		35	
2	Bajos de Balsamar	1004 1010	4 4		
3	El Jilguero- Puerto El Gallo	3192 3230 3232		3 3 2	
4	Escalerilla - La Laguna	1840			1
5	Escalerilla - La Laguna	2085			4
6	Escalerilla - La Laguna	2615		19	
7	Filo de Caballo	2286 2321			1 1
8	Filo de Caballo	2497		3	
9	Filo de Caballo	2667		4	
10	La Laguna - Asoleadero	2215			2
11	La Ola	1867 1910	6 4		
12	La Ola – San Antonio de Texas	2200 2207			3 1
13	La Ola - San Antonio Texas	2418			1
14	La Ola - San Antonio Texas	2685			1
15	La Ola- El aguacate	1920	1		
16	Omitemi	2145 2188			6 3
17	San Antonio Texas	2127			2
18	San Antonio Texas	2229	3		5

1.3 Phenotypic Plasticity

Many of the species in the *S. formosus* group are weakly defined and diagnosed by combinations of morphological characteristics that are not constants within species and often tend to occur with some frequency in other species (e.g. number of canthal and dorsal scales, and coloration patterns). *Sceloporus* exhibit enormous phenotypic plasticity which makes it difficult to rely on morphological characters to describe any new species. The usual diagnostic characters tend to be extremely homoplastic causing great confusion in identification. Smith (2001), proposed a phylogenetic hypothesis for speciation within the *S. formosus* group including a combined matrix of molecular and morphological data. When looking at this phylogeny (Fig. 1.4), a high degree of homoplasy in the morphological data becomes evident: clade A contains relatively terrestrial species that are found in comparatively dry areas at moderate to high elevations; clade B contains *E. clarki*, and *S. lundelli*, highly arboreal species from very dry areas; clade C possesses species from moderate to high elevations occurring in more mesic situations; clade D includes species from very humid areas at usually moderate to high elevations, most of them very arboreal and from humid areas, but from mostly low and moderate elevations. These results suggest that the morphology of these lizards is extremely plastic to environmental conditions and several clear patterns appear to be evident. For example, according to Smith (2001), scalation and coloration, tend to be associated with adaptation to environmental pressures, and much of the group variation can also be found within single populations of one species. *S. adleri* and *S. scitulus* have been confused with *S. stejnegeri* at relative low elevations because of the high degree of

scale fusion and low number of scales that they acquire in relatively hotter and drier environments. On the other hand, *S. tanneri* has been mistaken for *S. formosus* at high elevations, where they are darker, smaller and have higher scale counts (Smith, 2001).

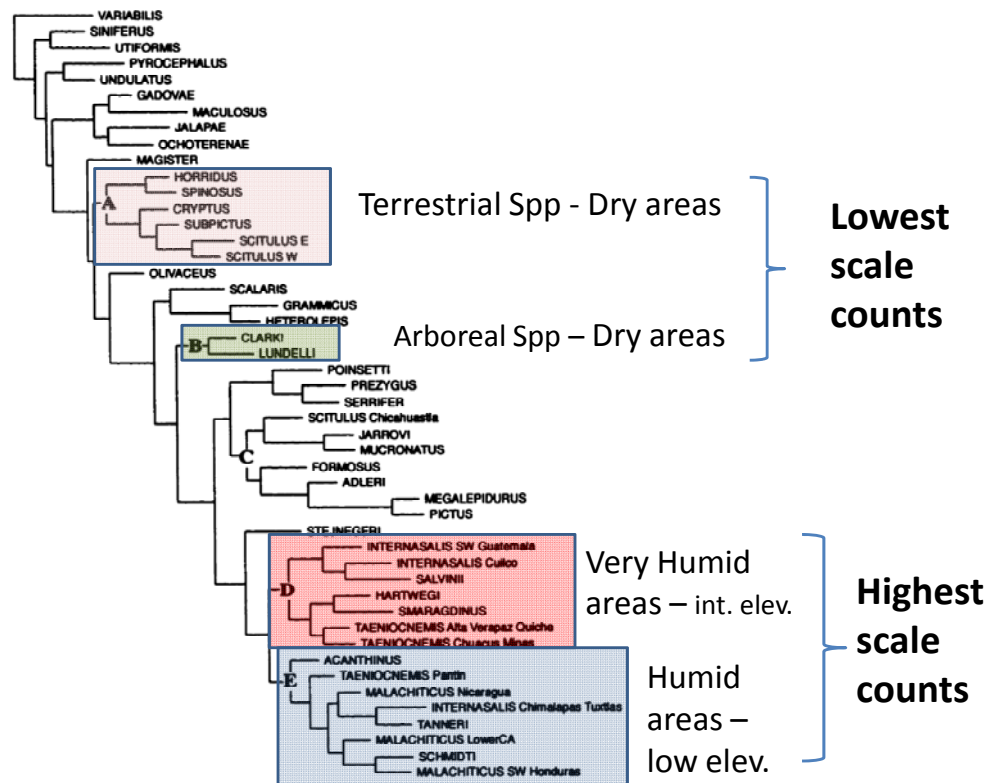


Figure 1.4 Shortest parsimony tree with morphological data 48 OTUs, based on 52 morphological characters: scalation, color pattern and morphometrics (Smith, 2001).

1.4 Objectives

A fair number of studies suggest that the number and size of scales influence water balance (Horton, 1972; Soule' and Kerfoot, 1972; Thorpe and Baez, 1987, 1993; Malhotra and Thorpe, 1997). A possible explanation is that for species occurring in high humidity areas, water loss through inter-scale areas is less critical than for those

occupying arid environments. Having more but smaller scales may increase rates of dehydration by exposing more of the skin surface areas between scales. Conversely, for species inhabiting hot/dry environments, having fewer but larger scales may better retard water loss by reducing the amount of interscalar skin exposed (Dmi'el, 2001; Neilson, 2001). Decreasing the number of scales could reduce flexibility and negatively affect locomotory performance, on the contrary, possessing high numbers of smaller scales may enhance performance by increasing skin flexibility and movement.

In this study, I investigate three taxa belonging to the *Sceloporus formosus* group (*S. adleri*, *S. scitulus* and *S. druckercolini*) to see if water loss and sprinting performance are part of a trade-off system influencing scale number. The purposes of this investigation are the following: first, to determine if there is significant variation in scale counts in relation to environmental conditions; second, to examine how water loss varies in relation to scalation; third, to evaluate the assumption that possessing more and smaller scales could enhance performance; and fourth, to see if a tradeoff between locomotion and water loss exists and controls the number of scales.

CHAPTER 2

PHYLOGENETIC RELATIONSHIPS WITHIN THE *Sceloporus formosus* GROUP

2.1 Introduction

Despite being a model system organism, the phylogenetic relationships of *Sceloporus* have remained poorly known. Although there have been several important systematic studies (Hall 1973, Smith 1939), the only explicitly cladistic analysis addressed the relationships of only 19 species (Mindell et al., 1989). The most recent definition of the group, based mostly in chromosomal evidence, was given by Flores-Villela et al. (2000), but there have been no comprehensive analyses of the genus using modern systematic techniques. Smith (2001), more recently, proposed a phylogenetic hypothesis for speciation within the *S. formosus* group based on mitochondrial DNA sequence, life history characters and morphological characters. The study included a total of 700 specimens collected in 100 localities from Mexico and Central America.

In this paper, the phylogenetic placements of the newly described species, *Sceloporus druckercolini*, and a possible new species (*Sp. nov* 1), collected in Guerrero, Mexico, are inferred using mtDNA (ND4 protein-coding gene, and associated tRNA genes; 895 bp total).

2.2 Methods

DNA was extracted from muscle tissue from one specimen of *Sp. nov* 1 collected in the road, and six more DNA's samples were obtained for six specimens of *S.*

druckercolini, corresponding to three different populations. All the new sequences included in this analysis came from specimens collected during June 2007, except for two *S. druckercolini* sequences (Fig. 2.1 ●), collected in El Balcon. Tissues from these two specimens were provided by J.C. Blancas.

DNA extractions were done with a DNeasy Extraction kit. To compare with Smith's (2001) work, the mitochondrial gene ND4 (895 bp) was amplified. The primers were described by Arevalo et al. (1994) for *Sceloporus grammicus*. Adjacent tRNA's corresponded to 186 bases of the sequence were also included. PCR products were sequenced and resulting DNA sequences were edited using MEGA 4 (software package). The new edited sequences were added to the previous data set, Smith (2001), and the same multiple outgroups were used to assure the recovery of the ingroup. A total of 126 OTUs (119 OTUs + 7 OTUs) were aligned using Clustal W. To reconstruct the phylogeny, three criteria were applied: UPGMA, NJ and maximum parsimony (MP). All analysis were run in both MEGA 4 and PAUP* 4.0. Branch support was evaluated with non-parametric bootstrap using heuristic searches due to the large amount of data.

2.3 Results

All the trees obtained from each of the analyses (UPGMA, NJ and MP) showed the same topology arrangement and the seven added OTUs were observed in three different clades. To simplify the visualization of a consensus tree from a maximum parsimony analysis containing 126 OTU, a subtree with 52 OUT was delimited to show the phylogenetic position of *S. druckercolini* (Fig. 2.1). *Sceloporus nov 1*(▼) claded with *S. adleri*, and *Sceloporus druckercolini* was spread in two distant clades, one that came

from Bajos de Balsamar and La Ola (▲ and ■, respectively) and a second one (●) from El Balcon. By observing the bootstrap values, the monophyly of the group was recovered being consistent with Smith's (2001) results. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option). There was a total of 675 positions in the final dataset, out of which 318 were parsimony-informative.

2.4 Discussion

The analyzed mtDNA data provided strong support for the monophyly of the *Sceloporus formosus* group. Reeder's (1995) analyses of mitochondrial ribosomal DNA sequences also supported the monophyly of the *S. formosus* group, as subsequently did Reeder and Wiens (1996) and Wiens and Reeder's (1997) phylogenetic analyses of morphological and molecular data, which included many species of *Sceloporus* from various groups. *Sceloporus druckercolini* shows a great divergence forming two different clades (Fig. 2.1). Specimens from the first clade (●) were collected at 2500 m, while specimens in the second clade (▲ and ■) ranged between 1000 to 1900 m in altitude. There is no overlapping across elevations; however, a similar coloration pattern is present in both clades.

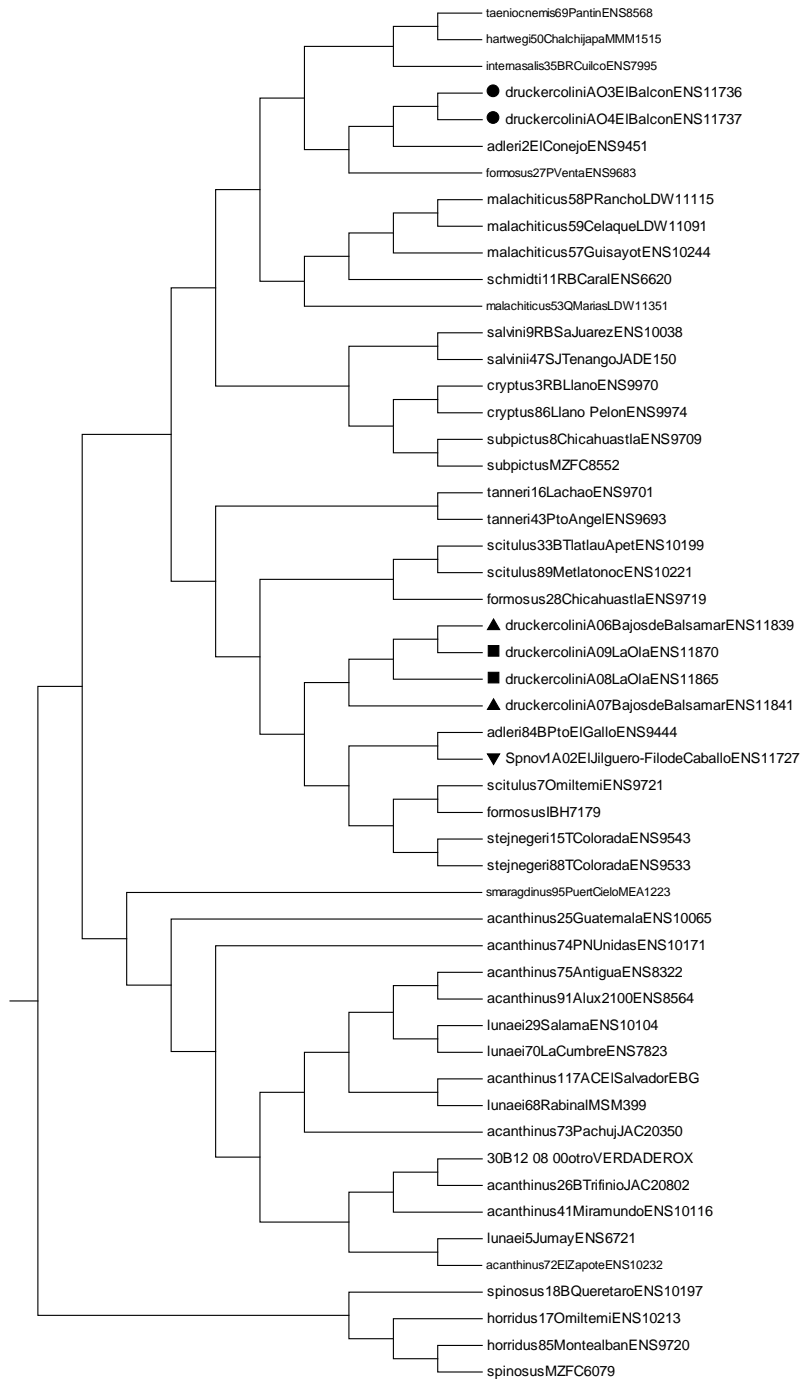


Figure 2.1 Relationships within the *S. formosus* group based on 895 bp of ND4 sequence data of 36 most parsimonious trees, derived from a subset of 126 OTU using Maximum Parsimony optimality criterion. Symbols represent localities from six new sequences of *S. druckercolini* (El Balcon (●), Bajos de Balsamar (▲), La Ola (■)) and one of a possible new species *Sp. nov 1* (El Jilguero- Filo de Caballo (▼)).

Due to the high phenotypic plasticity observed in many species of the genus *Sceloporus*, a more detailed morphological and molecular comparisons (including nuclear markers) needs to be done in order to conclude that the two clades formed by *S. druckercolini* correspond to two different species. *Sceloporus nov 1* (▼), appeared in the same clade with *S. adleri*, which might suggest that these two specimens found in different populations are sharing the same haplotype, being most probably the same specie with different coloration patterns.

CHAPTER 3

VARIATION IN SCALATION AGAINST CLIMATIC VARIABLES

3.1 Introduction

The keratinized scales of reptiles have served as a protective shield against injuries, and were a key innovation in the radiation of amniotes that invaded land (Alibardi, 2003). Across different taxa of squamates a common observation regarding scalation is that many species living in arid habitats often have large, thick and overlapping scales, while those inhabiting more humid conditions have smaller and smoother scales with more exposed inter-scale skin area, though there are exceptions to this general pattern (e.g. the small granular scales of desert geckos). These patterns seem also to be constant within (Soule and Kerfoot, 1972; Calsbeek et al., 2006) and between (Hellmich, 1951; Calsbeek et al., 2006) species.

The correlation between climate and scalation has been frequently documented over the past 60 years. Hellmich (1951) carried out one of the earliest studies that addressed this relationship. He demonstrated that South American lizards in the genus *Liolaemus* have greater numbers of smaller scales at lower temperatures and high precipitation localities. Consistent changes in scalation have now been reported to be correlated with changes in a multitude of ecological variables [e.g. latitude and elevation (Thorpe and Baez, 1987), temperature (Soule' and Kerfoot, 1972), degree of habitat openness (Lister, 1976a) and rainfall (Malhotra and Thorpe, 1997a)]. In all of these

studies, scale numbers changed with microclimate but not always in the predicted direction – increasing scale numbers with increasing precipitation and elevation and decreasing temperature. Klauber (1941) is the first to study this ecomorphological association; however, the relationship he found contradicted the common trend mentioned above. He studied various snake taxa and found scale counts decreasing with precipitation and increasing with temperature.

So, do elevation, temperature and precipitation variables influence whether individuals will have fewer larger scales, or greater numbers of smaller scales? It appears that for the *formosus* group of *Sceloporus* scalation and coloration, tend to be associated with adaptation to environmental conditions. For example, *Sceloporus internasalis*, *S. smargadinus* and *S. malachiticus* have been confused with *S. acanthinus* at relatively low elevations because of the high degree of scale fusion and low number of scales that they acquire in relatively hotter and drier environments. On the other hand, the species *S. acanthinus* and *S. taeniocnemis* have been mistaken for *S. smaragdinus* at high elevations, where they are darker, smaller, and possess higher scale counts (Smith, 2001). Based on the former observations and previous studies on *Liolaemus* (Hellmich, 1951), *Sceloporus graciosus* (Soule and Kerfoot, 1972) and *Anolis sagrei* (Calsbeek et al., 2006) suggesting that changes in scalation may be related to microgeographic variation in climate, I expect inter and intraspecific variation in scalation to be positively correlated with elevation and precipitation but negatively correlated with temperature.

Few studies of scale count variation have considered the issue of elevation. Elevation by itself does not seem to have a biological significance, and although

correlated, is an indirect measure of temperature and precipitation. However, in most cases, higher temperatures are associated with lower elevations where conditions may be generally drier. Due to this geographic pattern and the trend previously observed in some species of the *S. formosus* group, I have included elevation as an environmental source for the variation in scale count.

3.2 Methods

Measurements of body mass were taken right after collecting the animals. For morphological data, snout–vent length (SVL) was recorded from preserved specimens; SVL was measured from the tip of the snout to the anterior margin of the vent. Measurements were made with an electronic calliper to the nearest 0.01 mm. Body mass was recorded to the nearest 0.001g. Sex was recorded as well.

The number of dorsal scales was counted in a straight line from the interparietal scale to the posterior level of the insertion of the hind limbs. A single count per specimen was taken. For *S. adleri*, in addition to the specimens collected in the field ($n = 69$), SVL and dorsal scale counts were taken from 35 specimens (15 males and 20 females) from the Amphibian and Reptile Diversity Research Center collection at UTA. Thus, a total of 104 specimens of *S. adleri* were used for dorsal scalation analyses. For all the 122 specimens collected in the field, the number of lateral scales was counted. To do this, a picture was taken of the side of each lizard. Then using Photoshop, a circle with diameter 4.5 mm was drawn on the side of the lizard and the number of scales inside of the circle was recorded. A scale was counted if at least one third of it was inside the circle (Fig. 3.1).

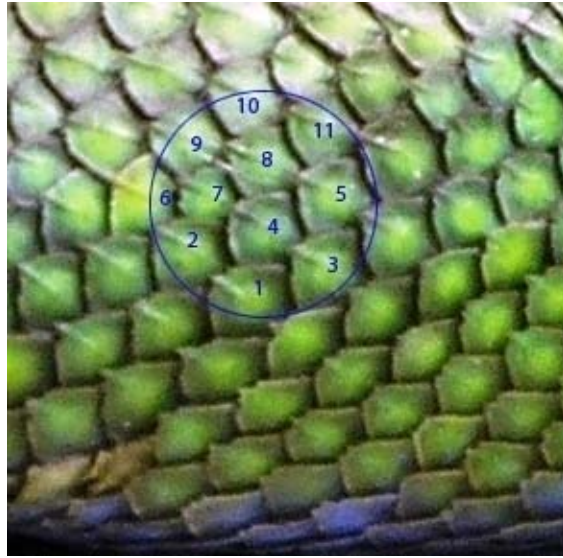


Figure 3.1. Lateral scale count. On the side of the lizard, the number of scales inside a circle with diameter 4.5 mm was counted. The circle was drawn in the same place where the water loss readings were taken.

Climate layers for Mexico, including 19 different parameters for temperature and precipitation, were downloaded from the 50-year (1950 – 2000) WORLDCLIM dataset (1 km² resolution, Hijmans et al., 2004). Using Maxent 3.3 (Phillips et al. 2004), the value of each environmental variable was matched to the corresponding geographic coordinate recorded in the field. Maxent is a correlative modeling tool that interpolates up to 19 climatic parameters for any location for which the latitude and longitude are known. Maxent can be used for describing the environment in which a species has been recorded and for identifying locations where the species may reside (Beaumont et al 2005).

3.2.1 Statistical analysis

Kolmogorov-Smirnov test was used to test for normality. Only lateral scales needed to be log transformed; all other variables were approximately normally distributed and met the criteria for parametric statistics. Because the number of scales is predetermined during the lizard's development, there was no a priori reason to believe that scale count should change as a function of growth. However, to corroborate this assumption, multiple linear regressions of the dependent variable dorsal scale count on the independent variables SVL and mass, were performed for all three species. As it was expected, no correlation was found for *S. scitulus* ($p = 0.093$) but for *S. adleri* ($p = 0.02$) and *S. druckercolini* ($p = 0.044$), the regressions were marginally significant. To remove the effect of body size in both species, dorsal scale count was regressed against SVL and the residuals were used in subsequent analyses. Independent variables were tested for correlation and transformed to minimize multicollinearity problems. Since lateral scalation was taken as an indirect measure of the size of the scale, the size effect was removed for all three species and the residuals of the transformed variable were used as the new variable.

Because climate variables are highly correlated to one another, a principal component analysis (PCA) was performed in order to identify which are the most important gradients among the 19 parameters that WORLDCLIM provides. The results of this statistical test show that Isothermality (BIO3) and Precipitation of the warmest Quarter (BIO18) have the maximum variability (Fig. 3.2). As a result, these two variables were selected to see how number of scales varies against temperature (BIO3)

and precipitation (BIO18). Isothermality is calculated from dividing the Mean Diurnal Range [Mean of monthly (max temp – min temp)] by the Temperature Annual Range (Max temperature of warmest month – Min temperature of coldest month).

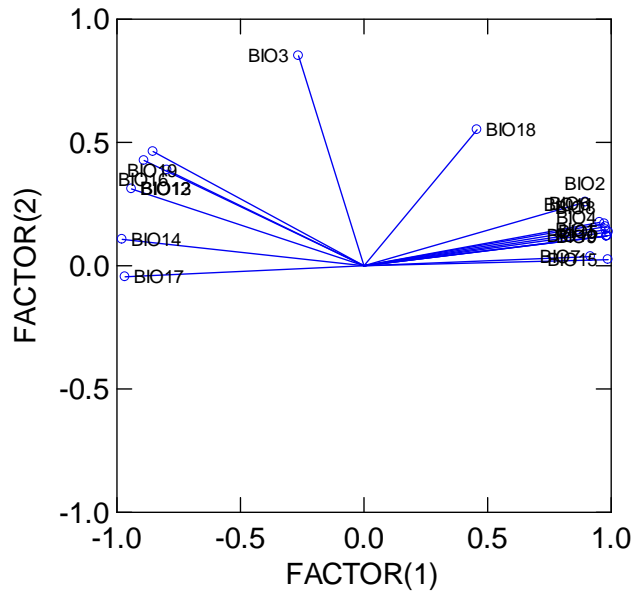


Figure 3.2. Principal Component Analysis (PCA) used to select one parameter for temperature (°C) and one for precipitation (mm). Isothermality (BO3) and precipitation of the warmest quarter (BIO18) were the two chosen variables.

To verify for possible correlations between the two variables selected above and elevation, Pearson correlation values were examined (Table. 3.1) and a correlation matrix (Fig 3.3) was performed.

Table 3.1 Pearson correlation matrix between climatic variables, $n = 122$.

	ELEVATION (m)	TEMPERATURE (°C)	PRECIPITATION(mm)
ELEVATION (m)	1.000		
TEMPERATURE (°C)	-0.028	1.000	
PRECIPITATION (mm)	-0.557	0.087	1.000

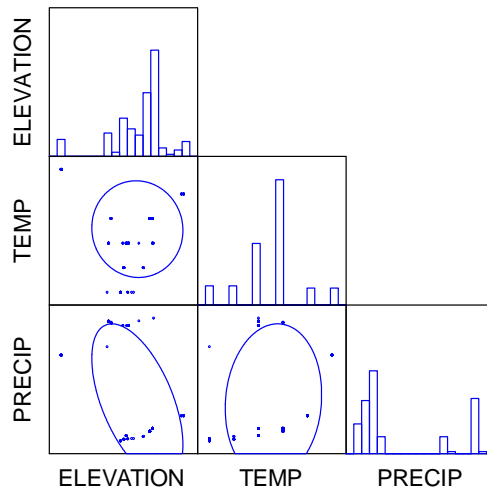


Figure 3.3 Correlation Matrix between climatic variables: elevation (m), temperature (TEMP, °C) and Precipitation (PRECIP, mm); $n = 122$.

To test for differences in scalation between sexes, two-way ANOVAS were made for each species. For *S. adleri*, measurements of scalation did not differ by sex ($F_{1,104} = 1.91$, $p = 0.170$ and $F_{1,69} = 0.35$, $p = 0.55$ for dorsal and lateral scales, respectively) and I pooled data from both sexes for all subsequent analyses. For *S. druckercolini*, no differences between sexes were found either (ANOVA $F_{1,22} = 0.08$, $p = 0.77$ and $F_{1,22} = 0.47$, $p = 0.49$ for dorsal and lateral scales, respectively). *Sceloporus scitulus* was the only species for which dorsal scalation differed by sex (ANOVA $F_{1,31} = 6.12$, $p = 0.019$ and $F_{1,31} = 0.00$, $p = 0.986$ for dorsal and lateral scales, respectively). I tested the associations between scalation (dorsal and lateral scales) and bioclimatic variables (elevation (m), precipitation (mm) and temperature (°C)), using multiple linear regressions. All analyses were performed using SYSTAT 11.

3.3 Results

When regressing scalation against environmental variables, pooling all species together, a significant relationship with both dorsal ($r^2 = 0.407$, $p = 0.001$, $n = 157$) and lateral scales ($r^2 = 0.0100$, $p = 0.006$, $n = 122$) was found only with elevation. This result is consistent with the expected pattern where scalation is positively correlated with elevation (Fig. 3.4 A).

Significant correlations with temperature were also found for dorsal ($r^2 = 0.05$, $p = 0.011$, $n = 157$) and lateral scale count ($r^2 = 0.03$, $p = 0.042$, $n = 122$). However, for this association, the number of dorsal scales increased with temperature, contrary to the expected direction (Fig. 3.4 B).

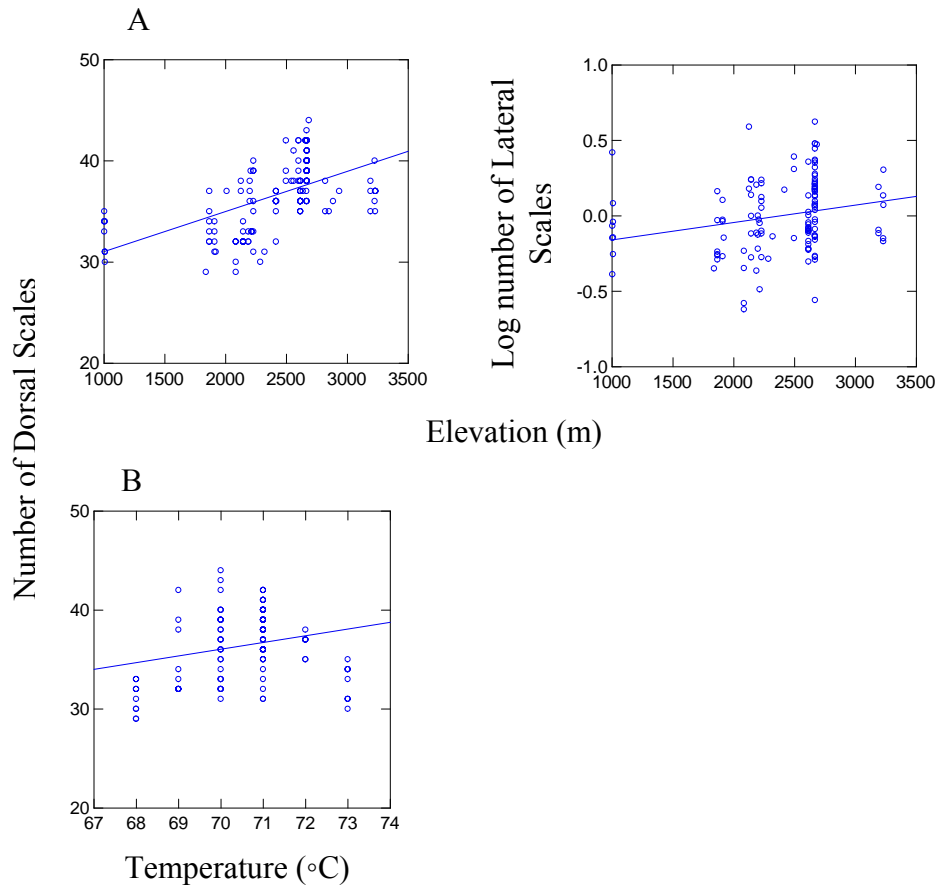


Figure 3.4 Interspecific regressions between scalation and climatic variables. The number of dorsal and lateral scales varied significantly with elevation (A). Hence, lizards found at higher elevations are associated with a higher scalation. Dorsal scale number was also significantly correlated with temperature (B), but opposed to the expected pattern. Thus, more and smaller scales were present in warmer climates.

3.3.1 *Sceloporus scitulus*

When pooling both sexes I found support for intraspecific dorsal ($r^2 = 0.867$, $p = 0.001$, $n = 31$) and lateral scalation ($r^2 = 0.267$, $p = 0.041$, $n = 31$) increasing with elevation and precipitation. The correlation between scalation and temperature was not

significant but; as with species pooled, number of dorsal scales increase with temperature (Fig. 3.5).

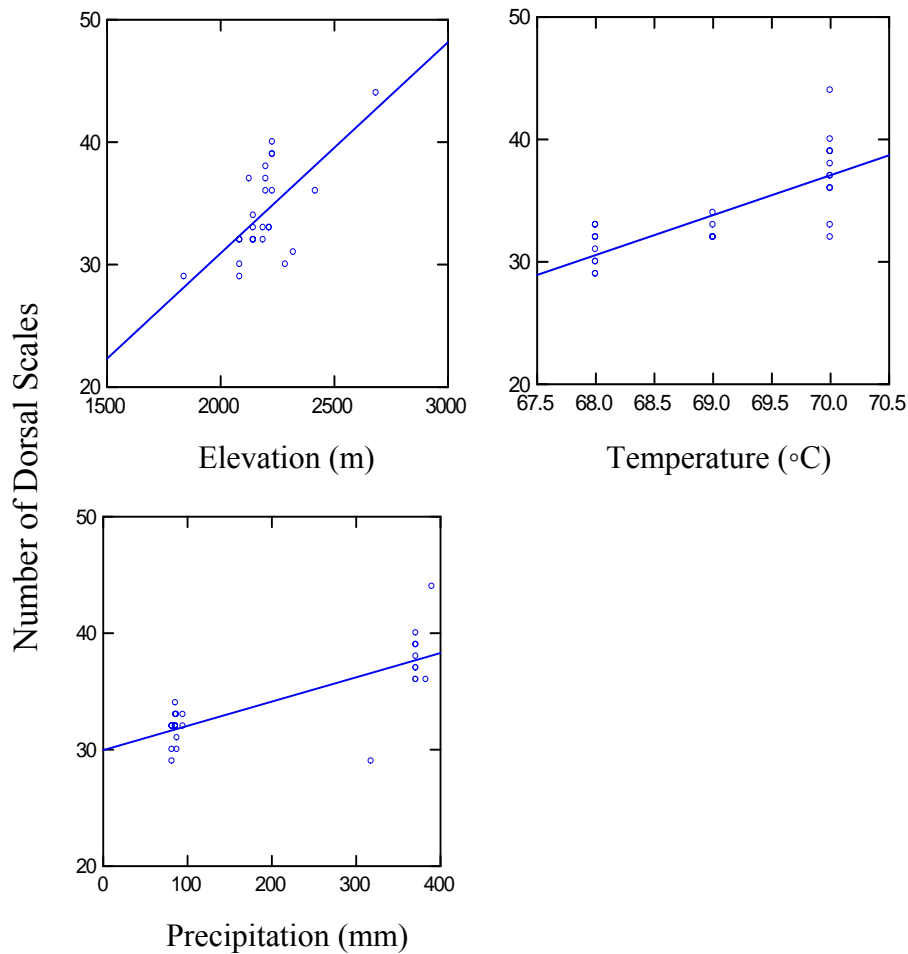


Figure 3.5. For *Sceloporus scitulus*, the number of dorsal scales increased significantly with elevation and precipitation. Although, dorsal scalation did not vary significantly with temperature, there is a tendency of having more dorsal scales with higher temperatures.

The correlations for males and females between scalation and the three climatic variables, followed in every case, the same patterns as when pooling sexes together. However, none of the multiple regressions were significant.

3.3.2 *Sceloporus adleri*

For dorsal scales, the number increased with elevation and precipitation and decreased with temperature as expected, but only the correlations with elevation and temperature ($r^2 = 0.491$, $p = 0.001$, $n = 104$) were significant (Fig. 3.6 A).

For lateral scalation no correlations were observed with elevation or precipitation ($r^2 = 0.089$, $p = 0.105$, $n = 69$); however, the association with temperature was significant and negatively correlated ($r^2 = 0.03$, $p = 0.035$, $n = 69$), observing fewer lateral scales with increasing temperature (Fig. 3.6 B).

3.3.3 *Sceloporus druckercolini*

For dorsal scales, the number increased with elevation and precipitation and decreased with temperature, as expected. However, none of the relationships were significant ($r^2 = 0.198$, $p = 0.253$, $n = 22$). No significant patterns were observed with lateral scale counts ($r^2 = 0.232$, $p = 0.203$, $n = 22$).

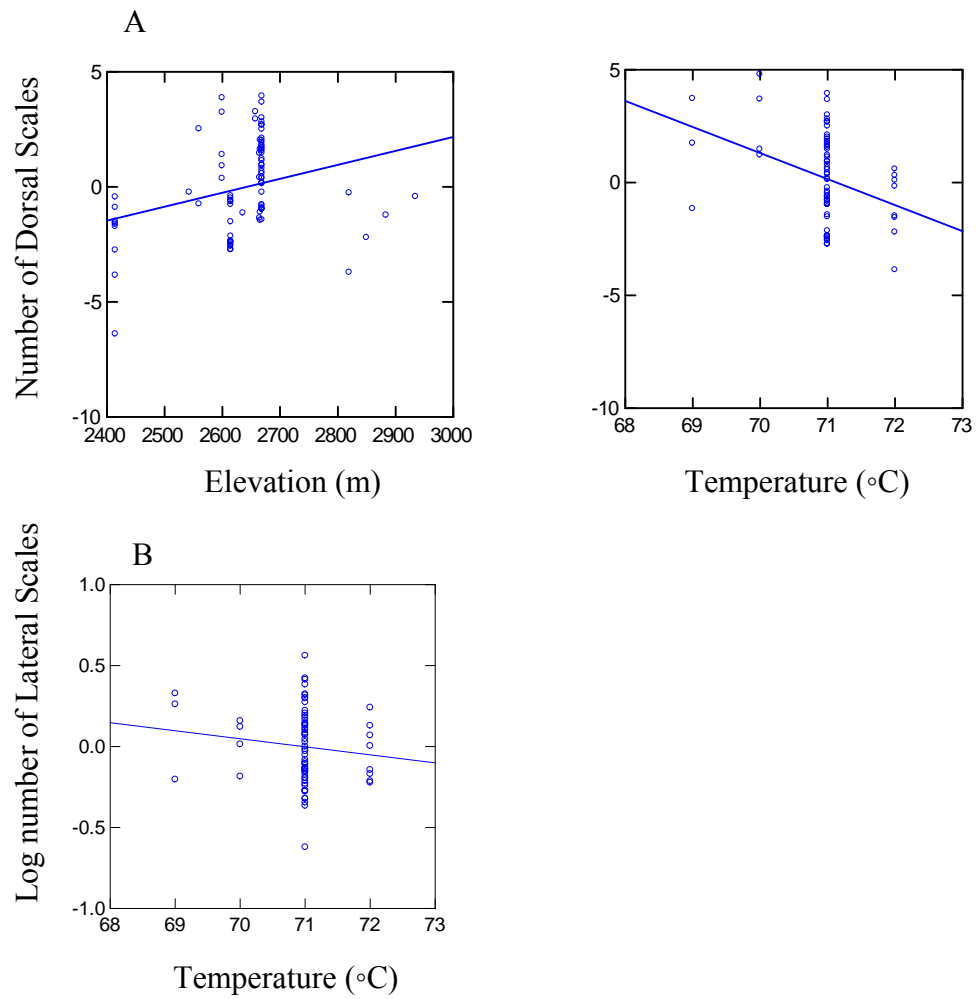


Figure 3.6. For *Sceloporus adleri* the variation in dorsal scalation followed the anticipated pattern (A). Lateral scale count also had a significant inverse relationship with temperature, as it was expected to be (B). Residuals for dorsal and lateral scales were plotted against climatic variables.

3.4 Discussion

A handful of studies have suggested that changes in scale number are correlated with microgeographic variation in climate. Here, I present new evidence on three species

of the *Sceloporus formosus* group, suggesting that scalation is indeed a highly plastic morphological character that may be under ecological selective pressures.

Geographic variation in scalation was found to be strongly correlated with elevation. Interspecific comparisons (*S. scitulus*, *S. adleri* and *S. druckercolini* pooled together), seems to indicate that scale number may be under selection pressure for fewer and larger scales in lowlands. Likewise, intraspecific comparisons showed that number of both dorsal and lateral scales decrease at lower elevation. Although this pattern was observed in the three species, it wasn't significant for *S. druckercolini*, possibly, due to the small number of individuals reported in this study. These results are supported by Thorpe and Baez (1987); who reported for the lizard *Gallotia galloti* a strong variation (stepped cline) between dorsal scalation and elevation.

When examining the correlation between scalation and precipitation for all species together, the number of dorsal scales decreased at lower precipitations. Within species; the same positive correlation was constant; however, significant results were obtained only for *S. scitulus*. Studies conducted in *Sceloporus graciosus* (Soule and Kerfoot, 1972), *Liolaemus* (Hellmich, 1951), *Crotalus mitchellii* (Meik, 2008) and *Anolis sagrei* (Calsbeek et al., 2006) have found the same pattern between scalation and precipitation. Hence, favoring the hypothesis that selection for fewer (larger) scales in drier, warmer climates reduces area of exposed interstitial skin, minimizing water loss through evaporation caused by high temperatures (Calsbeek et al., 2006). This suggests that selection may act on scalation based on differences in local climate favoring fewer numbers of scales in hot and/or dry conditions.

According to the above hypothesis, I expected that lizards will have less scales at higher temperatures. This was precisely the tendency observed for *S. adleri* and *S. druckercolini*, and these results also support the patterns observed by Smith (2001) for other species of the *formosus* group (*S. internasalis*, *S. smaragdinus*, *S. acanthinus* and *S. taeniocnemis*) where the number of dorsal scales decreases with low elevations and high temperatures. This pattern is also supported in previous studies of scalation in *Anolis sagrei* (Lister, 1976; Calsbeek et al., 2006), *Crotalus mitchellii* (Meik, 2008) and *Sceloporus graciosus* (Soule and Kerfoot, 1972) where 84 % percent of the geographic variation in scale number can be accounted for precipitation and temperature. According to this tendency, where the number of scales increase with elevation and precipitation, and decrease with temperature, it will be reasonable to suggest that scalation represents a general adaptation to prevent water loss in arid habitats (Soule and Kerfoot, 1972), since fewer number of scales in hotter and drier conditions would minimize the amount of interstitial skin exposed to the atmosphere (Horton, 1972). Since the same pattern has been observed in smooth and no overlapping scales (*Anolis sagrei*), as well as keeled and overlapping scales (*S. graciosus*), it can be concluded that the degree of overlapping is not influencing the variation in scale count with climatic factors.

For this same correlation (variation in scalation with temperature), my results also suggest, that for the three species together, the number of dorsal scales increases at higher temperatures (Fig. 3.4 C). This opposite pattern reported here is consistent with a previous study of scalation in *Parias* (Sanders et al., 2004), *Crotalus* (Klauber, 1941) and *Anolis oculatus* (Malhotra and Thorpe, 1997). Sanders et al (2004) hypothesized that an

increase in scale number results in a tighter fit between scales, reducing the surface area of exposed interstitial skin, and hence facilitating more efficient water retention with higher temperatures. An alternative interpretation for this positive correlation between scalation and temperature is that the waterproofing function of squamates reptiles is also attributable to sheets of lipids deposited in the stratum corneum and not in the scale per se (Lillywhite, 2006). This hypothesis will be further explored in the next chapter.

CHAPTER 4

THE LINK BETWEEN WATER LOSS AND SCALATION

4.1 Introduction

The shape and arrangement of scales have been implicated in various functions. For example, scales serve as a mechanical protection, as *Phrynosoma*, where the spines on the back and sides are modified scales. In some snakes, scales have been modified over time to form specialized structures such as 'eyelash' fringes, protective covers for the eyes, and the most distinctive modification, the rattlesnakes' rattle. Some other functions are to aid in locomotion, serve as visual signals (ornaments), sound production, substrate interactions, thermoregulation and provide complex coloration patterns which help in camouflage and anti-predator display. Scale coloration has also been known for contributing to thermoregulation functions through labile reflectivity (Porter and Norris, 1969). However, a quite controversial function of the scales has to do with water balance capabilities. The importance of scalation to water balance arises because the size and number of scales impact the surface area of interstitial skin that is exposed to the atmosphere. Fewer and larger scales are generally keeled and overlapping (Soule' and Kerfoot, 1972), reducing interstitial skin exposure and hence the amount of evaporative water loss. Smaller, more granular scales increase the exposed tissue between scales and

increase rates of dehydration (Dmi'el, 2001; Neilson, 2002). Thus, in addition to protection against injury, scales are likely to influence water balance (Alibardi, 2003).

Reptilian scale development involves emergence of a complex architecture of dermal collagen fibers, unique among living amniotes (Maderson and Alibardi, 2000). It differs from mammals in having two different types of keratin, α and β (Fig. 4.1).

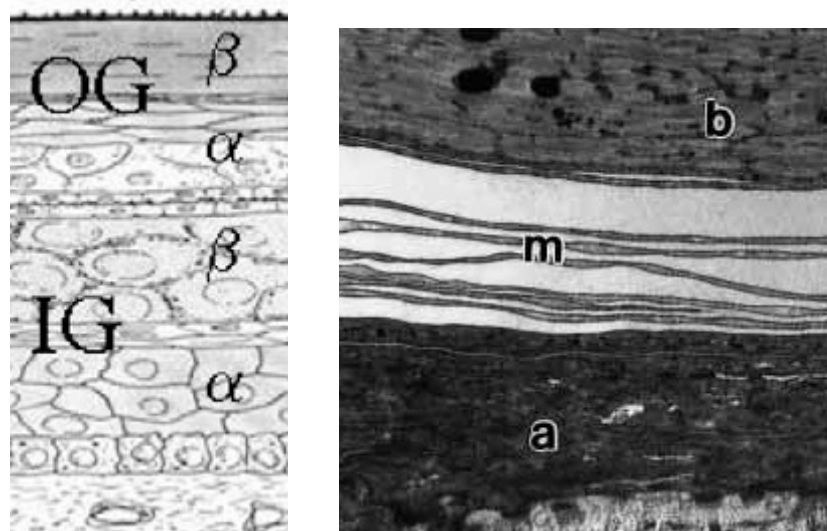


Figure 4.1 Features of the squamate integument. On the left side, OG and IG indicates outer and inner epidermal generations that might be present in a squamate reptile prior to skin shedding. Right side shows the β (b) and α (a) keratin with the mesos layer (m) in between. Sheets of laminated lipids can be observed within the mesos layer. Modified from Lillywhite (2006) and Lillywhite and Tu et al. (2002), respectively.

In lepidosaurs the entire surface of the body is covered by β -keratin beneath which is a tissue of α -keratin. Between these two layers of keratin is the mesos layer, a specialized cell type derived from α -keratins where the laminated lipids are located (Fig. 4.1). The β layer adds rigidity to the integument and is relatively thick on the outer

surface of the scale, but is represented by only a single layer of cells, the *Oberhäutchen*, on the inner surface and in the region between scales also known as hinge region (Fig. 4.2). The α layer has uniform thickness over the entire body (Lillywhite and Maderson, 1982).

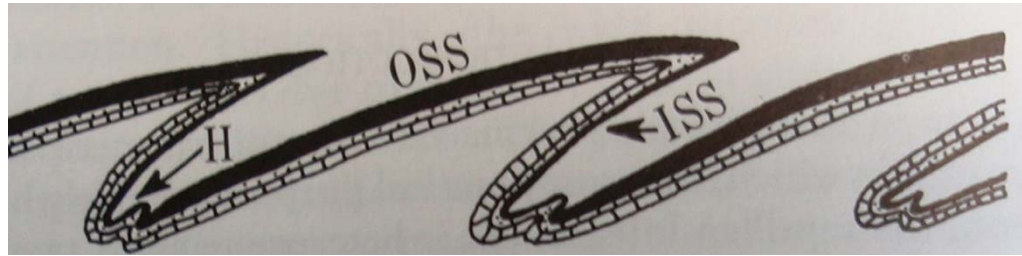


Figure 4.2 Organization of the reptile integument showing outer scale surface (OSS), inner scale surface (ISS) and hinge region (H). Adapted from (Lillywhite and Maderson, 1982).

Based on the studies of Horton (1972), Soule' and Kerfoot (1972), and Malhotra and Thorpe (1997), which emphasize the importance of scalation in water balance, I expect that skin resistance (R_s) should be negatively correlated to number of scales. Thus, a higher skin resistance (R_s) should be observed with less lateral scales. Likewise, since the rate of water that can be lost through evaporation is dependent on climatic conditions, and based on patterns observed in lizards from the *S. formosus* group (Smith, 2001), I also expect R_s increasing with temperature and decreasing with elevation and precipitation.

4.2 Methods

Water loss was measured using a device called VapoMeter (designed by Delfin Technologies). It consists of a closed cylindrical chamber that contains a sensor at one

end for relative humidity (RH) and temperature. This sensor, with a diameter of 4.5 mm, was placed against the scales on the right side of the lizard, between the foreleg and the hind leg (Fig. 4.3). After approximately 15-20 seconds, a value for transepidermal water loss (TEWL; $\mu\text{g} / (\text{cm}^2 \cdot \text{h}^{-1})$) was calculated from the increase in RH% sensed by the instrument within the measurement chamber. With the purpose of having as much precision as possible, I repeated the measurements with each specimen until obtaining relatively consistent values, and then used the average.

A TEWL average was calculated for every specimen and then transformed to obtain a skin resistance (R_s) measurement. Skin resistance ($\text{s} \cdot \text{cm}^{-1}$) was computed from the following equations (Tu et al., 2002):

$$(1) R_t = \{((T_s * 1.3) - 9.43) - [(((T_a * 1.3) - 9.43) * RH)]\} / (TEWL / 3600)$$

$$(2) R_s = R_t - R_b$$

where:

R_t : total resistance

T_s : skin temperature ($^{\circ}\text{C}$)

T_a : air temperature ($^{\circ}\text{C}$)

R_b : boundary layer resistance ($R_b = 2.7$)

The boundary layer resistance (R_b) was determined utilizing measurements of TEWL from a water saturated paper towel that evaporated as a free water surface. Thus, by replacing the corresponding values in the first formula above, R_t was obtained and this was our R_b value ($R_b = 2.7$). Skin resistance to water vapor transfer (R_s) was the variable used to examine the variation in water loss because its susceptibility to experimental conditions is considered very low (Eynan and Dmi'el, 1993).



Figure 4.3 Measurement of the skin resistance to water loss using a VapoMeter device. All readings were taken on the right side of the lizard's body.

Before taking the VapoMeter readings, each lizard was placed individually in an open plastic container for about 30 minutes with the purpose of eliminating any extra moisture transferred to a lizard from handling it. All measurements were taken in the same controlled environment and I took note of every time a lizard was shedding. Due to the keeled scales of these lizards, there was the possibility of not having a perfect seal between the body of the lizard and the sensor of the VapoMeter, which could have interfered with the accuracy of the readings. Therefore, special care was taking during each reading to avoid any possible gap. Whenever there was doubt of the accuracy of the measurement, the datum was deleted and the reading was repeated.

As an indirect measure of the size of the scale, I counted the number of lateral scales per unit area. This count was made on the same spot where the VapoMeter

reading was taken (Fig. 3.1). The same environmental variables used for scalation were utilized to assess the correlation between skin resistance and climate.

To determine from what body part VapoMeter values should be taken, readings from 6 specimens (two of each species) were taken from the head, hind limbs, dorsal, ventral and side of the lizards. The side (55.89 ± 11.44) and ventral (48.35 ± 10.20) areas showed the lowest standard deviations and lowest variances. The side of the lizards was chosen over ventral since this area is more exposed to water loss and a better seal was guaranteed over the head, hind limbs and dorsal areas.

4.2.1 Statistical analyses

Unless otherwise stated, all variables were approximately normally distributed and met the criteria for parametric statistics. Since the lateral scale count was taken as an indirect measure of scale size, there was no need to remove the size effect, since what we want to test is precisely, how water loss rates varies with the number of scales. Differences in R_s between sexes were tested with two-way ANOVAS. No significant differences were found for any of the three species ($F_{1,31} = 0.63$, $p = 0.434$; $F_{1,69} = 0.42$, $p = 0.838$ and $F_{1,22} = 4.37$, $p = 0.52$ for *S. scitulus*, *S. adleri* and *S. druckercolini*, respectively). To test the hypotheses for water loss, multiple linear regressions were performed between the dependent variable skin resistance (R_s) and the independent variables lateral scale count, elevation, precipitation and temperature.

4.3 Results

Across the three species, the relationship between water loss and lateral scale count was as expected; there is a stronger skin resistance to water loss (R_s) with less

number of lateral scales ($r^2 = 0.196$, $p = 0.001$; Fig. 4.4 A). Intraspecific correlations were found to be significant in *S. druckercolini* ($r^2 = 0.180$, $p = 0.039$, $n = 22$; Fig. 4.4 B) and in *S. adleri* ($r^2 = 0.140$, $p = 0.002$, $n = 68$; Fig. 4.4 C). *S. scitulus*, also presented the expected pattern but the correlation was not significant ($r^2 = 0.001$, $p = 0.855$, $n = 31$).

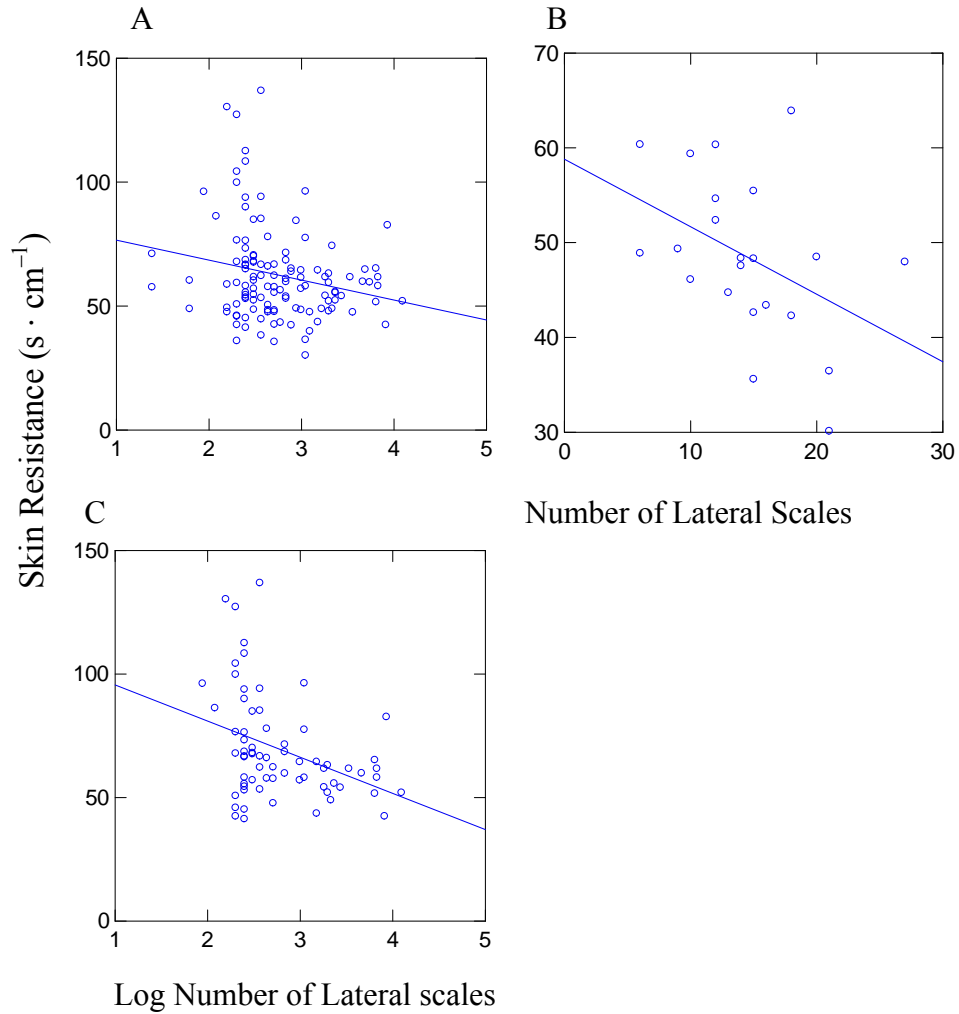


Figure 4.4. Skin resistance varied significantly with the number of lateral scales when pooling the three species together (A, $n = 122$) and with *S. druckercolini* (B, $n = 22$) and *S. adleri* (C, $n = 31$). Number of lateral scales was Log transformed for A and C.

For all the three species, the relationship involving water loss and precipitation was as expected, observing the lowest resistance to water loss in lizards occurring in moist habitats with higher precipitation rates ($r^2 = 0.179$, $p = 0.017$, $n = 119$; Fig. 4.5 A). Contrary to the expected tendency, the higher rates of skin resistance were observed in lizards present at highlands ($r^2 = 0.179$, $p = 0.025$, $n = 119$; Fig. 4.5 B). The correlation of R_s and temperature did not show any trend ($r^2 = 0.179$, $p = 0.268$, $n = 119$; Fig. 4.5 C).

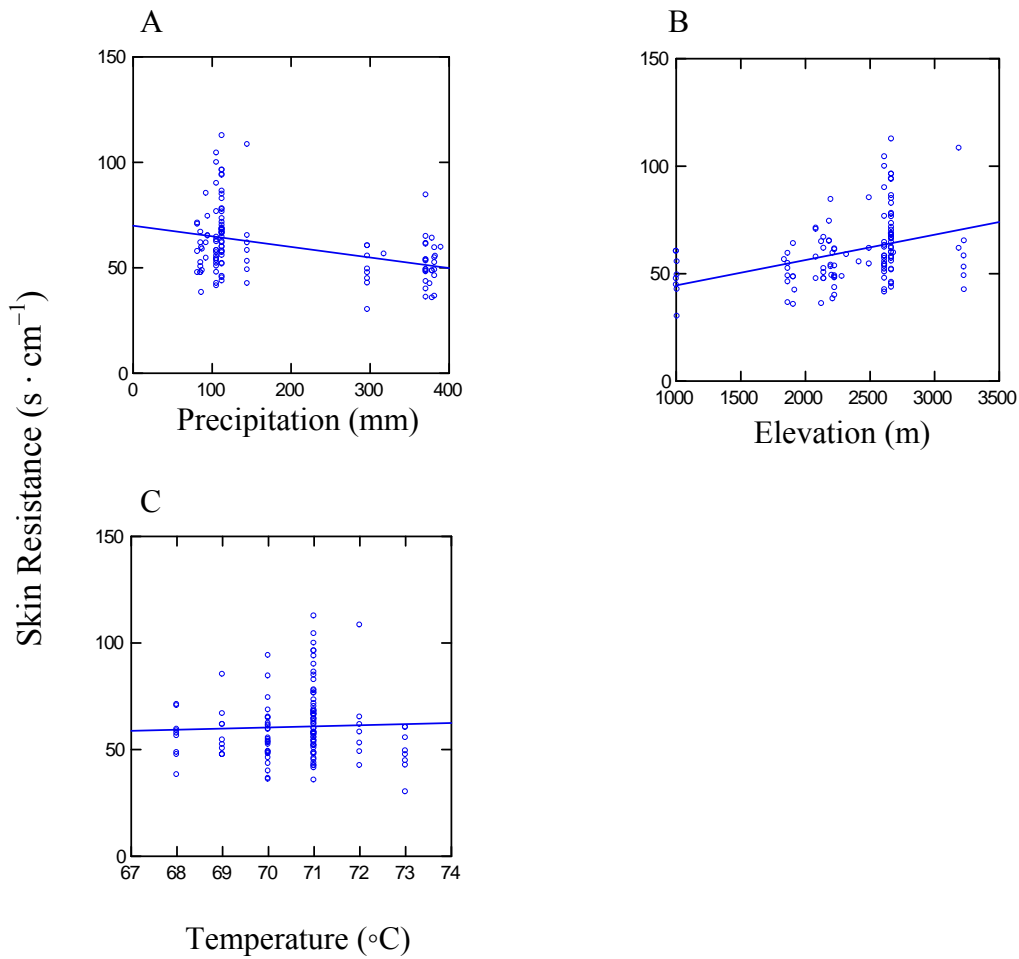


Figure 4.5 Among species, a stronger skin resistance (R_s) was observed with lower precipitation values (A). Skin resistance (R_s) was not inversely correlated with elevation, as expected (B). No relationship was found between R_s and temperature (C).

For the relationship between R_s and the climatic variables, no significant relationships were observed within species.

4.4 Discussion

The diverse patterns in the variation of scalation with climatic variables across different taxa of squamates have long been attributed to the assumed capacity of the scale to control for water loss (Alibardi, 2003). Since the earliest studies, it has been proposed that larger scales in drier, warmer climates minimize the area of exposed interscalar tissue decreasing rates of dehydration (Dmi'el, 2001; Hellmich, 1951; Soule and Kerfoot, 1972; Calsbeek et al., 2006; Kerfoot, 1968; Thorpe and Baez, 1987; Horton, 1972). The results from this study support this hypothesis. For interspecific and intraspecific correlations, skin resistance to water loss increased notably with decreasing number of lateral scales. This pattern was constant among all species and correlations were significant for all except *S. scitulus*. Such a strong pattern is in agreement with our results obtained in the previous chapter for the variation in scales size and number. For the three species of the *S. formosus* group, lizards with fewer and therefore bigger scales are occurring in lowlands with low precipitation rates, and consistently, the skin resistance to water loss increased when fewer scales were present. Thus, our results also support what numerous studies have demonstrated about the reptilian skin: rates of water loss vary inversely with habitat aridity (Dmi'el 1998; Neilson, 2002; Gans et al., 1968; Lahav and Dmi'el, 1996; Mautz, 1982; Soule and Kerfoot, 1972; Roberts and Lillywhite, 1980; Dawson et al., 1966; Bentley & Schmidt-Nielsen, 1966; Claussen, 1967; Gans et al., 1968; Prange &

Schmidt-Nielsen, 1969; Tu et al., 2002), and this variation can be attributed to the number of scales.

The pattern observed in these lizards of the *S. formosus* group also allows to suggest that the main route for water loss is not the scale *per se*, but the skin between scales, in which case the more scales, the greater the surface area of interstitial skin, and the greater the evaporative water loss. As a result, the inverse pattern is observed at interspecific and intraspecific levels: lower skin resistance with higher number of lateral scales.

Licht and Bennett (1972) questioned the role of scales in limiting water loss following an experiment on a scaleless snake (*Pituophis melanoleucus*). They measured transepidermal water loss (TEWL) and compared the structure of the integument between the scaleless specimen and a normal one of almost identical proportions. No differences were found for water loss between individuals; however, histological comparisons revealed that in the scaleless specimen, the width of α -keratin layer was essentially normal but, the outer (presumably β -) keratin layer was particularly thin. In general, the skin of the scaleless snake seemed most like the flexible hinge region that exist between scales in the normal snake (Licht and Bennett, 1972). Thus, the former study suggests that in the process of limiting transepidermal water loss, β -keratin, the main component of the scale *per se*, is not the main player, but the interstitial skin might be.

Studies conducted in *Elaphe obsoleta* (Roberts and Lillywhite, 1980) and *Anolis carolinensis* (Kattan and Lillywhite, 1989) provides another explanation for a possible mechanism limiting water exchange in the reptilian integument. Comparative

measurements of water loss in both species showed that permeabilities differ as expected without any manipulation of the integument. However, when lipids were extracted from the dorsal skin, rates of evaporation increased and the differences in water loss disappeared. Although these two studies have proved that the site of skin permeability barrier in *Elaphe obsoleta* and *Anolis carolinensis* is the mesos layer that contains lamellar lipids, this result should not be extrapolated to all squamates.

First, none of the studies that have measured evaporative water loss in scaleless versus normal specimens have examined how the mesos layer, and therefore the lipids content, varies as well. Second, the morphological features of the scales of *Anoles* and snakes like *Elaphe* and *Pituophis* are quite distant from the keeled and imbricated scales in *Sceloporus*, also known as “spiny lizards”. Undoubtedly, numerous influences have given rise to the varied morphologies in scalation present today (Lillywhite and Maderson, 1982), and such variation cannot be ignored. Moreover, none of those studies have looked at the role of lipids in determining R_s for *Sceloporus*. Thus, further studies in or closer related species to *Sceloporus* that addresses the content and distribution of the lipids, and hopefully, utilizing the same technology for measuring water loss, needs to be conducted before disproving a very well known association between scalation and water loss.

It can be concluded that the correlation between water loss and scalation is not occurring by chance, and a close examination of *Sceloporus*' integument remains to be done. Certainly, lipids are vital to the water barrier function, but the interstitial skin has also proven to control for water loss. It remains possible that the interstitial skin, if

exposed, it might have a less effective, maybe thinner, lipid layer than do the outer scale surface. This is still an unresolved issue and there may well be differences in different taxa of reptiles. Therefore, caution should be used in the interpretation of certain morphological features of squamates reptiles as an adaptation or not to the restriction of water loss.

CHAPTER 5

HOW SCALATION COULD INFLUENCE LOCOMOTORY PERFORMANCE

5.1 Introduction

Sprint speed is the most commonly studied aspect of reptilian locomotor abilities. Differences in body size and in relative limb length seem to be the most important causal factors for the variation in sprinting. Within populations of lizards and snakes, sprint speed generally increases with body size (Garland 1985, Huey and Hertz 1982, Sinervo 1990) and many studies have investigated whether the predicted positive relationship between limb length and sprint speed exists in lizards including *Sceloporus* (Sinervo and Losos 1991; Miles, 1994). However, the relationship between other morphological variables and sprint speed has been less explored (Garland and Losos, 2004), either between or within species. Future studies should, therefore, attempt to determine what other morphological, physiological or mechanical factors may account for additional variation in sprint speed (Bonine and Garland 1999).

To the best of my knowledge, no studies have reported the effect of scalation in lizard's locomotory ability. It would be logical to assume that for a lizard covered with big and thick plates of keratin, its flexibility and ease of movement could be limited more so than if it had smaller and thinner scales that allow for more elasticity. Thus, one of the

effects of having a higher scale count could be having more flexibility resulting in a better performance.

With this in mind, I proposed a third hypothesis relating the morphology with the performance of the lizards. I predicted that a higher number of dorsal and lateral scales will be positively correlated with sprint speed.

5.2 Methods

Once a lizard was captured, its body temperature was recorded immediately and then was allowed to become acclimatized for a period of three to four days. Before each trial, lizards were left undisturbed for about 2 hours until they reached a body temperature of 34 °C. Lizards reached this temperature by simply leaving them inside plastic containers without the lid in a 30 °C average room temperature. Body temperature was verified using a thermocouple thermometer with a skin sensor. Maximal sprint speed (cm s^{-1}) was measured using a 180 cm long x 20 cm wide racetrack with a cork sprinting surface. Lizards were chased and filmed laterally with a Hi-8 video camera (SONY DCR-DVD403) at a constant 30 frames s^{-1} . Every lizard was chased individually three times, consecutively (following Braña, 2003). A small stick was used to chase the animals. Videos were recorded in a VOB format and then captured in an .avi format in order to be analyzed using the program Adobe Premier. Sprint speed was calculated by counting the number of frames that the lizard took to travel a measured distance without stopping. The best performance of all three trials was taken as the maximum sprint speed (Max SP). Maximum sprint speed and the average of the three trials (mean sprint speed) were the two dependent variables used to assess performance.

5.2.1 Statistical analyses

I performed simple and multiple linear regressions of the dependent variables maximum (Max SP) and mean (Mean SP) sprint speeds against number of dorsal and lateral scales. To remove effects of body size, Max SP, Mean SP and lateral scalation, were regressed against SVL, and the residuals were used as the new variables. Differences in performance between sexes were tested with two-way ANOVAS and no significant differences were found for any of the three species.

5.3 Results

Among species, no significant relationships were observed. Within species, only *S. scitulus* presented a positively significant correlation: maximum ($r^2 = 0.472$, $p = 0.001$, $n = 26$; Fig. 5.1 A) and mean sprint speed ($r^2 = 0.326$, $p = 0.011$, $n = 26$; Fig. 5.1 B) increased with a higher number of dorsal scales. Performance also increased with lateral scalation; however, this relationship was not significant (Fig. 5.1 C).

Since the dorsal scalation was significantly different for males and females, separate regressions were made for each sex. Females presented a positive and significant relationship when regressing mean sprint speed against dorsal and lateral scale counts ($r^2 = 0.628$, $p = 0.031$, $n = 10$). Maximum sprint speed presented the same pattern with dorsal and lateral scalation ($r^2 = 0.785$, $p = 0.005$, $n = 10$).

For males, all the associations between performance and scalation followed the expected tendency: mean and maximum sprint speed increased with dorsal and lateral scale count. However, only a significant correlation was obtained between mean sprint speed and the number of dorsal scales ($r^2 = 0.384$, $p = 0.021$, $n = 15$). No relationship

was observed between performance (mean and maximum sprint speed) and lateral scalation.

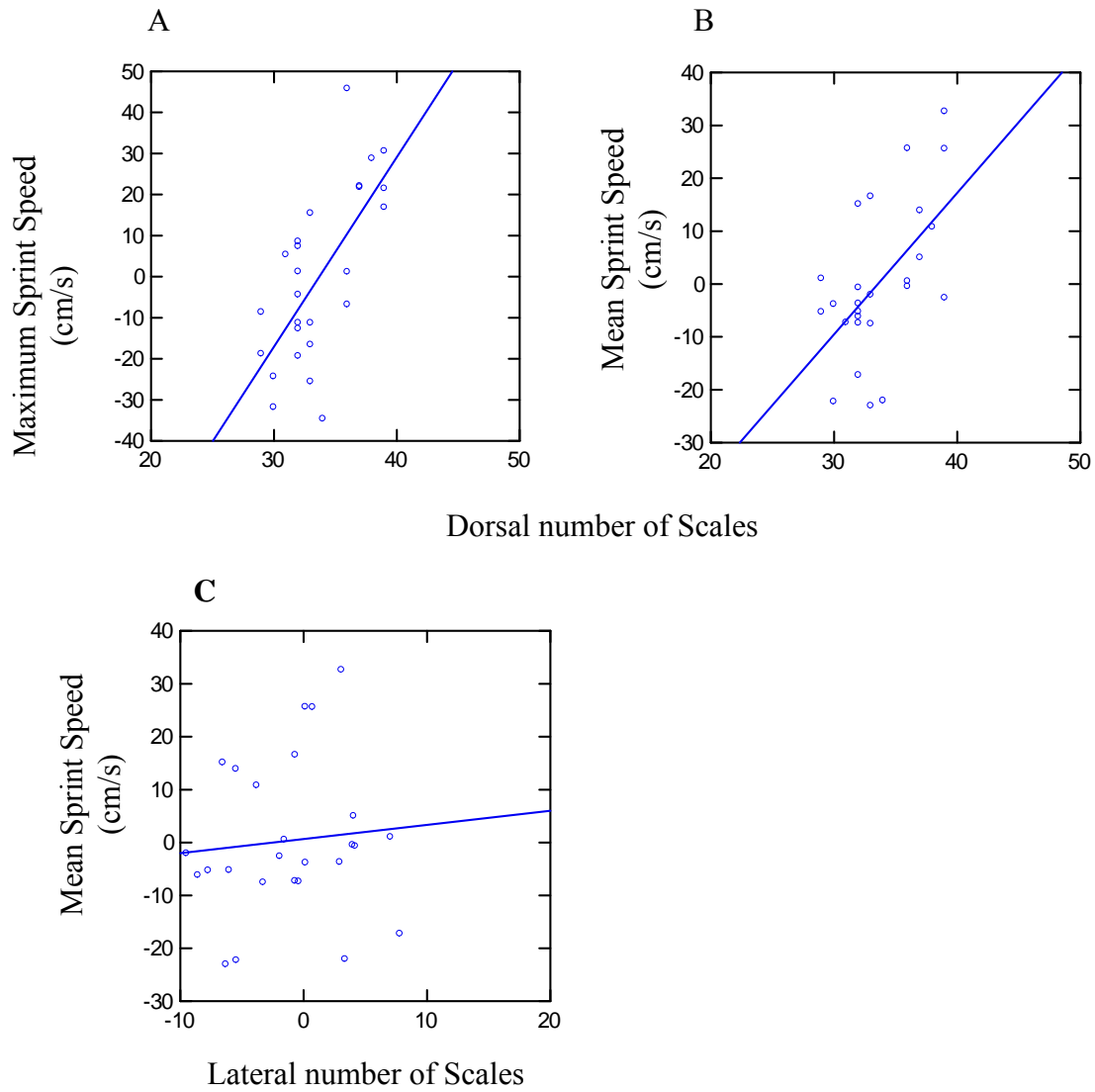


Figure 5.1 For *S. scitulus*, maximum (A) and mean sprint speed (B) increased with a higher number of dorsal scales. Mean sprint speed also increased with lateral scalation; however, this relationship was not significant (C). To remove the effect of body size, residuals for Max SP, Mean SP and lateral scalation were used as the new variables.

5.4 Discussion

A central idea of ecomorphological studies is the link between morphology and performance, but, surprisingly few analyses demonstrate this relationship. In this study we correlated the number of dorsal and lateral scales with lizards' locomotory abilities. In the reptilian integument, flexibility derives from the hinges regions (Fig. 4.2) where only a single layer of cells containing β -keratin is present. Assuming that this area of interstitial skin increases proportionally with the number of scales, we expected that a higher number of scales would provide more flexibility to the lizard's movement improving its sprint speed. Our results supported the proposed hypothesis but only within one species, *Sceloporus scitulus*, being out of the three species the one with the broadest distribution; therefore, exposed to more diverse environmental conditions.

Within *S. adleri* and *S. druckercolini* and the three species combined, locomotory performance varied inversely with number of scales, a number of factors could have influence the results. We already know that performance normally increase with body size, so juveniles sprint more slowly than do adults due to their smaller size. If both categories occupy the same niche, the smaller size of the juveniles will make them more vulnerable to predators and may limit the size of the preys that they can consume, making them less competitive compared to adults (Husak, 2006). Such circumstances suggest that natural selection on performance traits (like hind limb length) may be stronger in juveniles than adults (Carrier, 1996). This could also reflect a minimum range of speeds that individuals must attain for success in escaping and/or foraging (Husak, 2006). As a result, juveniles might be using a higher percentage of their maximal sprint speed

capacity than adults when escaping predators (Irschick, 2000; Irschick et al., 2000; Husak, 2005). Thus, behavioral or physiological variation, could have affected the performance measurements. Nonetheless, repeated testing of individuals and use of the fastest trial(s) as an index of maximal speed may help circumvent this potential problem (Garland and Losos, 2004).

The altitudinal factor could also have influenced the sprint speed of the lizards. Performance trials were held all at the same elevation (1250 m), however, lizards were collected between 1010 and 3232 m (Table 1). Potentially, animals raced at a higher elevation from where they were originally found, could have been disadvantageous compared to animals that were brought from a higher to a lower site.

Perhaps, one of the factors with the most biological significance in the sprinting capacity of the lizards is the type of scale selected to assess the variation in performance, in this case, dorsal scales. These scales, besides being a very common morphological character to separate species, represent an important fraction of the total surface area. However, considering the lateral movement involved in the locomotion of these lizards, scales on the side of the body may be under a stronger selective pressure to facilitate locomotion.

5.4.1 A possible trade-off

Potential trade-offs and constraints are of major concern in evolutionary biology (Sinervo and Licht, 1991); however, they are not always easy to predict. At the early stages of this study, we considered if water loss and performance were part of a trade-off system dependent on scale number. In low and dry environments, fewer but larger scales

may function in retarding water loss through interstitial skin, but with the cost of reducing flexibility and decreasing locomotory ability.

From the three species considered in this study, our results suggest that this trade-off could be occurring in *S. scitulus*. For this species, a higher number of dorsal and lateral scales present at lowlands and drier conditions may be successfully limiting the amount of water loss by observing a stronger resistance to water loss (R_s) with low scale counts. This beneficial relationship could be hindering the sprinting capabilities by having a poor performance caused by less number of scales and therefore, less flexibility. However, despite observing an inverse relationship between R_s and scalation, this correlation was not significant.

5.5 General Conclusions

Ours results provide strong evidence suggesting that the morphology of these lizards is extremely plastic to environmental conditions. Dorsal and lateral scale counts seem to be under elevation, precipitation and temperature selective pressures. The altitudinal component of the variation in scalation among and within species is extremely evident. However, elevation is an indirect measure of precipitation and temperature and these two climatic variables can fluctuate depending on seasonality. Likewise, diurnal and annual variation in temperature becomes more extreme in higher altitudes. Consequently, this could be a possible explanation for the presence of both patterns in the variation of scale number with temperature.

The present study suggests that the commonly held view regarding the physiological role of the scales of reptiles is correct. Skin resistance to water loss appears

to be varying as a function of scale count. Nonetheless, the characteristics of the scale itself (i.e., the thick β -keratin on the scale surface, the compact epidermis and the underlying supportive superficial dermis) are not sufficient to be the main or sole barrier to water loss. Therefore, further studies in *Sceloporus* showing the role of lipids in the integument needs to be conducted.

Finally, the adaptation in scalation to differences in local climate may account for much of the taxonomic confusion among *Sceloporus*. The ecological convergence in traits used for classification, like seen in scalation, is found to have important implications for species identification where in groups that are widely distributed over varying environments.

REFERENCES

- Calsbeek R., Knouft, J.H., Smith, T.B. 2006. Variation in scale numbers is consistent with ecologically based natural selection acting within and between lizard species. *Evolutionary Ecology* 20: 377-394.
- Dmi'el, R. 2001. Skin resistance to evaporative water loss in reptiles: a physiological adaptive mechanism to environmental stress or a phylogenetically dictated trait? *Isr. J. Zool.* 47, 55–67.
- Eynan, M., and R. Dmi'el. 1993. Skin resistance to water loss in agamid lizards. *Oecologia* 95: 290–294.
- Fitch, H. S. 1970. Reproductive cycles in Lizards and Snakes. Univ. Kans. Mus. Nat. Hist., Misc. Publ. 52:1-247.
- Hall, W. P. 1937. Comparative population cytogenetics, speciation and evolution of the crevice-using species of *Sceloporus* (Sauria: Iguanidae). Ph.D diss., Harvard Univ., Cambridge
- Hall, W. P. 1977. A Population Analysis of Two Species of Streamside Salamanders, Genus *Desmognathus*. *Herpetologica*. 33 (1): 109-113
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. and Jarvis A. 2004. The WorldClim interpolated global terrestrial climate surfaces. Version 1.3. Available at <http://www.biogeography.berkeley.edu/>.

- Horton, D.R. 1972. Lizard scale size and adaptation. *Syst. Zool.* 21, 441–443.
- Köhler, G. and Heimes, P. 2002. Stachelleguane. *Herpeton.* 175 p
- Lister, B.C. 1976. The nature of niche expansion in West Indian *Anolis* lizards II: evolutionary components. *Evolution* 30, 677–692.
- Malhotra, A. and Thorpe, R.S. 1997. Microgeographic variation in scalation of *Anolis oculatus* (Dominica, West Indies): a multivariate analysis. *Herpetol.* 53, 49-62.
- Mendez de la Cruz, F.R., Guillette, L.J., Jr, Villagran-Santa Cruz, M. & Casas-Andreu, G. 1988. Reproductive and fat body cycles of the viviparous lizard, *Sceloporus mucronatus* (Sauria: Iguanidae). *J. Herpetol.* 22: 1–12.
- Neilson, K.A. 2002. Evaporative water loss as a restriction on habitat use in endangered New Zealand endemic skinks. *J. Herpetol.* 36, 342–348.
- Reeder, T. W. 1995. Phylogenetic relationships among phrynosomatid lizards as inferred from mitochondrial ribosomal DNA sequences: Substitutional bias and the information content of transitions relative to transversions. *Molecular Phylogenetics and Evolution* 4: 203-222.
- Reeder, T. W., and J. J. Wiens. 1996. Evolution of the lizard family Phrynosomatidae as inferred from diverse types of data. *Herpetological Monographs* 10:43-84.
- Sites, J. w., Jr. W. Archie, C.J. Cole, and O Flores-Villela. 1992. A review of phylogenetic hypothesis for lizards of the genus *Sceloporus* (Phrynosomatidae): Implications for ecological and evolutionary studies. *Bull. Amer. Mus. Nat. Hist.* 213:1-110

- Smith E. N. 2001. Species boundaries and evolutionary patterns of speciation among the malachite lizards (formosus group) of the genus *Sceloporus* (Squamata: Phrynosomatidae). PhD. Dissertation. University of Texas at Arlington.
- Smith, H. M 1939. The Mexican and Central American lizards of the genus *Sceloporus*. Zool, Ser. Field Mus. Nat. Hist 26: 1-397
- Soule', M. and Kerfoot, W.C. 1972. On the climatic determination of scale size in a lizard. Syst.Zool. 21, 97–105.
- Thorpe, R.S. and Baez, M. 1987. Geographic-variation within an island – univariate and multivariate contouring of scalation, size, and shape of the lizard *Gallotia galloti*. Evolution 41, 256–268.
- Thorpe, R.S. and Baez, M. 1993. Geographic-variation in scalation of the lizard *Gallotia stehlini* within the island of Gran-Canaria. Biol. J. Linn. Soc. 48, 75–87.
- Tu, M. C., Lillywhite, H. B., Menon, J. G. and Menon, G. K. 2002. Postnatal ecdysis establishes the permeability barrier in snake skin: New insights into lipid barrier structures. J. Exp. Biol. 205, 3019-3030.
- Wiens, J. J., and T. W. Reeder. 1997. Phylogeny of the spiny lizards (*Sceloporus*) based on molecular and morphological evidence. Herpetological Monographs 11:1-101.

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