PHYLOGEOGRAPHY AND GEOGRAPHICAL VARIATION OF BEHAVIORAL

AND MORPHOLOGICAL CHARACTERISTICS IN

PARUROCTONUS BOREUS

by

ABRAHAM MILLER

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS AT ARLINGTON

August 2008

ACKNOWLEDGEMENTS

ABSTRACT

PHYLOGEOGRAPHY AND GEOGRAPHICAL VARIATION OF BEHAVIORAL

AND MORPHOLOGICAL CHARACTERISTICS IN

PARUROCTONUS BOREUS

Abraham Miller, PhD.

The University of Texas at Arlington, 2008

Supervising Professor: Daniel Formanowicz

Specimens of *Paruroctonus boreus* were collected from twenty-four sites to assess the geographic variation in behavioral, morphological and molecular traits. Statistical analyses support the observation that males are attracted towards females from the same population but not females from different populations, and that males avoided males from the same population. Pectine morphology was measured with SEM and demonstrated sexual dimorphism among and within populations. This difference was most apparent in mature individuals. Sequence data from mitochondrial 16S and COI genes suggest there is structure among the populations but this structure does not support a north and south clades or east and west clades.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

GENERAL INTRODUCTION

Scorpions are a moderately speciose group with approximately 1500 described species worldwide (Fet et al. 2000b). Scorpion diversity is highest between 20-40º latitude and they are found on all continents except Antarctica (Polis 1990a). Scorpion species have been recorded as far north as 55º latitude in the northern hemisphere (Polis 1990a). They are conserved morphologically as demonstrated by the minimal change in body appearance since the middle Silurian and are most likely derived from the Eurypterida, which are the extinct water scorpions (Kjellesvig-Waering 1966).

Extant scorpions are viviparous and the females demonstrate parental care for the young (Polis and Sissom 1990). Once the first instars are born they climb up the mother's legs onto her back where they stay until after their first molt (Polis and Sissom 1990). All scorpion species are venomous. In the United States, most species are found west of the Mississippi River and peak diversity is observed in the desert southwest (Polis 1990a, Fet et al. 2000a). Scorpions generally are nocturnal and active during the summer months once the ground has warmed up. During the winter they stay in their burrow or underneath rocks in a scrape (Warburg and Polis 1990). Until recently most species were seen infrequently as they rarely leave their burrow during the day. Scorpions fluoresce underneath UV light, which has made collection of burrowing species more achievable (Stahnke 1972).

Mating is highly ritualized in scorpions and appears to be conserved as the mating sequence is similar across taxa (Polis and Sissom 1990). Typically, males search for the females and begin courtship behavior with juddering in the nearby vicinity (Alexander 1956, Polis and Farley 1979, Polis and Sissom 1990). Juddering is rapid quivering movement by the male that creates vibrations in the substrate and functions as a sex or species recognition

1

behavior so the female is stimulated to cooperate in mating behavior (Polis and Sissom 1990). The male grasps the palps of the female and leads her through an elaborate mating dance (Polis and Farley 1979, Polis and Sissom 1990). Once the female is subdued, the male locates a suitable place to deposit his spermatophore. Once the spermatophore is cemented to the substrate, the male pulls the female over the spermatophore, which inserts into her genital operculum and releases his sperm (Polis and Farley 1979, Polis and Sissom 1990).

Scorpions are predacious and employ a sit-and–wait strategy of foraging for prey (Shachak and Brand 1983, Polis 1990a). They have low metabolic rates, high biomass within desert communities and have the ability to conserve water so they can persist in the desert environment (Hadley 1990, Polis 2001). Their nocturnal and burrowing behavior helps them to conserve water. Scorpion activity is seasonal and only a small percent of adults are active on a given night (Tourtlotte 1974, Polis 1980a). It is unknown if scorpions maintain home ranges or territory, but individuals are seldom found in close proximity to each other or at high densities (Tourtlotte 1974, Polis 1980a). This is likely caused by the cannibalistic behavior of scorpions (Polis 1980b, Polis 1981, Polis and Sissom 1990).

With the exception of some medically important species, little is known about the distributions of scorpion species. Most studies have focused their research at one site and made little attempt to sample across the distribution of their study organism. Few distribution maps exist for scorpion species and they are usually underrepresented by sites. Museums have far fewer specimens of scorpions than they do insects or vertebrates (personal observation), so it is difficult to produce maps with many locations.

Herein, I investigated the distribution of *Paruroctonus boreus* in the western US, and some associated variation in behavioral responses, morphology and mitochondrial DNA. This species is one of the most widespread scorpion species in North America, it inhabits a geographically diverse region, and little is known about its biology.

2

CHAPTER 2

DISTRIBUTION OF *PARUROCTONUS BOREUS*

<u>2.1 Introduction</u>

Biogeography is the study of spatial patterns of biodiversity past and present, and the related patterns of variation we see (Brown and Lomolino 1998b). The widespread availability of molecular analysis has brought about a related field of study known as phylogeogarphy. Phylogeography deals with the phylogenetic components of spatial distributions of gene lineages, usually from closely related species or populations (Avise 2001). Both biogeographers and phylogeographers attempt to explain the underlying processes and patterns of distribution in extant and extinct organisms. Investigations can focus on the distribution of a particular genus of organism, such as the spider genus *Homalonychus* (Crews and Hedin 2006), or it may focus on the distribution of a single species, like the scorpion *Paruroctonus mesaensis* (Yamashita and Fet 2001). Fundamental to all cases is establishing a range map for the species of interest (Brown and Lomolino 1998a). These maps are useful for other scientists to carry out biogeographical studies. These maps represent locations where the species has been collected and areas where they likely occur. In theory, the areas where they are likely to occur but have not been collected represent the realized niche of the species albeit a rough representation of the organism's niche. In 1957 Hutchison defined realized niche as the multidimensional space of environmental variables that a species inhabits after its interactions with other species (Vandermeer 1972). Map boundaries are thought to represent the areas where one or more factors are actively limiting the distribution of the species. These factors may be abiotic, such as temperature, or biotic through predation or competition. To date, there has not been a map created showing areas where *Paruroctonus boreus* is likely to occur.

Paruroctonus boreus Girard, 1854, has the most extensive latitudinal and elevational range of any North American species of scorpion, and yet, it is under-sampled across its range. *Paruroctonus boreus* is the most northerly occurring scorpion species in the western hemisphere with a distribution that extends from northern Arizona northward into southern Canada (Gertsch and Soleglad 1966, Tourtlotte 1974). Museum records indicate that its distribution may be limited by the Sierra Nevada and Cascade mountains in the west and to the east, it extends into the lower valleys of the Rocky Mountains. *Paruroctonus boreus* is unlikely distributed continuously throughout its range, since it does not occur at lower elevations in the southern extent of the range (personal observation). This may have led to isolated populations likely to have diverged behaviorally, morphologically and/or molecularly from each other. I hypothesize that the distribution of *P. boreus* is fragmented across its range and that southern populations are more isolated from each other by dispersal barriers.

2.2 Materials and Methods

Field collecting was done for approximately two hours each night, starting approximately one hour after sunset, to coincide with the peak abundance and activity of scorpions (Warburg and Polis 1990). Collections were made between May and August of 2003-2007. An effort was made to sample the populations described in the literature (Fig. 2.1) and to locate new populations of *Paruroctonus boreus*. Once collected, populations were georeferenced with a handheld GPS unit with a minimum accuracy of 10 meters. Twenty-four populations were sampled as shown in Figure 2.2. These were populations with known longitude and latitude originally reported in degrees, minutes and seconds. Each locality was then transformed into degree-decimal format so that they could be added to ArcMap. Names were given to each locality reflecting a nearby, designated place.

Map layers were obtained from www.worldclim.org to determine the altitude, annual precipitation and the extent of rain zone area of each site in the western US where the populations are found. The ecoregions were obtained from www.nationalatlas.gov along with

4

annual temperatures. The data were then added to ArcMap and spatially joined to each site using the spatial analyst tool within ArcMap.

In order to model the geographic distribution of *Paruroctonus boreus*, I used the MaxEnt program for maximum entropy modeling species' geographic distributions (Phillips et al. 2006). This program requires at least 20 localities to be considered accurate, therefore it was appropriate for this analysis. The generated map allows us to identify isolated populations and test hypotheses regarding the potential versus realized niche of the species. All climate data were obtained from www.worldclim.org. Environmental variables included in this analysis were annual mean temperature, mean diurnal temperature (Mean monthly Max-Min), max temperature in the warmest month, minimum temperature in the coldest month, annual precipitation, precipitation seasonality (coefficient of variation) and altitude.

2.3 Results

Individuals were collected from twenty-four populations across the range of *Paruroctonus boreus* (Fig. 2.2). Sampling at lower elevations around these sites yielded no specimens of *P. boreus*, instead, other scorpion species like *Vaejovis confusus* were collected. Scorpions were not found at higher elevations than *P. boreus* across its distribution. Four of the twenty-two populations were found in sympatry with other species. The Mt. Charleston (5) and Wendover (22) populations were found in sympatry with *Anuroctonus phaiodactylus* and *Vaejovis confusus*. The Flat Tire (3) and Bruneau Sands (6) populations were found in sympatry with *Hadrurus spadix* and *V. confusus*. *Vaejovis confusus* is a similarly sized species within the family *Vaejovidae* and is known from Arizona, California, Idaho, Nevada and Utah (Sissom 2000). *Hadrurus spadix* is a large (many times larger than the other species) species recorded from Arizona, California, Colorado, Idaho, Nevada, Oregon and Utah (Sissom and Fet 2000). *Anuroctonus phaiodactylus* has very robust palps and is the second largest species found and is known from California, Nevada and Utah (Sissom and Fet 2000). The latter two species were collected far less frequently than either *P. boreus* or V. confusus.

5

Approximately equal numbers of *Paruroctonus boreus* and *Vaejovis confusus* were collected at the Wendover, Flat Tire and Bruneau Sands populations. However, the species' apparent abundances varied greatly at the Mt. Charleston population. Mt. Charleston is a fairly steep mountain that rises from the desert floor outside of Las Vegas, NV. The desert floor has an approximate elevation of 500 m. I collected along an elevational transect at this location and found *P. boreus* between 1925 - 2265 m elevation. *Paruroctonus boreus* and *A. phaiodactylus* are found at the highest elevation with *V. confusus* never being found above 1965 meters in elevation. Between 1925 - 1965 m in elevation, all three species occur in sympatry. In the area with all three species *P. boreus* becomes less common and *V. confusus* becomes the dominant species as you move down in elevation until only *V. confusus* and *A. phaiodactylus* are found.

The elevational distribution of *Paruroctonus boreus* is negatively correlated with latitude (p <0.001, Fig. 2.3) but not correlated with longitude (p > 0.1, Fig. 2.4). Their distribution is not associated with eco regions (not shown) and temperatures are correlated with precipitation.

The resulting map from the MaxEnt program is shown in Figure 2.5. It suggests that *Paruroctonus boreus* should be very widespread and across its distribution with few dispersal barriers. Annual precipitation and precipitation seasonality contributed 46% of the explained variation in the model. Mean annual temperature contributed 36% of the variation and altitude contributed 10% of the variation in the model. The other variables accounted for less than 5% of the variation combined. The map does not give resolution at a fine enough scale that would be useful in identifying suitable habitats for populations like Mt. Charleston (5), which appear to have restricted distributions.

In ArcMap, I created a map showing areas that receive precipitation amounts of 10 - 20 inches per year (Fig. 2.6). This better represents the likely distribution of *Paruroctonus boreus* when including sampled localities, where *P. boreus* was not found. This map shows areas of suitable habitat surrounded by areas with unsuitable habitat, suggesting they are isolated habitat patches that may contain equally isolated populations of *P. boreus*. Figure 2.7 shows an up close view of Mt. Charleston and the associated areas which receive 10 - 20

6

inches of precipitation a year. In this location, *P. boreus* is only found in the areas that receive

17.5 in/yr as determined by the elevational transect.
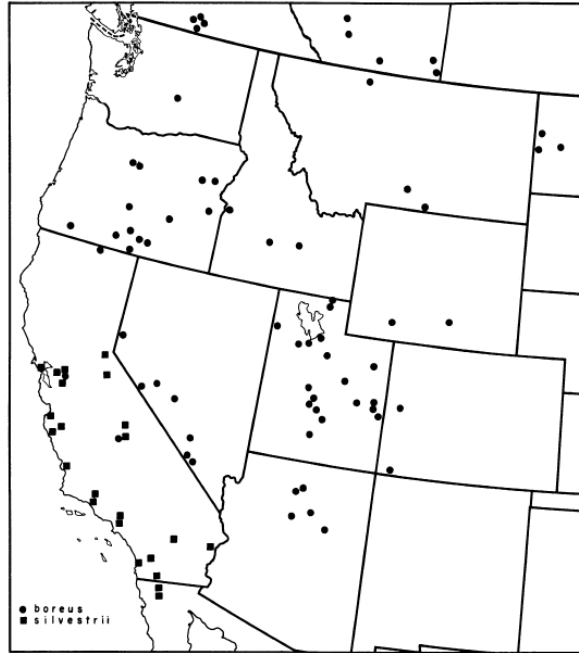
Fɪɢ. 1. Distribution map of *Vejovis boreus* and *silvestrii.*

Fig. 2.1. Map from Gertsch and Soleglad (1966) showing all known localities of Vejovis boreus (now Paruroctonus boreus) and Vejovis silvestrii (now Paruroctonus silvestrii).
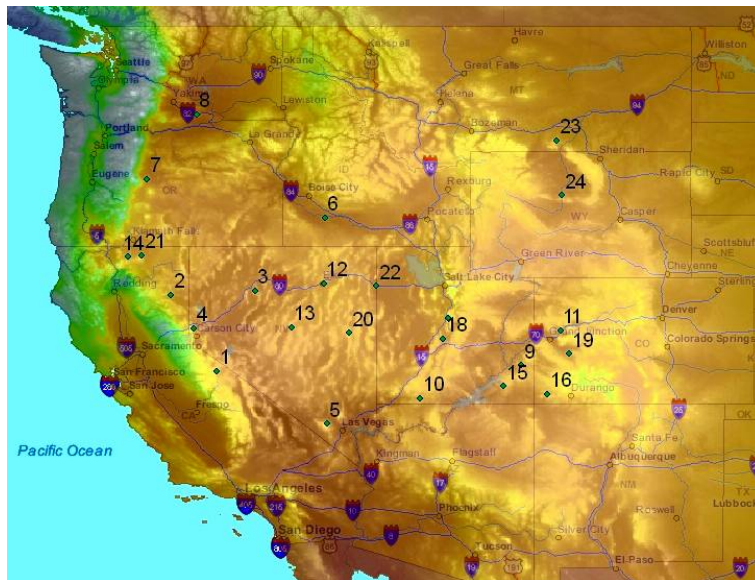
Fig. 2.2. Map showing locations of new populations collected between 2003-2007 that were used in this investigation.

Elevation of Populations as a Function Latitude

R² = 0.8795
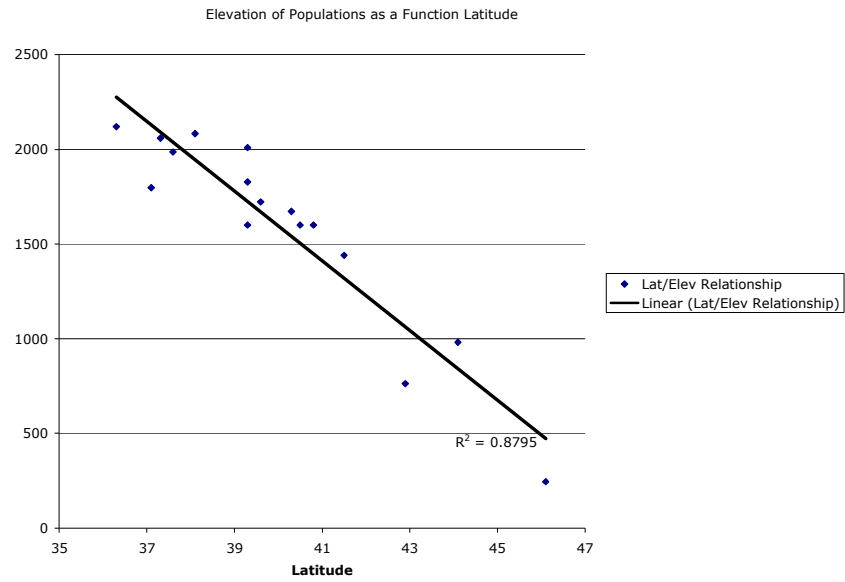
Fig. 2.3. *Paruroctonus boreus* altitudinal distribution is significantly correlated with latitude in the western United States (p < 0.01).
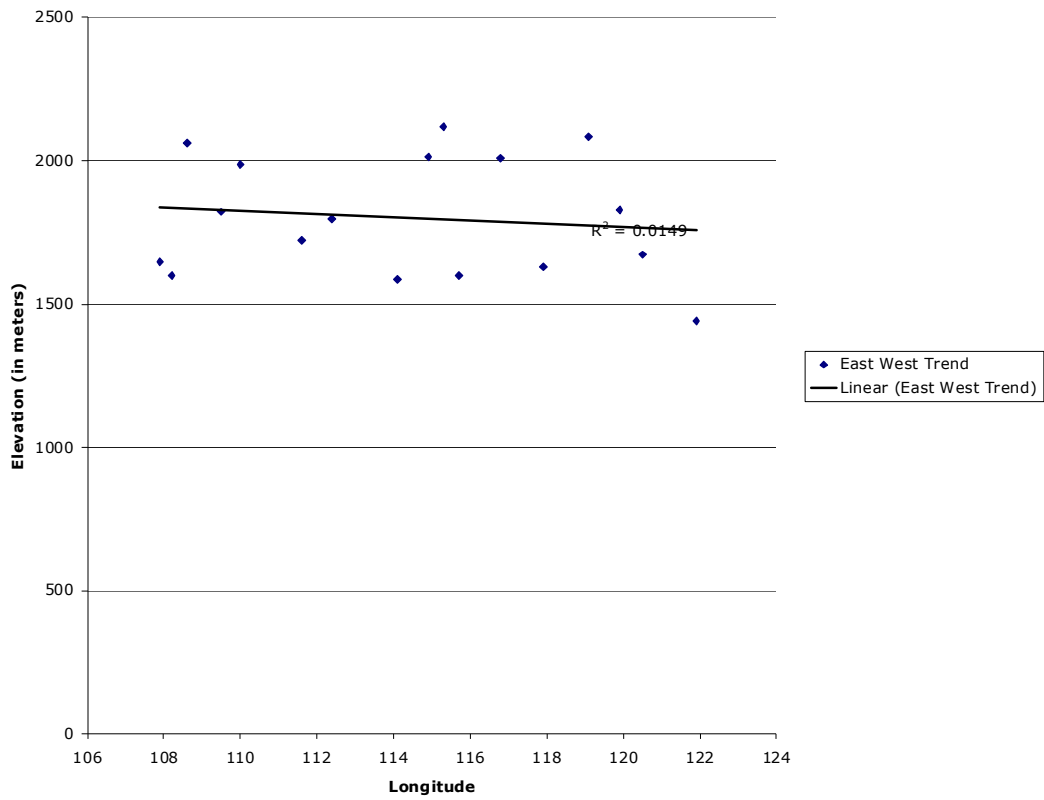
Fig. 2.4. *Paruroctonus boreus* altitudinal distribution is not significantly correlated with longitude in the western United States (p < 0.01).
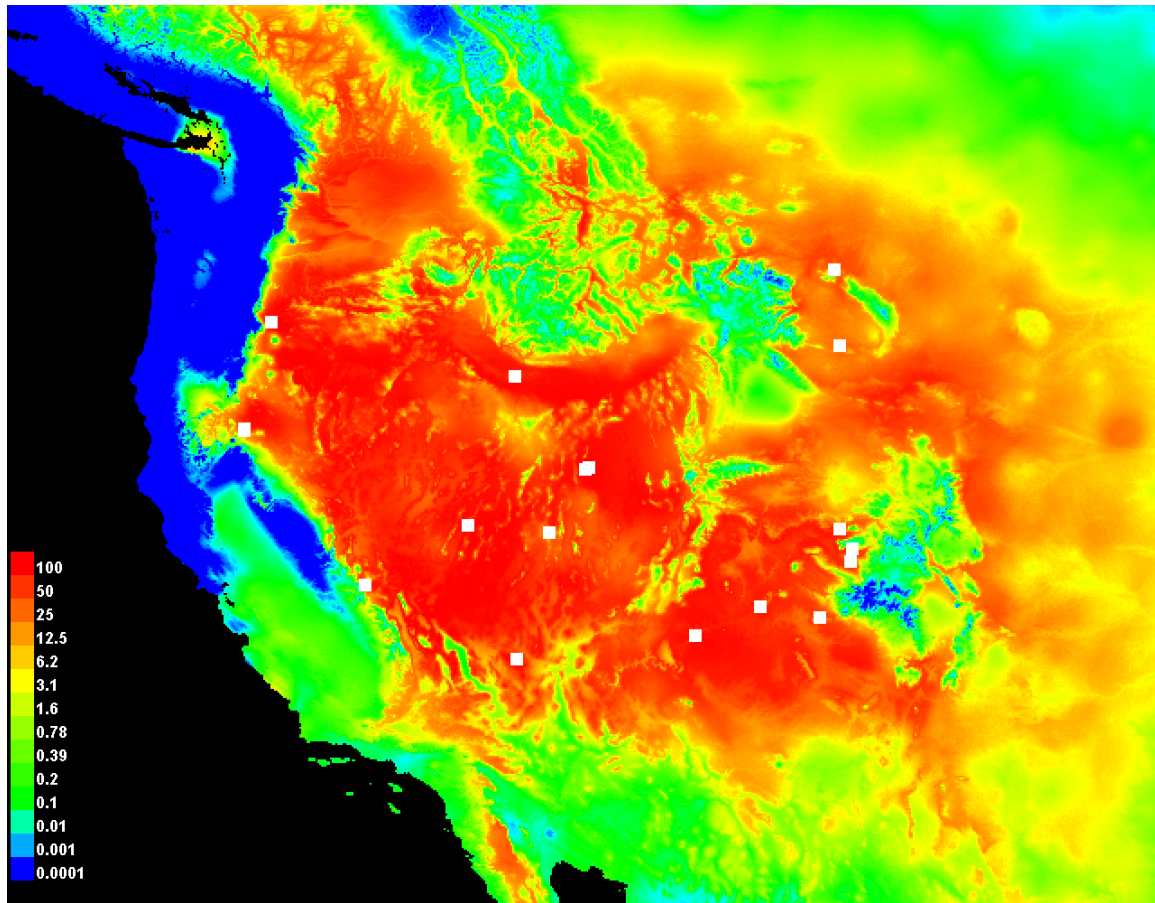
Fig. 2.5. Map showing the results of the maximum entropy modeling species' geographic distributions (Phillips et al. 2006) for *Paruroctonus boreus* in the western US. Areas in red represent areas with favorable habitat based on modeling climatic variables. Squares represent localities used in building the model.

Fig. 2.6. Map of the western US showing areas that receive between 10 - 20in of precipitation a year. The red dots represent localities of Paruroctonus boreus. This is an example of the output generated by GIS that can be used as a layer in building niche models.

Fig. 2.7. Isolated population (5) of *Paruroctonus boreus* at Mt. Charleston, outside of Las Vegas, NV. The population is to areas that receiving 17.5 in precipitation per year. The surrounding areas at lower elevation receive less rainfall, acting a dispersal barrier. This isolation is characteristic of southern populations of *P. boreus*.

2.4 Discussion

Paruroctonus boreus inhabits patches of suitable habitat in the western US, which occur at lower elevations as latitude increases. There is not a similar east to west pattern. This elevational cline of *P. boreus* causes southern populations to be more isolated from each other than northern populations. This is best demonstrated in Figure 2.7 by the Mt. Charleston (5) population, which is surrounded by what is presumed to be unfavorable habitat. The relatively small area in which *P. boreus* can be found at this location can promote divergence due to a lack of gene flow. This population may also serve as an environmental indicator as human urban construction has increased in the area in the last five years. Furthermore, scientists may be able to monitor the elevational distribution of this population to track climatic change over time.

The map generated using the MaxEnt program, overestimates the distribution of *Paruroctonus boreus* by a large margin. Perhaps biotic interactions are the most important factors defining and further restricting to the distribution of *P. boreus*. However, it is just as likely that the program does not work as well at this scale in an area, like the western US, which is characterized by steep elevational changes. These steep changes in elevation influence most other abiotic factors at a fine scale, but these are not reflected in a broad-scale analysis. Based on the sampled distribution of this species, the map showing areas that receive between 10-20 in/yr of precipitation, likely represents areas of suitable habitat (Fig. 2.6). Both maps suggest there are areas of unsuitable habitat in between sampled localities. Areas of unsuitable habitat may act as dispersal barriers for *P. boreus* reducing gene low among populations.

CHAPTER 3

BEHAVIORAL RESPONSE OF *PARUROCTONUS BOREUS* TO SYMPATRIC
AND ALLOPATRIC CONSPECIFICS

3.1 Introduction

Much attention has been given to pheromone signaling in insects (Kaissling 1997, Wyatt 2003), however, fewer studies have addressed the possible importance of such signaling in arachnids (Sonenshine 1985, Gaskett 2007). Among those studies, the majority have been with spiders and pheromones associated with the silk of conspecifics (Foelix 1982b, Gaskett 2007). It is thought that pheromones are responsible for directing movement and eliciting courtship behavior in spiders (Foelix 1982b, Roberts and Uetz 2004) and mites (Sonenshine 1985).

Species that demonstrate no postzygotic or morphological isolating mechanisms and only behavioral prezygotic isolating mechanisms, have been termed ethological species or simply "ethospecies" (Uetz and Denterlein 1979, Roberts and Uetz 2004). These species may exhibit some morphological variation but not with regard to the genitalia and not enough to reproductively isolate the species. Much of the arachnid behavioral research on reproductive isolation has used spiders, most often members of the family Lycosidae (Foelix 1982a, Ayyagari and Tietjen 1986, Persons et al. 2001). However, scorpions differ from spiders, especially in their poor vision and lack of silk glands, and less is known about the behavioral mechanisms that may be involved with reproduction or reproductive isolation.

The eyes of scorpions are sensitive to changes in light (Fleissner 1977, Fleissner and Fleissner 2001) but lack the visual acuity of many insects and spiders (Locket 2001). Consequently, scorpions must rely on tactile localization (Brownell 1977, Brownell and Farley 1979) and possibly chemical cues to explore their environment (Gaffin and Brownell 1992). Subsequently, unlike many spiders, scorpions cannot visually communicate with potential mates

to signal their interest (Locket 2001). Scorpions respond to vibrations in the substrate but usually only those within a meter (Brownell 1977, Brownell and Farley 1979). Scorpions rarely reach population densities that would render vibrational cues useful (Polis 1990a) for attracting mates and it seems unlikely that males rely on random walks to find mates. For this reason, this chapter will focus on the behavioral responses of male scorpions to chemical cues (pheromones) deposited by conspecifics on the substrate.

The use of chemical communication by scorpions has only been investigated a few times (Gaffin and Brownell 1992, Gaffin and Brownell 2001, Melville et al. 2003, Steinmetz et al. 2004) and the functional molecules have not been isolated. Gaffin and Brownell (1992) and Mellville *et al.* (2007) found that males exhibited positive responses to areas that had been exposed to female conspecifics. However, males did not demonstrate a preference in the third study, although the authors suggested that males changed their behavior in areas previously exposed to female conspecifics. Additionally, (Melville et al. 2003) examined the response of males to conspecific males and found that they exhibited a non-significant preference for areas exposed to conspecific males over the control areas.

The pectines and their associated peg sensilla are thought to be responsible for sensing substrate-borne chemical cues, such as pheromones, in scorpions (Brownell 2001). Pairs of pectines are located on the ventral side of all scorpions and are actively moved across the substrate. The pectines have multiple teeth at their distal ends, which usually are sexually dimorphic and species specific. The tips of their teeth contain many peg sensilla, which are the functional chemosensory structures in scorpions (Gaffin and Brownell 1997, Gaffin and Brownell 2001). These structures have been suggested to be responsible for locating mates and prey (Krapf 1986, Gaffin and Brownell 2001), although the detection of prey remains less certain. Males actively sweep their pectines across the substrate when the substrate has been exposed to conspecific females (personal observation), (Gaffin and Brownell 2001) and usually exhibit an increase in sweeping during pre-mating behaviors (Polis and Sissom 1990, Gaffin and Brownell 1992). (Gaffin and Brownell 2001) demonstrated that experimental removal of the pectines

14

resulted in males that do not respond to the substrate exposed to conspecific females, supporting the idea that pectines are actively involved in substrate borne chemoreception.

Investigations into pheromonal detection in scorpions are still in their infancy. All previous studies have examined the pheromonal response to conspecifics from the same locality (population) and responses were not universal (Gaffin and Brownell 1992, Melville et al. 2003, Steinmetz et al. 2004). To date, these studies have only tested responses of three species of scorpions from North America; *Paruroctonus mesaensis* (family Vaejovidae), *Hadrurus arizonensis* (family Iuridae) and *Centruroides vittatus* (family Buthidae) (Gaffin and Brownell 1992, Melville et al. 2003, Steinmetz et al. 2004). The former two species are more closely related to each other than the latter (Stockwell 1992) and demonstrate similar responses to female conspecifics. This might suggest pheromonal response to conspecific females is a familial attribute. However, more investigations need to be done to assess pheromonal response across different scorpion species and different scorpion families to assess if this is a familial attribute. (Melville et al. 2003) is the only study to date to look at the pheromonal response of males to conspecific males. There has not been a published investigation of variation in pheromonal responses among populations within a species of scorpion.

The scorpion *Paruroctonus boreus* has a disjunct distribution (Chapter 2), which can be a precursor to local adaptation and speciation among populations. Local adaptation to the environment might be expected and can be tested with various physiological and behavioral techniques (ref) but identifying speciation would require far more genetic research. Alternatively, I could test speciation by mating individuals from different populations, and assessing the viability or fertility of their offspring. However, the behavior among populations in the field may actually preclude reproduction between populations that artificially reproduce in the lab (Uetz and Denterlein 1979). This is the first study that investigated the response of male scorpions to same population and different population females. I also investigated the response of male *P. boreus* to same population males, to determine if response to chemical cues was sex-specific. I hypothesized; (1) males would positively respond to females from the same

15

population but not respond to females from other populations and, (2) males would not respond to other males from the same population.

<center>3.2 Materials and Methods</center>

3.2.1 General Collecting and Housing

Field collecting was done for approximately two hours each night, starting approximately one hour after sunset, to coincide with the peak abundance and activity of scorpions (Warburg and Polis 1990). Collections were made between May and August of 2003 - 2007 and localities are shown in Chapter 2 Figure 2.1. Scorpions were contained in the field in small plastic vials within a cooler. Scorpions were transferred to large plastic deli cups (diameter 110 mm, 60 mm depth) in the lab located in the basement at the University of Texas at Arlington. Each container had 1 cm of sandy soil and scorpions were given water weekly and fed every 2 - 3 weeks. Scorpions were housed at 20 - 25°C on a reverse light cycle (16 hrs light, 8 hrs dark) so behavior experiments could be carried out during the day.

3.2.2 Behavioral Responses

Collected animals were measured and sexed according to (Haradon 1985), and only animals judged to be adults of reproductive age were used in the behavioral experiments. Four experimental Y-mazes were constructed based on (Melville et al. 2003). The mazes consisted of a rectangular acclimation area that was divided from the stimulus and control arms by a removable plastic divider. The Y-mazes (Fig. 3.1) measured 145 mm (base width), by 250 mm (base length), by 80 mm (arm width), by 250 mm (arm length) by 100 mm (depth). The mazes were cleaned with a 20% bleach water solution between each trial and lined with new filter paper before each trial to remove any pheromonal traces from previous trials. Sand substrate was not used because of the difficulty in cleaning the sand between trials. The control arm of the Y-maze was lined with new filter paper and was left untreated. The stimulus arm of the Y-maze was lined with new filter paper, like the control side, and a stimulus animal was confined to that arm by a plastic divider. Stimulus animals were the animals assigned to one of the arms as the treatment. The selection of the arm to be used for the stimulus treatment was

<center>16</center>

randomized at a 50% frequency between the two arms. Test animals in this experiment were the individuals housed and confined to the acclimation area by a plastic divider, then allowed to move freely in the Y-maze during the experiment and for which data were gathered. Test and stimulus animals were placed in the mazes 24 hours before the experiments were run. A black light was placed directly overhead to avoid shadows and was turned on when the white lights were turned off. The experiments were started approximately 30 minutes thereafter. Half the mazes were oriented in a westerly direction, half in an easterly direction in the basement at the University of Texas at Arlington Biology Department. The stimulus animals were removed just prior to the start of the experiment, which was followed by the removal of the plastic dividers. The test subject was then allowed to move freely without interruption for two hours, to coincide with the peak activity of scorpions in the field (Hadley and Williams 1968, Polis 1980a, Warburg and Polis 1990). The data collected were total time spent in each area (acclimation area, control arm and stimulus arm) of the Y-maze.

All four experiments were run simultaneously and recorded in real time using a stopwatch. Animals were randomly assigned to be stimulus or test animals. Ten different populations were represented in the sympatric male-female response test, eight populations in the allopatric male-female response test, and five populations were represented in the sympatric male-male response test (Table 3.1). Once they had been used as test animals, they were not used again as test animals for the same experiment. Animals used in the experiments had carapace lengths greater than 4.3mm for males and greater than 4.5mm for females. These lengths were used to insure the animals were of reproductive size. The data were not normally distributed so a Wilcoxon's signed-ranks test, a nonparametric equivalent to the paired t-test, was performed (Sokal and Rohlf 1995). A Wilcoxon's signed-ranks test was applied on the total time spent in the control and stimulus arms, and the alpha value was set at 0.05.

3.3 Results

Males spent significantly more time in the stimulus side of the Y-maze than the control side when the stimulus was a female from the same population ($n = 28$; p = 0.034; Fig. 3.2, Table 3.2). Males on average spent 51 minutes in the treatment arm, 34 minutes in the control arm and 35 minutes in the acclimation area. Some of the males were observed juddering and swaying, indicating a pre-mating behavioral response (Polis and Farley 1979, Polis and Sissom 1990), in the stimulus arm during the trials with same population females, but never in the control arm. Males did not demonstrate a significant preference for either arm of the Y-maze when the stimulus was an allopatric female ($n = 20$; p = 0.7; Fig. 3.2, Table 3.2). Males demonstrated significant avoidance of areas exposed to other males from the same population ($n = 12$; p = 0.03; Fig. 3.3, Table 3.2). On average 21 minutes were spent in the treatment arm, 57 minutes in the control arm and 41 minutes in the acclimation area.

Table 3.1. A list of the different populations of males and females used in the tests of male behavioral response to chemical cues from sympatric females, allopatric females and sympatric males. Males in the left columns were the test animals and individuals in the right columns were the stimulus animals.

Sympatric		Allopatric		Sympatric	
Male	**Female**	**Male**	**Female**	**Male**	**Male**
Pop 1	Pop 1	Pop 1	Pop 4	Pop 4	Pop 4
Pop 2	Pop 2	Pop 1	Pop 9	Pop 4	Pop 4
Pop 3	Pop 3	Pop 2	Pop 4	Pop 4	Pop 4
Pop 4	Pop 4	Pop 4	Pop 7	Pop 4	Pop 4
Pop 4	Pop 4	Pop 4	Pop 5	Pop 4	Pop 4
Pop 4	Pop 4	Pop 4	Pop 5	Pop 4	Pop 4
Pop 4	Pop 4	Pop 4	Pop 6	Pop 6	Pop 6
Pop 4	Pop 4	Pop 4	Pop 10	Pop 6	Pop 6
Pop 4	Pop 4	Pop 4	Pop 1	Pop 10	Pop 10
Pop 5	Pop 5	Pop 6	Pop 12	Pop 10	Pop 10
Pop 6	Pop 6	Pop 6	Pop 1	Pop 12	Pop 12
Pop 6	Pop 6	Pop 10	Pop 13	Pop 15	Pop 15
Pop 6	Pop 6	Pop 10	Pop 2		
Pop 6	Pop 6	Pop 12	Pop 4		
Pop 7	Pop 7	Pop 12	Pop 6		
Pop 7	Pop 7	Pop 13	Pop 1		
Pop 10	Pop 10	Pop 13	Pop 12		
Pop 10	Pop 10	Pop 13	Pop 12		
Pop 10	Pop 10	Pop 13	Pop 2		
Pop 10	Pop 10	Pop 15	Pop 7		
Pop 10	Pop 10				
Pop 11	Pop 11				
Pop 11	Pop 11				
Pop 11	Pop 11				
Pop 12	Pop 12				
Pop 12	Pop 12				
Pop 12	Pop 12				
Pop 12	Pop 12				
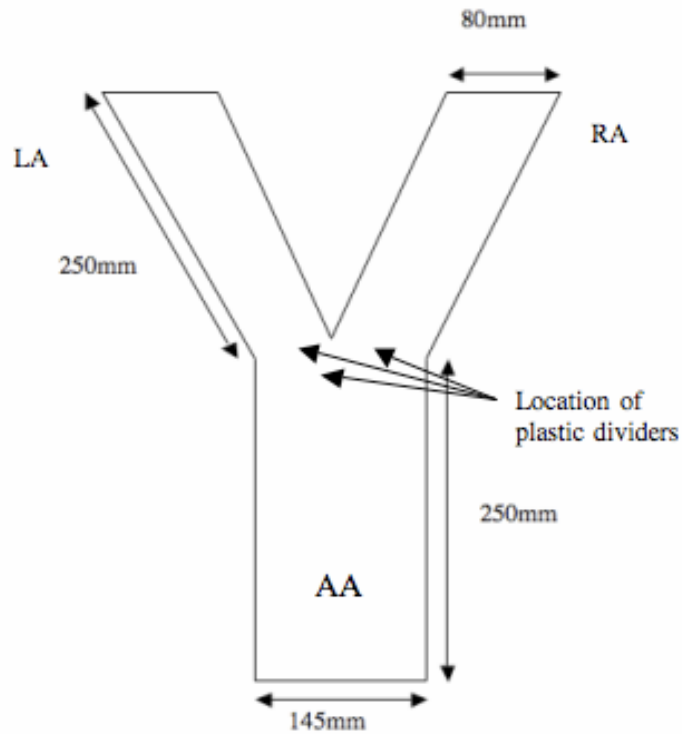
Fig. 3.1 Y-maze consisted of an acclimation area (AA) for the test subject, the left arm (LA) and the right arm(RA). The arrows indicate the dimensions of the arena and location of the plastic dividers. The height/depth of the maze was 100 mm.

Table 3.2. Male *Paruroctonus boreus* demonstrated positive responses to areas exposed to sympatric females, actively avoided areas exposed to sympatric males and demonstrated no responses when exposed to allopatric females. Data reported are mean time spent in minutes (+/- standard error) in each arm of the Y-maze.

Experiment	Control	Stimulus	P-value
Male-Female Sympatric	34.23 (4.3)	51.4 (4.3)	**P = 0.034**
Male-Female Allopatric	39.75 (5.5)	39.1 (5.95)	P = 0.526
Male-Male Sympatric	57.5 (8.43)	21.38 (7.88)	**P = 0.028**
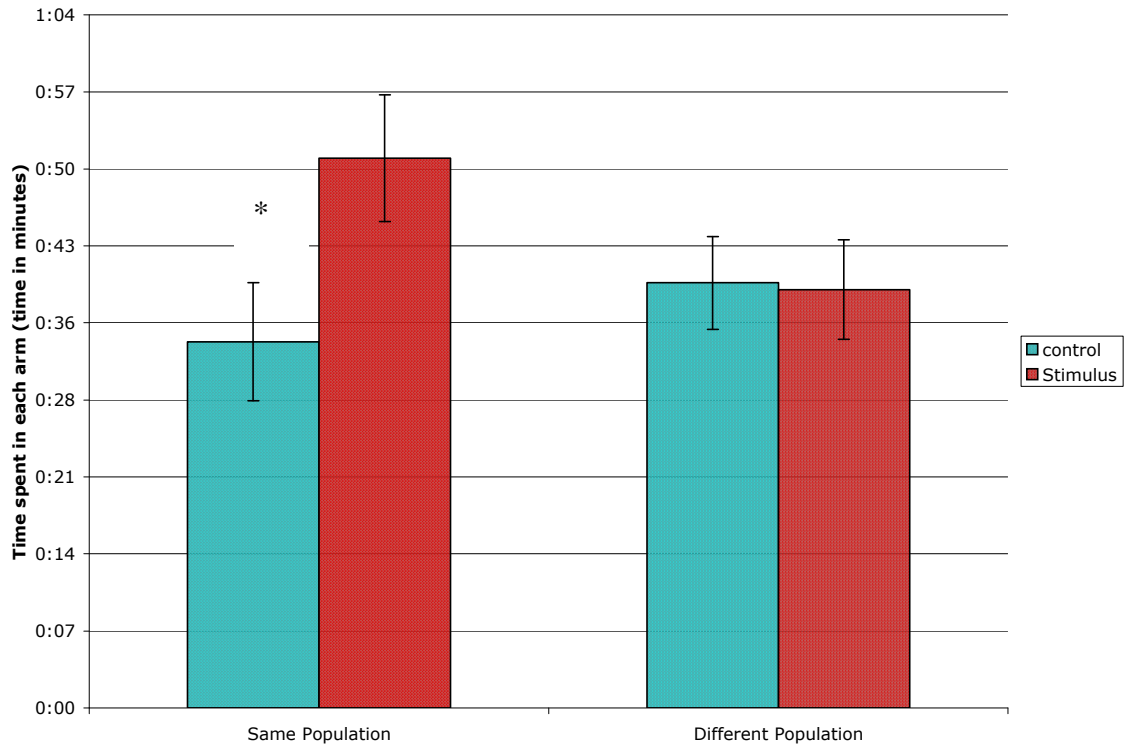
Fig. 3.2. Mean (+/- s. e.) time spent in the arms of the Y-maze by males when the treatment arm was exposed to females from the same population and females from different, allopatric populations. The asterisk indicates a significant difference between the time spent in the control and treatment arms (p = 0.03) and bars represent +/- 1 standard error.
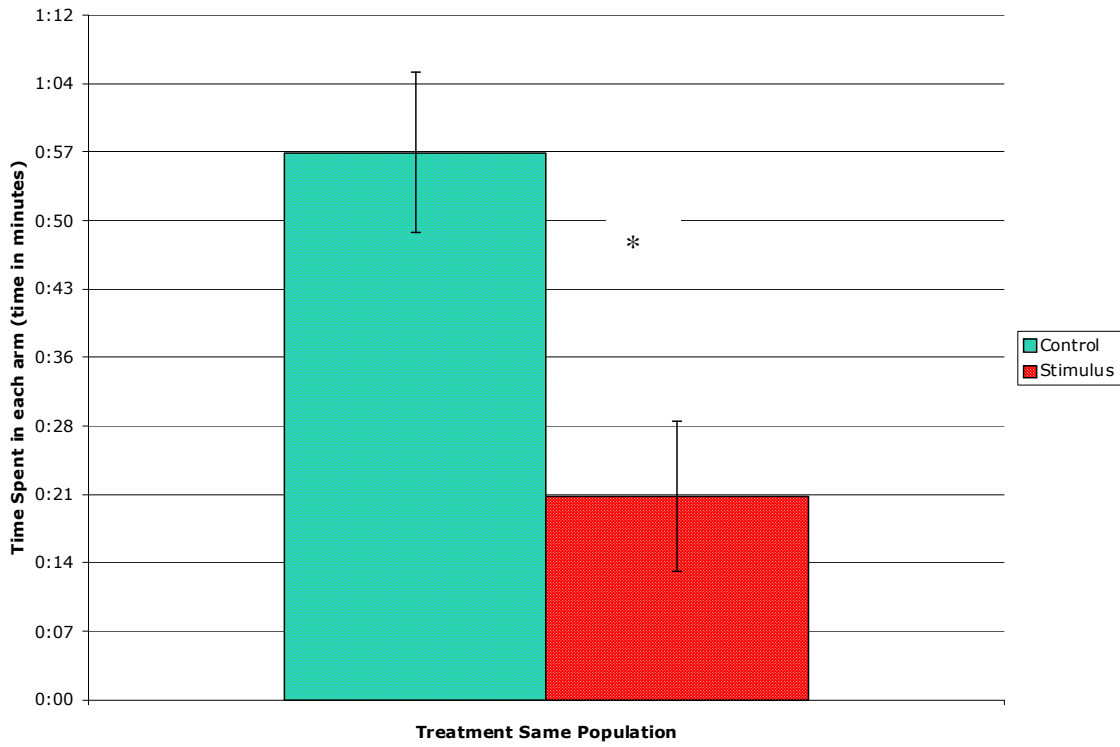
Fig. 3.3. Mean (+/- s. e.) male time spent in the arms of the Y-maze when the treatment arm was exposed to males from the same population. The asterisk indicates a significant difference between the time spent in the control and treatment arms (p = 0.03) and bars represent +/- 1 standard error.

Mate recognition would be beneficial for non-communal animals such as scorpions, since they rarely occur within close proximity. Males are usually the more vagile sex and are thought to actively search for females during the reproductive season (Polis and Farley 1979). The use of pheromones would likely increase the reproductive success of both sexes by increasing the number of encounters between the sexes. This study provides evidence that males are attracted to the pheromones of females from the same population. Considering males respond to female pheromones, and this likely increases their reproductive success, I expect strong selection pressure on the ability for males to recognize females. Selection pressure over time could create populations that could not recognize mates from other populations by chemical cues alone. These findings suggest that this has occurred in *Paruroctonus boreus*. I did not investigate the mating success rates between individuals from different populations, so I cannot determine if populations have already become reproductively isolated. However, I speculate that males and females from different populations maintain compatibility, as I have not observed any other isolating mechanisms but males would be less successful at locating and mating with females from other populations. Consequently, isolated or geographically distant populations (Chapter 2, Fig. 2.1) of *P. boreus* may become reproductively isolated populations due to reduced gene flow.

Just as mate recognition is beneficial for reproduction, it may also be beneficial for survival and competition. Outside of the reproductive season, male scorpions rely on a sit-and-wait strategy to obtain food (Polis 1990b). However, during the mating season, male *Paruroctonus boreus* can be found actively walking in the open (personal observation). Similarly, (Polis and Farley 1979) observed that male *Paruroctonus mesaensis* become more vagile during the mating season and suggested this was to search for mates. Males in this study positively responded to the pheromones of females, suggesting that they follow chemical trails in the field to find mates. This would increase encounters with potential mates during the

23

breeding season, especially if densities are comparable to those of *Paruroctonus utahensis*, a closely related species estimated to have densities of 0.06 - 0.27 individuals per square meter (Polis 1990a). An explanation for why males avoid areas exposed to conspecific males would be that they avoid the negative cost associated with an antagonistic interaction. It is plausible that multiple males are following the same female trail, which would likely end in an aggressive encounter between the males. Male-male encounters in *P. boreus* can be very aggressive (personal observation) and (Polis and Farley 1979) found that 9% of the diet of *P. mesaensis* were conspecifics. Consequently, intraspecific encounters within this genus may be associated with a high cost. An attribute that would allow males to increase the number of encounters with potential mates, while reducing the number of intrasexual encounters, would be favored. I suggest male *P. boreus* actively avoid males from the same population to reduce the cost associated with male-male interaction. Alternatively, males may avoid areas that have previously been exposed to males because females may be less likely to mate if they have previously been mated. Future research will investigate the response of males to allopatric males.

CHAPTER 4

MORPHOLOGICAL VARIATION IN STRUCTURES ASSOCIATED WITH THE
REPRODUCTIVE ECOLOGY OF *PARUROCTONUS BOREUS*

4.1 Introduction

Investigations of morphological variation in scorpions has primarily focused on adult size and color variants across a given geographical area or the number of pectinal teeth (Brown 1996, Myers 2001). Few studies have looked at characteristics beyond this. It has been suggested that observed variation is genetic on the basis of common rearing experiments between populations (Myers 2001). However, except for systematic investigations, few have looked at intraspecific variability in scorpions.

Systematic studies on scorpions have relied extensively on the chelicerae, trichobothria, coxosternal region, leg spination, telson and reproductive structures (Sissom 1990). In the family Vaejovidae, Stahnke (1973) primarily used cheliceral and trichobothrial patterns to differentiate genera. However, these patterns can rarely be used alone to distinguish relationships at the species level (Sissom and Lourenco 1987) and should be used with caution to look at relationships (Francke 1981). Some closely related, but distinctly different species, exhibit the same trichobothrial patterns (Francke 1981) and the number and location is often fixed within a species (Francke and Jones 1982). For this reason, scorpion species are delimited by size ratios, coloration, or variability in internal and external characters, such as metasomal crenulation or reproductive structures (Gertsch and Soleglad 1966, Haradon 1984a, 1985, Sissom and Lourenco 1987, Sissom 1990).

Few morphological studies have been done on *Paruroctonus boreus*, and those studies have admittedly been inexact and a bit random (Gertsch and Soleglad 1966). Distinguishing characters tend to be qualitative and difficult to discern without large samples of specimens to examine. In the key to *Paruroctonus* species, Gertsch and Soleglad (1966) distinguished *P.*

boreus from *P. bantai* by the amount of metasonal granulation. They identified *P. bantai* as having distinct keels that are irregularly granulate and *P. boreus* as having essentially obselete or non-granulate keels. This distinction is somewhat subjective and specimens were used from a restricted geographical area (Haradon 1985). Haradon (1985) described the inter- and intrapopulation variability within *P. boreus* to be considerable and that some of the characteristic traits, such as fucous pattern, have been essentially lost in some populations. To date, no further investigations of this variation have been done in *P. boreus*.

In order to quantitatively investigate interpopulation and intersexual variability, novel characters were chosen that I believe experience strong selection pressure based on their function (Chapter 3). The characters chosen were carapace length as an overall measure of size, 5th metasomal length, pectine tooth tip length, pectine tooth tip width and associated peg sensilla density. These characters have been associated with the reproductive and behavioral ecology of scorpions (Polis and Farley 1979, Gaffin and Brownell 1992, Gaffin and Brownell 1997, Tallarovic et al. 2000, Gaffin and Brownell 2001), yet of those chosen for this study, only the 5th metasomal segment and number of pectinal teeth have been used to investigate inter- and intraspecific variation in scorpions (Francke and Jones 1982, Sissom and Lourenco 1987, Brown 1996).

The 5th metasomal segment is the terminal, pre-anal segment, of the opisthosoma in scorpions (Hjelle 1990). This segment demonstrates sexual dimorphism in multiple species, with males having longer segments (Francke and Jones 1982, Brown 1996). During courtship, the male often grabs the female's 5th metasomal segment to subdue her and males use their metasoma to club the female during the courtship process (Polis and Sissom 1990, Tallarovic et al. 2000). The 5th metasomal segment may also be involved in male-male interaction as males will try to grab their opponents 5th segment in a battle (Formanowicz personal communication).

The pectines of scorpions are comblike structures located on the ventral side of the prosoma, which function as mechano- and chemoreceptors (Hjelle 1990, Gaffin and Brownell

1997, Gaffin and Brownell 2001). Each pecten has multiple teeth, the number of which is fixed at birth and often species and sex specific (Francke and Jones 1982). Males of the family Vaejovidae have a greater number of teeth than females and are used to distinguish the sexes (Gaffin and Brownell 2001). Each tooth contains many peg sensilla, the functional chemosensory structures of the pectines (Gaffin and Brownell 1997, Gaffin and Brownell 2001). These structures are swept across the substrate during courtship and are important to scorpions in finding mates (Gaffin and Brownell 1992, Melville et al. 2003) and identifying suitable substrate for spermatophore deposition (Hjelle 1990, Polis and Sissom 1990, Tallarovic et al. 2000). Swoveland (1978) found a significant increase in the number of peg sensilla in *Paruroctonus mesaensis* males as they matured. He also noted that males had a greater number of peg sensilla than females in chactoid species, which includes the family Vaejovidae. However, the area containing the peg sensilla, the density of peg sensilla, and the variation within a species have not been reported. Consequently, to date, there has not been an investigation into the variation of pecten morphology among populations.

I hypothesized that *Paruroctonus boreus* would demonstrate sexual dimorphism in the 5th metasomal segment, peg sensilla density, pecten tooth tip width and pecten tooth tip length. I also hypothesize that there will be interpopulation differences in these traits. Due to the behavior of males searching for females, I would expect the tooth tip size of the pectines in males to be larger and that peg sensilla density would be greater than in females.

4.2 Materials and Methods

A total of ninety-nine individuals were measured for carapace length (Fig. 4.1), 5th metasoma length (Fig. 4.1), peg sensilla density (Fig. 4.3), tooth tip length (Fig. 4.2) and tooth tip width (Fig. 4.3). The length of teeth #2, #3 and #4, as counted from the most distal tooth, were measured for each individual. Additionally, the number of teeth on the pectines, were counted for each measured individual. A minimum of two individuals from each sex and population were measured using scanning electron microscopy, with the exception of population 2, which only had one female measured and population 5 which only had one male measured.

Peg sensilla density (Fig. 4.3) was measured from a minimum of three teeth per individual, one distal tooth, one proximal tooth and one in between. A randomly sized polygon of known area was drawn over the peg sensilla on the tooth tip and the number of peg sensilla within the polygon, were counted. This gave the number of peg sensilla per square micrometer for each area measured. A minimum of two areas from each tooth, were measured and the mean density for each tooth was used for the analysis. An ANOVA was performed to test whether peg sensilla densities varied based on tooth location. Peg sensilla densities did not vary significantly with tooth location ($p > 0.1$) so all peg sensilla densities were analyzed together. The data were tested for normality using a Kolmogorov-Smirnov test and tested for equal variance using Levene's test of equality of variances and visually, using residual plots. I could not reject the null hypotheses for normal distribution and homogeneity so parametric tests were used. A Pearson correlation test was carried out to determine if carapace length, a measure of overall size, was correlated with peg sensilla density. There was a significant correlation between the two variables ($p < 0.01$, Fig. 4.4), so a two-way analysis of covariance was used to analyze peg sensilla density among populations and among the sexes, using carapace length as a covariate. To test the difference between the sexes within a population and between populations within a sex, residuals obtained from regressing peg sensilla density on carapace length were used in an analysis of variance.

28

Tooth tip length and width (Fig. 4.2 & Fig. 4.3) were measured from a minimum of three teeth per individual, one distal tooth, one proximal tooth and one in between. These measurements were taken from the same teeth as the peg sensilla densities. Although there was little variability in measuring tooth tip length and width (< 5%), the lengths were measured three times and the mean of each tooth was used for the analysis. The data were tested for normality using a Kolmogorov-Smirnov test and tested for equal variance using Levene's test of equality of variances and visually, using residual plots. The null hypothesis for normal distribution and equal variance was rejected so non-parametric tests were used on tooth tip length and width. A Kruskal-Wallis Test was performed to test whether these measurements varied based on tooth location. They did not significantly vary with location ($p > 0.05$) so all lengths were analyzed together. Tooth tip length and width were plotted against carapace length to explore the relationship between the two variables (Fig. 4.5 & Fig. 4.6). Both measurements have a positive relationship with carapace length, more so in males than females. A non-parametric form of a two-way ANCOVA was performed. The test appropriate for these data was the Scheirer-Ray-Hare test, an extension of the Kruskal Wallis test, with considerably lower power than a typical ANOVA (Dytham 2003).

Teeth #2, #3 and #4 were measured three times each and the means were used in the analysis. The data were tested for normality using a Kolmogorov-Smirnov test and tested for equal variance using Levene's test of equality of variances and visually, using residual plots. I could not reject the null hypothesis for normal distribution and equal variance, so parametric tests were used in this analysis. A Pearson correlation test was used to determine if carapace length, a measure of overall size, was correlated with teeth length. There were significant correlations between all three measurements and carapace length ($p < 0.01$, Fig. 4.7), so a two-way analysis of covariance was used to analyze tooth length among populations and among the sexes.

29

The 5th metasomal segment was measured once for all ninety-nine individuals. The data were tested for normality using a Kolmogorov-Smirnov test and tested for equal variance using Levene's test of equality of variances and visually, using residual plots. The null hypothesis for normal distribution could not be rejected; however, equal variance was rejected and a non-parametric test was used to analyze the 5th metasomal length between the sexes. Visual inspection of the data (Fig. 4.8) suggests a positive relationship with carapace length, so the 5th metasomal lengths were regressed against carapace lengths and the residuals were used in a Mann-Whitney U test to investigate sexual dimorphism in this trait.

4.3 Results

4.3.1 Peg Sensilla Density

The Pearson test for correlation revealed peg sensilla density exhibits a significant negative correlation with carapace length ($p < 0.01$, Fig. 4.4), with females having more peg sensilla on average per given area (Fig. 4.4). The ANCOVA revealed there was a significant population, sex and interaction effect ($p < 0.01$). The mean peg sensilla density for females was on average significantly higher than males. Females had a mean density of $2.374 * 10^{-2}$ (se $1 * 10^{-4}$) peg sensilla per square micrometer and males had a mean density of $1.978 * 10^{-2}$ (se $1 * 10^{-4}$) peg sensilla per square micrometer. There were significant among population differences for peg sensilla density ($p < 0.01$). Mean peg sensilla densities for populations are given in table 4.1. Due to the significant interaction between sex and population, a more meaningful result comes from looking at intrasexual differences within a population. As shown in Figure 6, 64% of the populations demonstrated significant sexual dimorphism in the density of their peg sensilla ($p < 0.025$). Populations that were sexually dimorphic were populations one, two, four, six, seven, eight, ten, twelve and fifteen ($p < 0.025$, Fig. 4.9, Table 4.1). In order to compare between populations, I compared the mean peg sensilla density among populations for each sex. There is more variability and more significant differences in peg sensilla densities among populations of females than there are of males (Tables 4.2 and 4.3). Six populations of

30

males were significantly different from other populations ($p < 0.025$). Of these populations, population four was significantly different from populations eight and nine, population five was significantly different from populations eight and nine, population seven was significantly different from population eight, population eight was significantly different from populations four, five, seven and fifteen, population nine was significantly different from populations four and five, and population fifteen was significantly different from populations eight and nine. All populations of females demonstrated a significant difference from at least one other population. Of these populations, population one was significantly different from populations four, five, six, eight, nine, eleven and fourteen, population two was significantly different from populations eight, nine and eleven, population three was significantly different from populations nine and eleven, population four was significantly different from populations one and nine, population five was significantly different from populations one and nine, population six was significantly different from populations one and nine, population seven was significantly different from populations eight, nine and eleven, population eight was significantly different from populations one, two, seven, twelve, thirteen and fifteen, population nine was significantly different from populations one through seven, ten and twelve through fifteen, population ten was significantly different from population nine, population eleven was significantly different from populations one, two, three, seven, twelve, thirteen and fifteen, population twelve was significantly different from populations eight, nine and eleven, population thirteen was significantly different from populations eight, nine and eleven and population fifteen was significantly different from populations one, eight, nine and eleven.

4.3.2 Tooth Tip Width

There was no significant difference in tooth tip width among populations ($p > 0.1$), nor was there a significant interaction effect between population and sex ($p > 0.1$, Table 4.4). However, there was a significant difference between male and female tooth tip width, adjusting for carapace length ($p < 0.001$, Table 4.4). Males across populations had a mean tooth tip

31

width of 51.5 μm (+/- s.e. 1), compared to females, which had a mean tip width of 29.7 μm (+/- s.e. 1). Figure 4.6 shows this difference is most pronounced in individuals with larger carapace lengths.

4.3.3 Tooth Tip Length

There was no significant difference in tooth tip length among populations (p > 0.1), nor was there a significant interaction effect between population and sex (p > 0.1, Table 4.5). Like tooth tip width, males had significantly longer tooth tip lengths than females (p < 0.001, Table 4.5). Mean tooth tip length for males across populations was 555.5 μm (+/- s.e. 11) and mean female tip length was 206.2 μm (+/- s.e. 11). Figure 4.5 again shows that the sexual dimorphism is more pronounced between individuals with larger carapace lengths.

4.3.4 Length of Teeth

All three variables demonstrated a significant population effect (p < 0.001) and sex effect (p < 0.001) but none of them exhibited a significant interaction between sex and population (p > 0.1). In all three measurements, males had significantly longer tooth lengths for teeth #2, #3 and #4 (p < 0.001, Table 4.6, Fig. 4.10). Tooth #2 was the longest measured, followed by tooth #3, then tooth #4. However, the difference between the teeth, were not significant (p > 0.1). Mean tooth length for tooth #2 in males was 248.9 μm (+/- 4.4), compared to females who had a mean tooth length of 230.2 μm (+/- 4.4). Mean tooth length for tooth #3 in males was 245.2 μm (+/- 4.2) and in females it was 221.1 μm (+/- 4.3). Mean tooth length for tooth #4 in males was 240.2 μm (+/- 4.1), compared to females who had a mean length of 220.1 μm (+/- 4.1).

4.3.5 Metasomal Length

Accounting for carapace length, males had on average, significantly longer 5th metasomal segments than females (p < 0.001, Fig. 4.8). This difference is most pronounced in males with large carapace lengths.
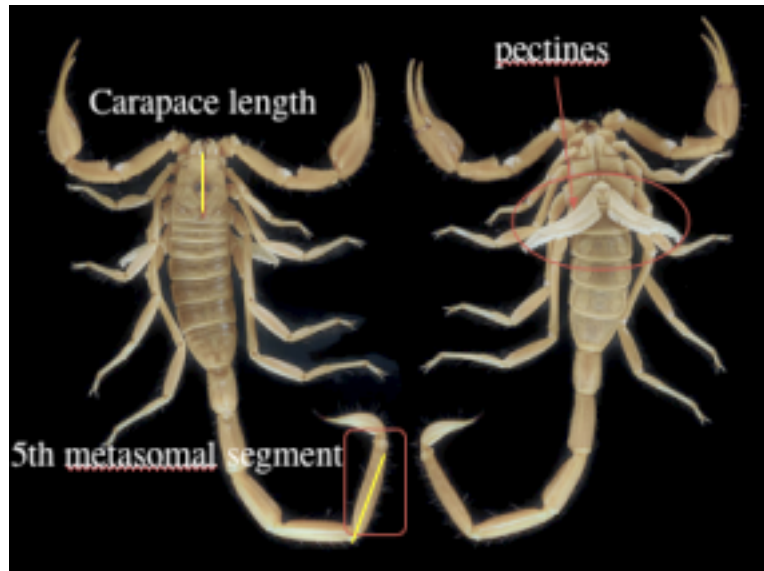
Fig. 4.1. Picture of *Paruroctonus* sp. showing the location of carapace length measurement, 5th metasomal length and the location of the pectines.
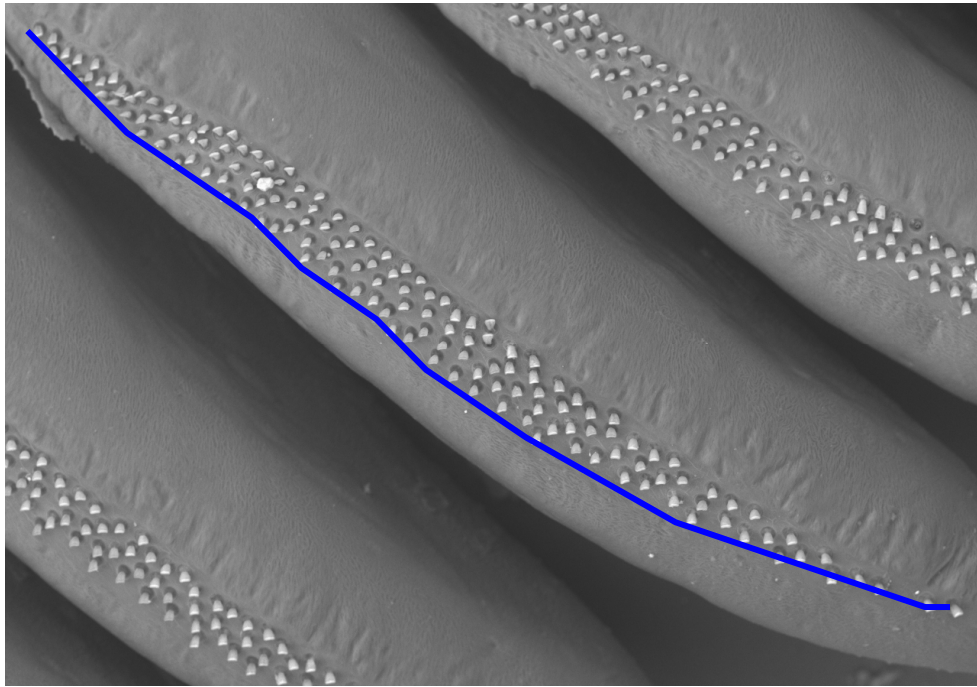
Fig. 4.2. Picture illustrating the measurements of tooth tip length taken from the pectines of *Paruroctonus boreus*, using scanning electron microscopy.
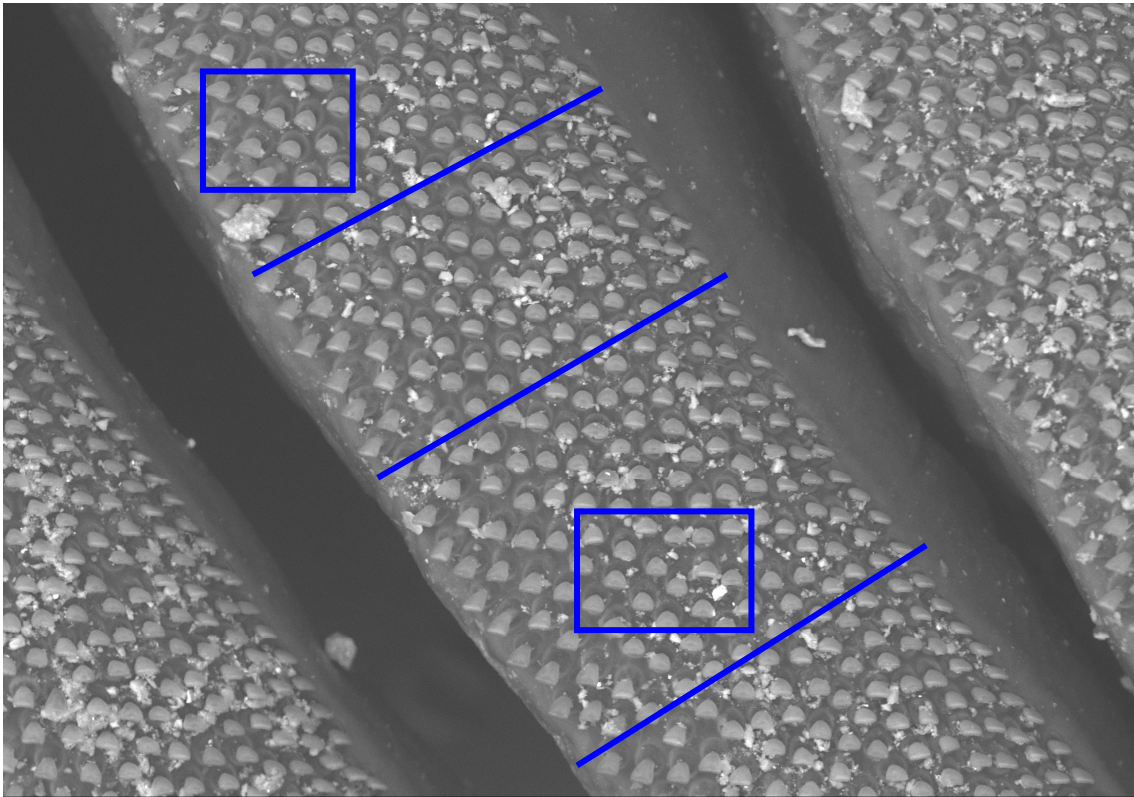
Fig. 4.3. Picture illustrating the measurements of tooth tip width and peg sensilla density, taken from the pectines of *Paruroctonus boreus*, using scanning electron microscopy.

Table 4.1. Comparison of sexually dimorphic marginal mean peg sensilla densities within populations of *Paruroctonus boreus*. Densities represent the number of peg sensilla per square micrometer x 10^{-2} (+/- standard error x 10^{-2}).

Population	Male	Female	P-value
1	1.95 (.1)	2.97 (.1)	P < 0.01
2	2.11 (.1)	2.96 (.2)	P < 0.01
3	2.55 (.1)	2.50 (.1)	P = 0.75
4	2.08 (.1)	2.47 (.1)	P = 0.02
5	2.14 (.2)	2.42 (.1)	P = 0.40
6	1.72 (.1)	2.35 (.1)	P < 0.01
7	1.96 (.1)	2.72 (.1)	P < 0.01
8	1.71 (.1)	2.02 (.1)	P = 0.01
9	1.60 (.1)	1.68 (.1)	P = 0.27
10	1.86 (.1)	2.48 (.2)	P < 0.01
11	1.73 (.1)	1.94 (.1)	P = 0.03
12	2.10 (.1)	2.54 (.1)	P = 0.01
13	2.11 (.1)	2.65 (.1)	P = 0.08
15	2.06 (.1)	2.63 (.1)	P < 0.01
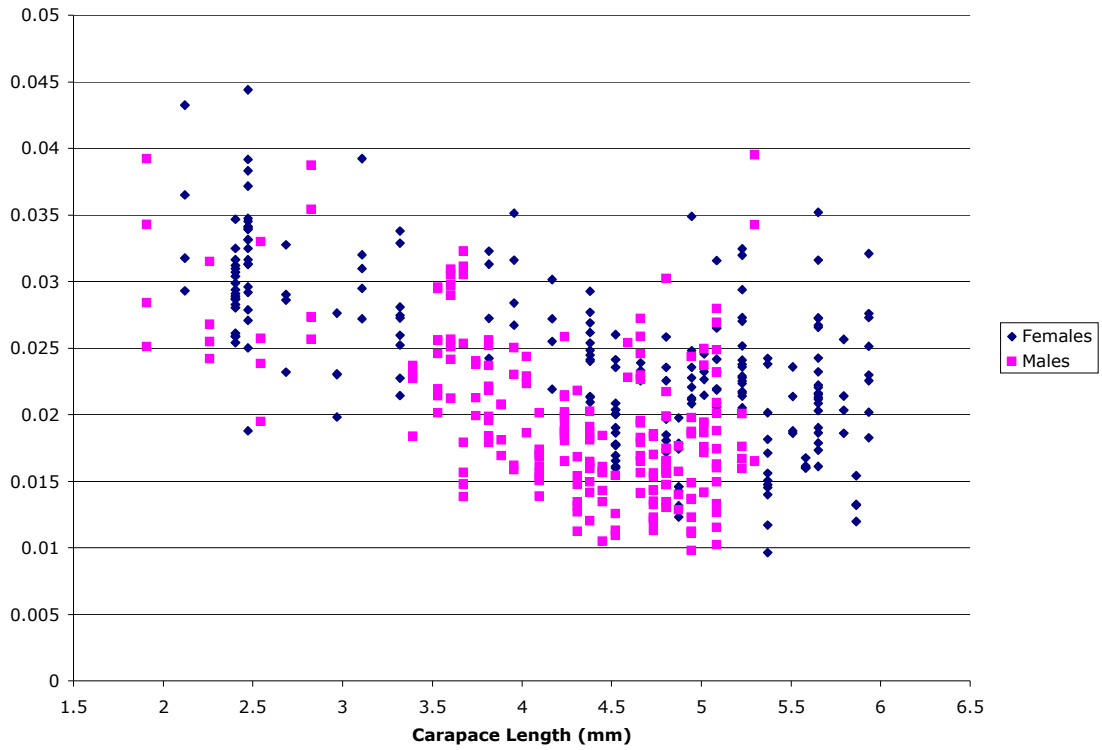
Fig. 4.4. Peg sensilla density (y-axis) is negatively correlated with carapace length (x-axis) in male and female *Paruroctonus boreus* (p < 0.01).
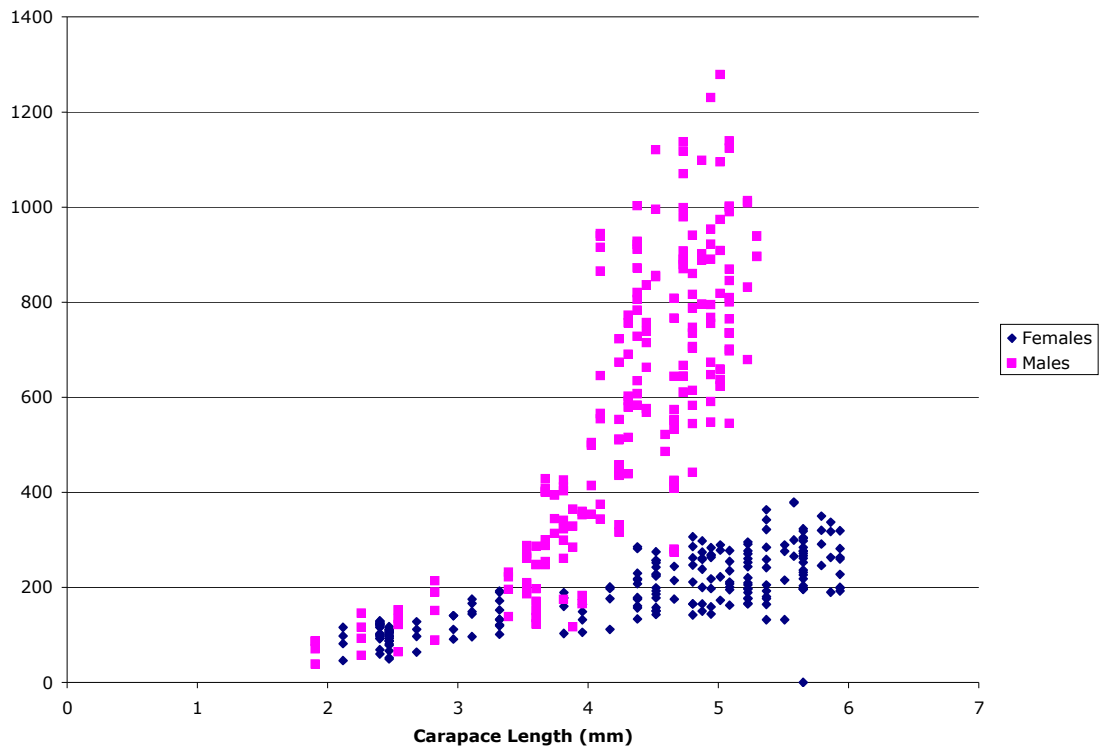
Fig. 4.5. Tooth tip length (y-axis) exhibits a positive relationship with carapace length (x-axis) in *Paruroctonus boreus*. This relationship is more pronounced in mature males (carapace length > 4.3 mm), than in females.
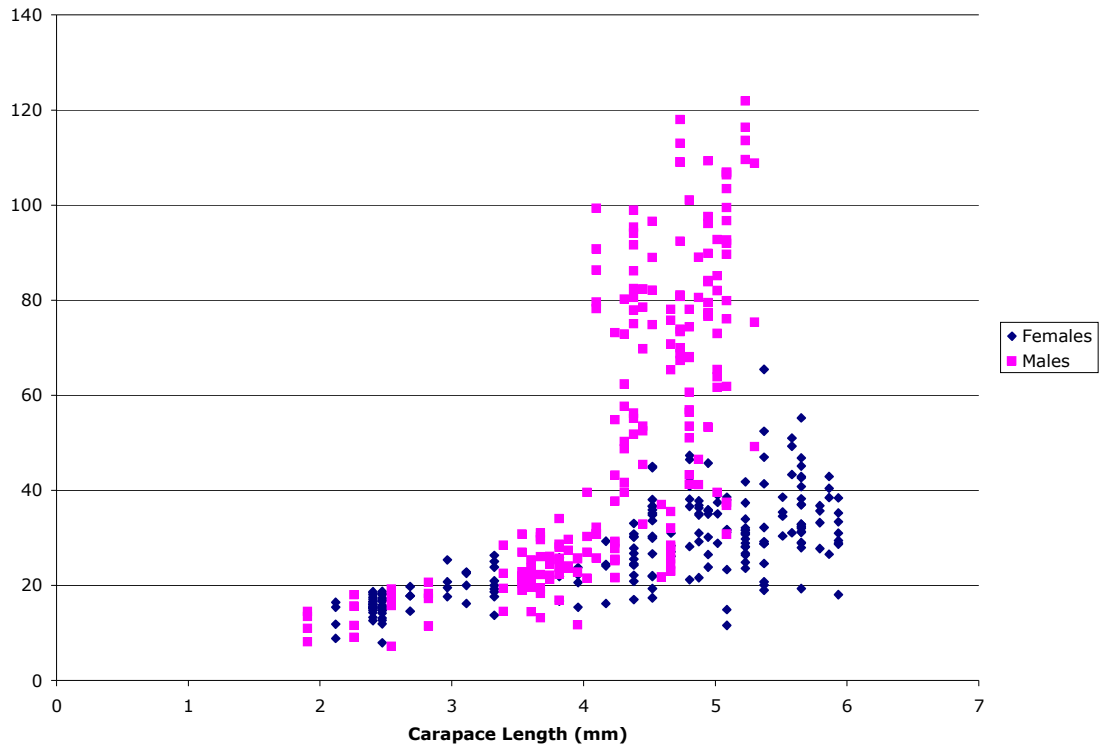
Fig. 4.6. Tooth tip width (y-axis) exhibits a positive relationship with carapace length (x-axis) in *Paruroctonus boreus*. Males show a steep increase in tooth tip width at the onset of maturity (carapace length > 4.3 mm)
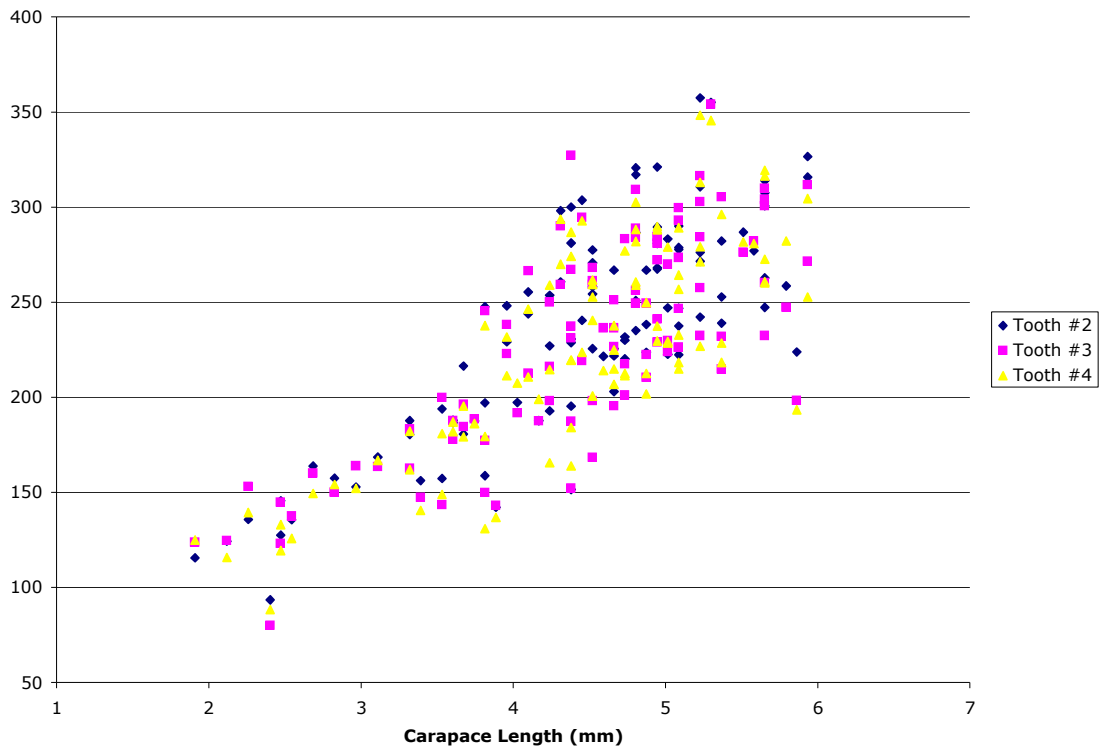
Fig. 4.7. Tooth length in *Paruroctonus boreus* increases with carapace length. There is not a significant difference between the lengths of tooth #2, #3 and #4 (p > 0.1).
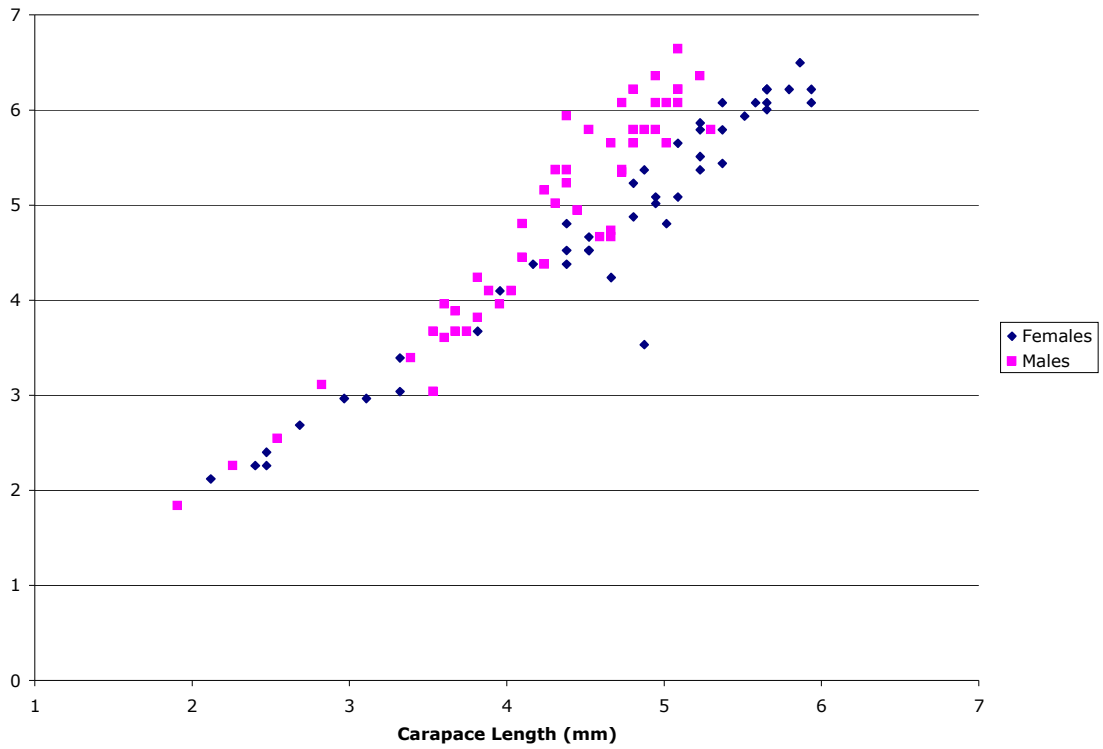
Fig. 4.8. The 5th metasomal segment in *Paruroctonus boreus* shows a positive relationship with carapace length. There is significant sexual dimorphism in their 5th metasomal segment (p < 0.01), especially at maturity (carapace length > 4.3 mm).
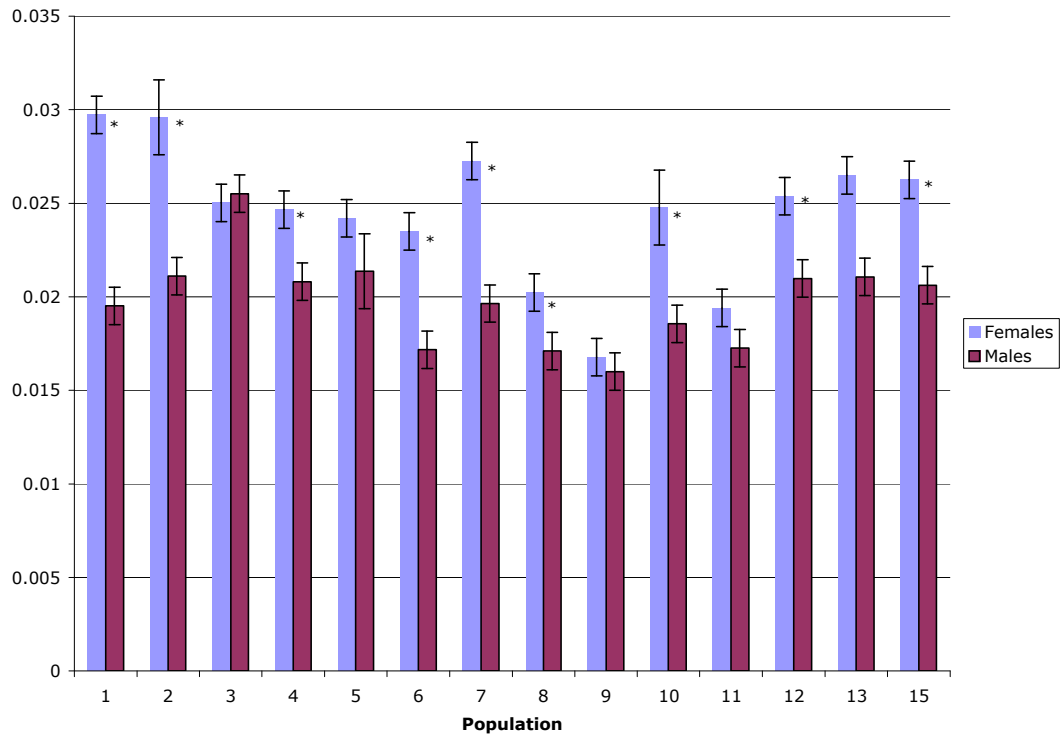
Fig. 4.9. Estimated marginal means of peg sensilla density in male and female *Paruroctonus boreus* across populations, using carapace length as a covariate. Bars represent one standard error and asterisks indicate a significant difference between male and female densities within a population, adjusting for multiple comparisons (p < 0.025).

Table 4.2. Pairwise comparison of female peg sensilla densities of *Paruroctonus boreus*. Boxes marked with an (*) indicate a significant difference between the populations (p < 0.025).

POP	1	2	3	4	5	6	7	8	9	10	11	12	13	15
1				*	*	*		*	*		*			*
2								*	*		*			
3									*		*			
4	*								*					
5	*								*					
6	*								*					
7								*	*		*			
8	*	*	*	*	*	*	*					*	*	*
9	*	*	*	*	*	*	*			*		*	*	*
10									*					
11	*	*	*				*					*	*	*
12								*	*		*			
13								*	*		*			
15	*							*	*		*			

Table 4.3. Pairwise comparison of male peg sensilla densities of *Paruroctonus boreus*. Boxes marked with an (*) indicate a significant difference between the populations (p < 0.025).

POP	1	2	3	4	5	6	7	8	9	10	11	12	13	15
1														
2														
3														
4								*	*					
5								*	*					
6														
7								*						
8				*	*		*							*
9				*	*									*
10														
11														
12														
13														
15								*	*					

Table 4.4. Results of the Scheirer-Ray-Hare Test for Tooth Tip Length in *Paruroctonus boreus*
(r-squared value of 0.836)

	SS	SS/MS$_{total}$	d.f.	P-value
CL	915843.77	15	1	**< 0.001**
Pop	225360.46	3.7	13	0.99
Sex	2110596.73	34.6	1	**< 0.001**
Pop*Sex	309150.677	5.1	13	0.97

Table 4.5. Results of the Scheirer-Ray-Hare Test for Tooth Tip Width in *Paruroctonus boreus*
(r-squared value of 0.782)

	SS	SS/MS$_{total}$	d.f.	P-value
CL	1083745.9	17.8	1	**< 0.001**
Pop	594406.4	9.7	13	0.71
Sex	718430.9	11.8	1	**< 0.001**
Pop*Sex	369919.5	6.1	13	0.94

Table 4.6. Comparison of sexually dimorphic marginal means for the length of teeth #2, #3 & #4 within populations of *Paruroctonus boreus*. Measurements are given in micrometers (+/- se). Asterisks indicates a significant difference between the sexes.

Population	Tooth #	Male	Female
1	2	236.5 (13.3)	211.6 (15.7)
	3	226.9 (12.8)	192.7 (15.2)
	4	221.6 (12.5)	186.2 (14.7)
2	2	228.1 (10.1)	246.6 (27)
	3	223.7 (9.8)	242.5 (26.2)
	4	215.9 (9.5)	211.1 (25.3)
3	2	225.6 (15.8)	197.4 (15.6)
	3	222.6 (15.3)	200.9 (15.1)
	4	224.1 (14.9)	201.3 (14.6)
4	2	237.3 (15.6)	247.5 (13.5)
	3	238.0 (15.1)	239.1 (13)
	4	235.9 (14.6)	237.3 (12.6)
5	2	243.4 (27.9)	229.5 (11.9)
	3	256.2 (27)	221.9 (11.5)
	4	248.2 (26.1)	224.0 (11.2)
6	2	277.3 (15.4)	219.3 (15.4)
	3	264.9 (14.9)	206.7 (14.9)
	4	276.1 (14.4)	216.8 (14.4)
7	2	241.4 (11.3)	232.9 (13.3)
	3	229.5 (10.9)	227.3 (12.9)
	4	234.2 (10.6)	235.1 (12.5)
8	2	300.4 (18.8)	242.2 (15.4)
	3	292.3 (18.2)	210.1 (14.9)
	4	280.5 (17.6)	234.4 (14.4)
9	2	212.0 (10.2)	188.0 (13.8)
	3	202.6 (9.9)	169.1 (13.4)
	4	195.4 (9.5)	163.2 (12.9)
10	2	239.8 (15.4)	238.4 (19)
	3	234.6 (14.9)	230.8 (18.4)
	4	226.7 (14.4)	227.5 (17.8)
11	2	287.1 (18.8)	251.7 (15.4)
	3	305.9 (18.2)	250.7 (14.9)
	4	276.0 (17.6)	248.9 (14.4)
12	2	253.4 (15.4)	256.9 (15.7)
	3	250.3 (14.9)	256.6 (15.2)
	4	246.1 (14.4)	256.4 (14.8)
13	2	284.9 (13.6)	250.6 (15.4)
	3	276.8 (13.2)	246.6 (14.9)
	4	275.8 (12.7)	238.7 (14.4)
15	2	218.2 (15.2)	209.8 (11.4)
	3	208.1 (14.9)	200.9 (11)
	4	206.4 (14.4)	200.5 (10.7)
Mean	2*	**248.9 (4.4)**	**230.2 (4.4)**
	3*	**245. 2 (4.2)**	**221.1 (4.3)**
	4*	**240.2 (4.1)**	**220.1 (4.1)**
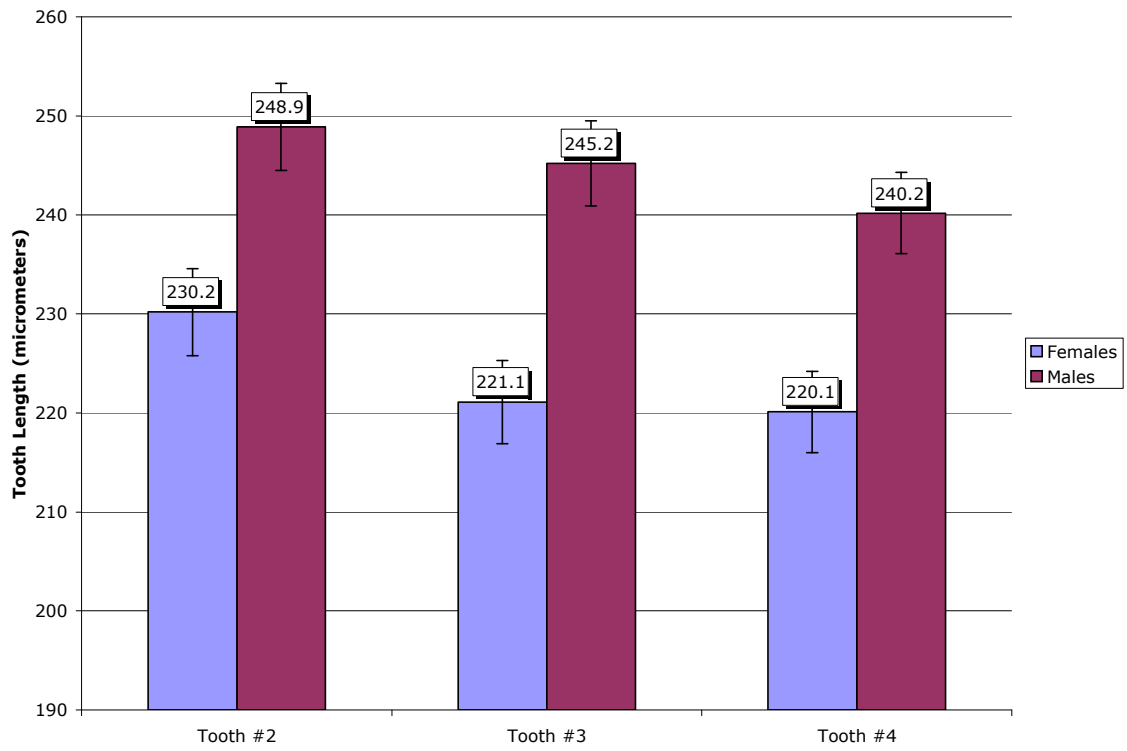
Fig. 4.10. *Paruroctonus boreus* exhibits significant sexual dimorphism in pectinal tooth lengths for tooth #2, #3 & #4 in all populations (p < 0.001). Numbers represent mean tooth length and the bars represent +/- one standard error.

Table 4.7. Comparison of sexual dimorphism of scorpion pectines among and within families, including data from *Paruroctonus boreus*. This table is adapted from (Gaffin and Brownell 2001) and new data are in bold.

Family	Species	Sex	No. Teeth/ 2 pectines	No. Pegs/ Tooth	Total no. Pegs	No. Pegs $\frac{}{}$
Vaejovidae	*Anuroctonus phaiodactylus*		19 / 13	201 / 115	3,819 / 1,495	2.55
	Nullibrotheas allenii		24 / 17	160 / 82	3,840 / 1,394	2.75
	Paruroctonus mesaensis		75 / 48	1,600 / 180	120,000 / 8,640	13.89
	Uroctonus mordax		27 / 20	1,100 / 153	29,700 / 3,060	9.71
	Vaejovis confusus		34 / 27	1,000 / 292	34,000 / 7,884	4.31
	Vaejovis spinigerus		46 / 37	1,000 / 310	46,000 / 11,470	4.01
	Paruroctonus boreus		**56** / **38**	**566** / **149**	**31,696** / **5,662**	**5.60**
Buthidae	*Centruroides exilicauda*		50 / 44	800 / 490	40,000 / 21,560	1.86
	Parabuthus pallidus		72 / 62	1,650 / 1,050	118,800 / 65,100	1.82
Chactidae	*Superstitionia donensis*		12 / 12	800 / 490	9,600 / 5,880	1.63
Diplocentridae	*Didymocentrus comondae*		17 / 15	1,050 / 152	17,850 / 2,280	7.83
Scorpionidae	*Pandinus gregoryi*		36 / 34	1,400 / 900	50,400 / 30,600	1.65

4.4 Discussion

Paruroctonus boreus demonstrates significant differences in peg sensilla density between populations, between sexes, and in their interaction. I reject the null hypothesis, that males would have a greater peg sensilla density than females. Females have a greater peg sensilla density on average. There are interpopulation differences, likely due to their isolation from each other. The more interesting result came from the interaction of population and sex. It suggests that populations are adapting or evolving independently of each other in response to localized selection or genetic drift. This is indicated by the presence of sexual dimorphism in some populations and not others. While there is definitely something influencing this change in peg sensilla density, between the populations, I cannot say what the mechanism is. There is no obvious pattern to the differences in peg sensilla density among populations. Peg sensilla density is not correlated with latitude, longitude, elevation or sympatry. However, I hypothesize the following. Populations seem to occupy habitats with similar abiotic factors (Chapter 2) but this analysis did not include soil type. Scorpions are usually habitat specialists and uniquely adapted to the substrate in which they live (Polis 1990a). However, Brownell (2001) showed that the spatial frequency of sand grains in the soil had a stronger relationship with pectine teeth than it did with peg sensilla, and suggested that the peg sensilla are better adapted for detecting substrate structure or detecting chemical cues, such as pheromones. The latter of the two may best explain the variation among the populations given their behavioral response to sympatric conspecifics and not their allopatric counterparts (Chapter 3). Therefore, male peg sensilla density change is driven by the pheromones of their conspecifics. Assuming that males experience strong selection on their ability to detect substrate borne chemical cues, we would expect less variation in peg sensilla density among populations than females. This is a likely scenario illustrated by greater among population variation in females than in males (Table 4.2, Table 4.3 & Fig. 4.9). Females are likely to experience less selection pressure on their peg sensilla density, since they do not actively search for males, and therefore, demonstrate more

49

variability among populations than males. The negative correlation between peg sensilla density and carapace length may be explained by the size of the pegs themselves. There are no data on the absolute size, but smaller individuals may have smaller peg sensilla diameter, therefore, a greater density per square area. Hjelle (1990) noted there were differences in the structures of the peg sensilla between the sexes in some scorpion families but did not indicate any size differences. As a result, differences in diameter of the peg sensilla may also explain sexual dimorphism in *Paruroctonus boreus*. Future studies could look at the size of the peg sensilla and the space between them to help explain the differences seen.

Males had on average significantly larger teeth as indicated by tooth tip width, tooth tip length and length of teeth #2, #3 and #4. The only measure of pectinal tooth size that demonstrated a difference between populations was tooth length. Similar to what Hjelle (1990) found in *Paruroctonus mesaensis*, *Paruroctonus boreus* males demonstrated a steep increase in the area of the teeth which contain peg sensilla at maturity. This sexual dimorphism, pronounced at maturity, supports the idea that the pectines are important to the reproductive biology of *P. boreus*.

The 5th metasomal segment has been implicated in courtship behavior and noted to be sexually dimorphic in other scorpion species (Francke and Jones 1982, Polis and Sissom 1990, Tallarovic et al. 2000). My results demonstrate that *Paruroctonus boreus* also exhibits sexual dimorphism in this trait, specifically at maturity. Perhaps a longer 5th metasomal segment facilitates the courtship process or indicates their size to potential mates or enemies. Future behavioral studies are needed to address the function and importance of this trait.

In conclusion, traits thought to be associated with the reproductive ecology in *Paruroctonus boreus* are all sexually dimorphic, with exceptions in a few populations that did not demonstrate sexual dimorphism in peg sensilla density. The degree of sexual dimorphism is greatest in mature individuals. Males did not demonstrate a dramatic change in their peg sensilla density at maturity, like the other traits did. However, the total number of peg sensilla is

higher in males and increases dramatically at maturity because of the increase in tooth tip width and tooth tip area. The tooth length of both sexes increased with size, with males having longer teeth in all size classes. Males have more teeth than females (Haradon 1985) and this did not vary with population. I was unable to accurately count all the peg sensilla on each tooth, but it is possible to obtain an estimate of the total number of peg sensilla per tooth, by multiplying the tooth tip width, tooth tip length and peg sensilla density. This provides an estimate of 566 peg sensilla per tooth in males and 150 peg sensilla per tooth in females and is within the range of other species in the family Vaejovidae (Table 4.7).

CHAPTER 5

PHYLOGEOGRAPHY OF *PARUROCTONUS BOREUS* IN THE WESTERN US

5.1 Introduction

The scorpion genus *Paruroctonus* Werner, 1934, currently contains 29 species, and is widely distributed in the Western US and Mexico. California has the most species with up to 16, primarily found west of the Sierra Nevada Mountains and south into desert regions (Sissom 2000). Many of these species are limited to sand dunes and isolated mountain ranges (Polis 1990a, Sissom 2000). Haradon (1983, 1984a, b, 1985) partitioned *Paruroctonus* into several small groups he believed to be monophyletic based on morphological characters. *Paruroctonus boreus*, the most wide-ranging species, was assigned to the *boreus* infragroup and smaller *boreus* microgroup. Haradon (1985) grouped *P. silvestrii* and *P. bantai* with *P. boreus* into the *boreus* microgroup, using morphometrics primarily associated with the chelicerae. It has been common practice for systematists to use these characters as well as trichobothria and setae patterns to identify scorpion species (Sissom and Francke 1981, Sissom 1990). However, molecular evidence suggests *P. boreus* and *P. utahensis* group sister to each other (personal, unpublished data). These species belong to different "monophyletic groups" as assigned by Haradon (1985), which suggests that the fundamental characters traditionally used in scorpion systematics may not resolve the true relationship at the specific or subspecific level.

Paruroctonus boreus appears to exhibit variation in size, color and setae patterns (Tourtlotte 1974, personal observations). This suggests populations are under different selection pressures or that they represent multiple species within *P. boreus*. Although this variation has been noted (Gertsch and Soleglad 1966), there have been no previous investigations of among population variation in morphological or molecular characters in this geographically widespread species.

Scorpions have a conservative body plan that has changed little since the Devonian period (Polis 1990), and using morphological characters in scorpion systematics may not reveal lineages that have diverged recently. Investigators have begun to use gene sequence information to infer evolutionary relationships of scorpions (Gantenbein et al. 1999, Yamashita and Fet 2001, Fet et al. 2003). The most commonly sequenced genes are the nuclear 18s and mitochondrial 16s, ribosomal genes, which have been valuable in revealing relationships at the genus level and above but are less informative at the species level, yielding weak support for relationships (personal observation). More recently, investigators have sequenced multiple genes or gene fragments to analyze phylogeography or intraspecific variability in scorpions (Gantenbein et al. 2003, Parmakelis et al. 2006). These two studies of Eurasian taxa are the first to use multiple genes to assess the biogeography or phylogeography of a species or species group in scorpions. To date, there has not been a similar study in a North American species. I used data from mitochondrial 16s and CoxI fragments to assess the phylogeography of *Paruroctonus boreus* in the Western US. Fundamental to this research is identifying any cryptic species. Is *P. boreus* one species and if so, do populations demonstrate variability among sites that exhibit geographical affinities, which can give insight into current distribution of the species.

5.2 Materials and Methods

Individuals from 15 populations were euthanized by freezing at - 20ºC for 1 hour and then immediately preserved in 95% ethanol. These populations serve as a representative sample of the populations on a north-south and east-west gradient. The left pedipalp was removed and genomic DNA was extracted within 48 hours of preserving. Specimens representing the populations are shown in Table 5.1 and Chapter 2, in this manuscript.

DNA was extracted above using DNeasy®Tissue Kit (250) Cat. No. 69506. Two mitochondrial genes, 16S rRNA (~ 290 bp) and cytochrome oxidase subunit I (~ 944 bp), were amplified from 51 specimens by polymerase chain reaction (PCR) in a PTC-100™ Thermal

Cycler (MJ Research). For the 16S fragment, a pair of scorpion specific primers were used as reported in (Gantenbein et al. 1999) and for the amplification of the COI gene, primers were used as described in (Vink et al. 2005). A negative control was used and the PCR product was run out on 1% agarose gel to visually inspect. Sequencing reactions were performed using standard ABI protocols and sequenced using an ABI 3130 XL Sequencer.

I used maximum likelihood (ML) in PAUP*4.0b10 (Swofford 1999) and Garli 0.951 (Zwickl 2006) and a Bayesian analysis in Mr.Bayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) to infer the phylogeny for each dataset (16S, COI, combined data) separately. The model of evolution for all analyses was chosen using the Akaike Information Criterion (AIC) in ModelTest 3.04 (Posada and Crandall 1998) by means of successive iterations. ML analyses were conducted using a heuristic search with the chosen model, TBR branch swapping, 10 random-addition replicates, and a neighbor-joining starting tree. Bootstrap support was calculated in Garli with default options using 100 replicates. The best ML tree was then used in MrModeltest 2.2 (Nylander 2004) to determine the parameters for a Bayesian analysis according to the AIC. These parameters were input into MrBayes 3.1.2 for 5,000,000-10,000,000 generations with four chains, two repetitions, and sampling every 1,000 generations. Sampling was stopped after the standard deviation of split frequencies was <0.01. The first 2,000,000-5,000,000 generations were discarded as burnin. Outgroup taxa used to root the tree were *Vaejovis confusus* (family Vaejovidae) and *Mesobuthus gibbosus* (family Buthidae). Pairwise distances were obtained from PAUP* for COI sequence information using the Hukes-Cantor model of substitution.

Sequences were obtained from fifty-one (14 populations) and fifty-five (15 populations) specimens for 16S and COI respectively. Sequence lengths for the 16S gene averaged 290 bp and 944 bp for COI for a combined analysis of 1234 bp. The total combined analysis yielded a 4 equally likely trees, from which a consensus tree was generated. The chosen model of evolution for both datasets was the GTR + I + G model. For ML analyses in PAUP*, I set all parameters to the values estimated by MrModeltest. For analyses conducted in Garli and MrBayes, the program estimated the parameters based on the general model derived from MrModeltest.

The analysis showed strong support for a clade consisting of individuals from population 3 grouping sister and basal to all other individuals (Fig. 5.1). However, two individuals (C 30 and C 4) from population 3 grouped within other populations. Working up the tree, all individuals from population 9 grouped together, sister to the other populations. The sister clade was supported by the Bayesian analysis but not the bootstrap analysis. Within this clade, there is strong support for a clade containing populations 5, 10, 11, 12 and 15. All individuals from population 5 grouped together with one individual from population 12 with strong support. Nested within this clade is a grouping supported by the Bayesian analysis but not the bootstrap analysis. The Bayesian analysis supports a clade containing all individuals from population 2 with one individual from population 11 and 14 as basal to a large polytomy. Within the polytomy, all individuals from population 1 group together with strong support.

Despite the broad geographic sampling (Chapter 2), there was little phylogenetic structure reflecting the geographical locations among the populations. Branch lengths were small within clades and individuals from multiple populations group together. Pairwise distances (Table 5.2) demonstrate population 3 is the most divergent, being on average 7% divergent from other populations based on the COI data. Populations of *Paruroctonus boreus* averaged 4% divergence from each other (range 0 – 8%). Eight of the fifteen populations

exhibited multiple haplotypes. The outgroup species *Vaejovis confusus* and *Mesobuthus gibbosus* demonstrated 15% and 28% divergence respectively.

Table 5.1. Specimens of *Paruroctonus boreus* sequenced for 16S and COI gene fragments. The population number corresponds to the Figures in Chapter 2, the letters correspond to the labeling of specimens on the tree and the coordinates of each population are given in degrees decimal.

Pop. Map #	Pop. Letter	Specimens	Pop. GPS Coordinates
1	A	A10,A16,A18,A19,A28,A3	N 38.05445 W 119.12532
2	B	B1, B16, B2, B36, B4, B9	N 40.46 W 120.57
3	C	C1, C14, C15, C16, C23, C30	N 40.601367 W 117.91349
4	D	D16, D20, D22, D3, D30, D33	N 39.393925 W 119.83883
5	E & H	E1, E15, E2, E33 H11, H15, H21, H4	N 36.3688 W 115.6287
6	K	K16, K18, K301, K36	N 42.902839 W 115.6927
7	L	L2, L3, L301, L5, L6	N 44.1312833 W 121.33235
8	M	MJF1, M4, M8	N 46.2 W 111.95
9	N	N24, N202, N209, N16, N13	N 38.226869 W 109.4582
10	O	O11, O16, O18, O33, O9	N 37.19 W 112.66
11	P	P1, P13, P16, P201, P21	N 39.33516667 W 108.203
12	Q	Q11, Q6, Q9	N 40.82 W 115.73
13	R	R10, R2, R3, R4, R9	N 39.4455 W 116.7561
15	S	S1, S501	N 37.5611 W 110.0087
14	T	T201	N 41.6726667 W121.94618

Fig. 5.1. The single most likely tree for *Paruroctonus boreus* inferred from the total combined sequence evidence information of the genes 16S and COI. Significantly supported branches are indicated by their bootstrap/posterior probabilities displayed above the branches. Outgroup branch lengths have been adjusted in order to display the tree in this manuscript.

Table 5.2. Pairwise distances among populations of *Paruroctonus boreus* inferred from the COI gene using the model of substitution JC69.

	pop1	pop2	pop3	pop4	pop5	pop6	pop7	pop8	pop9	pop10	pop11	pop12	pop13	pop14	pop15
pop1															
pop2	0.023														
pop3	0.076	0.064													
pop4	0.065	0.065	0.065												
pop5	0.054	0.038	0.077	0.05											
pop6	0.015	0.027	0.075	0.042	0.054										
pop7	0.019	0.027	0.081	0.032	0.058	0.023									
pop8	0.031	0.023	0.081	0.042	0.054	0.035	0.035								
pop9	0.038	0.031	0.067	0.042	0.035	0.035	0.042	0.046							
pop10	0.044	0.028	0.069	0.032	0.03	0.045	0.042	0.044	0.037						
pop11	0.039	0.022	0.039	0.034	0.028	0.036	0.04	0.044	0.035	0.029					
pop12	0.037	0.025	0.075	0.035	0.037	0.041	0.037	0.039	0.037	0.036	0.033				
pop13	0.021	0.019	0.078	0.041	0.05	0.03	0.03	0.027	0.034	0.042	0.037	0.03			
pop14	0.023	0	0.064	0.024	0.038	0.027	0.027	0.023	0.031	0.028	0.022	0.025	0.019		
pop15	0.052	0.036	0.07	0.046	0.015	0.052	0.056	0.052	0.04	0.024	0.028	0.04	0.048	0.036	

5.4 Discussion

The analyses show there is some structure among the populations. Most populations are grouping together as expected with the exception of populations 10, 11 and 12. These populations group within multiple clades with strong support. Population 10 and 11 are located near the southern and eastern extent of the range of *Paruroctonus boreus* but population 12 is centrally located within the distribution. This analysis suggests population 3 groups basal to the other populations. However, two individuals from this population grouped within other clades. This population is located in central Nevada, centrally located within the distribution of *Paruroctonus boreus.* The specimens that formed the basal clade may represent a primitive haplotype and specimens that grouped with other populations represent more recent haplotypes. Population 9, located in eastern Utah was strongly supported as basal to all populations except population 3.

Paruroctonus boreus was determined to be one species in this study for the following reasons. Genetic distances among the populations are consistent with the exception of population 3, which had large distances, but two individuals from this population grouped with other populations. On average, populations were only 4% divergent from each other and 15% divergent from the familial outgroup. The morphological differences among populations were minimal (Chapter 4). Although, there is some genetic structure among populations elevating any of them to species would be unjustified at this time.

Without molecular clock or fossil dating, I cannot assign a timeline to when *Paruroctonus boreus* may have evolved. I presume the family Vaejovidae evolved between the Jurassic period and the last ice age because the sister family Chactidae occurs in South America. Historical events likely to have affected the distribution of *P. boreus* would include the inundation of the Great Basin Region by salton seas, Lake Bonneville and Lake Lahontan. These lakes occupied a large area in western Utah and western Nevada. At the same time lower temperatures and glaciers may have excluded *Paruroctonus boreus* from the northern

59

part of their current range and the mountains that they currently inhabit in the southern part of their range. This scenario would support the origin of *P. boreus* in central Nevada. This corresponds to population 3, which groups basal to and within other clades.

Range expansion could have progressed rapidly as the seas dried up and the glaciers retreated, allowing population 3 to expand in all directions. As temperatures warmed, populations would have been forced northward or up in elevation. Multiple populations of *Paruroctonus boreus* in the south are restricted to isolated mountain habitats surrounded by unsuitable habitat. It is doubtful that these populations are part of a large panmictic population across the western US. I suggest populations are isolated and the lack of phylogenetic structure among populations can be explained by incomplete lineage sorting that occurred during rapid range expansion and secondary isolation. Riddle and Honeycutt (1990) found similar results among populations of grasshopper mice in the western US and suggested either post-isolation dispersal or recent isolating events caused the phylogenies to be unresolved. Their first explanation can almost certainly be ruled out for *P. boreus* as they have not been observed traveling long distances.

REFERENCES

Alexander, A. J. 1956. Mating in Scorpions. Nature **178**:867-8868.

Avise, J. C. 2001. The History and Purview of Phylogeography. Pages 3-108 *in* J. C. Avise, editor. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, Ma.

Ayyagari, L. R. and W. J. Tietjen. 1986. Preliminary Isolation of Male-Inhibitory Pheromone of the Spider *Schizocosa ocreata* (Araneae, Lycosidae). Journal of Chemical Ecology **13**:237-244.

Brown, C. A. 1996. Interpopulation and Intersexual Variation in Pectine Tooth Counts in *Centruroides Vittatus* (Scorpionida, Buthidae). Journal of Arachnology **24**:262-264.

Brown, J. H. and M. V. Lomolino. 1998a. Distributions of Single Species. Pages 61-94 *in* J. H. Brown and M. V. Lomolino, editors. Biogeography. Sinauer Associates, Sunderland, Ma.

Brown, J. H. and M. V. Lomolino. 1998b. The Science of Biogeography. Pages 3-38 *in* J. H. Brown and M. V. Lomolino, editors. Biogeography. Sinauer Associates Inc., Sunderland, Ma.

Brownell, P. 2001. Sensory Ecology and Orientational Behaviors. Pages 159-183 *in* P. Brownell and G. A. Polis, editors. Scorpion Biology and Research. Oxford University Press, New York, Ny.

Brownell, P. and R. D. Farley. 1979. Detection of Vibrations in Sand by Tarsal Sense Organs of the Nocturnal Scorpion, *Paruroctonus mesaensis*. Journal of Comparative Physiology A **131**:23-30.

Brownell, P. H. 1977. Compressional and Surface Waves in Sand: Used by Desert Scorpions to Locate Prey. Science **197**:479-482.

Crews, S. C. and M. Hedin. 2006. Studies of Morphological and Molecular Phylogenetic Divergence in Spiders (Araneae: *Homalonychus*) from the American Southwest, Including Divergence Along the Baja California Peninsula. Molecular Phylogenetics and Evolution **38**:470-487.

Dytham, C. 2003. The tests 1: tests to look at differences. Pages 66-164 *in* C. Dytham, editor. Choosing and Using Statistics A Biologist's Guide. Blackwell Science, Malden, Ma.

Fet, V., B. Gantenbein, M. Soleglad, V. Vignoli, N. Salomone, E. V. Fet, and P. J. Schembri. 2003. New Molecular and morphological data on the "Euscorpius carpathicus" species complex (Scorpiones: Euscorpiidae) from Italy, Malta, and Greece justify the elevation of *E. c. sicanus* (C.L. Koch, 1837) to the species level. Revue Suisse de Zoologie **110**:355-379.

Fet, V., W. D. Sissom, G. Lowe, and M. E. Braunwalder. 2000a. Catalog of Scorpions of the World (1758-1998). The New York Entomological Society, New York, Ny.

Fet, V., W. D. Sissom, G. Lowe, and M. E. Braunwalder. 2000b. Introduction. Pages 1-7 *in* V. Fet, W. D. Sissom, G. Lowe, and M. E. Braunwalder, editors. Catalog of the Scorpions of the World (1758-1998). The New York Entomological Society, New York, Ny.

Fleissner, G. 1977. The Absolute Sensitivity of the Median and Lateral Eyes of the Scorpion, *Androctonus australis* L. (Buthidae, Scorpiones). Journal of Comparative Physiology A **118**:109-120.

Fleissner, G. and G. Fleissner. 2001. The Scorpion's Clock. Pages 138-158 *in* P. H. Brownell and G. A. Polis, editors. Scorpion Biology and research. Oxford University Press, New York, Ny.

Foelix, R. F. 1982a. Biology of Spiders. Harvard University Press, Cambridge, Ma.

Foelix, R. F. 1982b. Reproduction. Pages 172-210 *in* R. F. Foelix, editor. Biology of Spiders. Harvard University Press, Cambridge, Ma.

Francke, O. F. 1981. The Family Iuridae Thorell (Arachnida, Scorpiones). Journal of Arachnology **9**:233-258.

Francke, O. F. and S. K. Jones. 1982. The Life History of *Centruroides gracilis* (Scorpiones, Buthidae). Journal of Arachnology **10**:223-229.

Gaffin, D. D. and P. H. Brownell. 1992. Evidence of Chemical Signaling in the Sand Scorpion, *Paruroctonus mesaensis* (Scorpionida: Vaejovida). Ethology **91**:59-69.

Gaffin, D. D. and P. H. Brownell. 1997. Response properties of chemosensory peg sensilla on the pectines of scorpions. Journal of Comparative Physiology A **181**:291-300.

Gaffin, D. D. and P. H. Brownell. 2001. Chemosensory behavior and physiology. Pages 184-203 *in* G. A. Polis, editor. Scorpion Biolgy and Research. Oxford University Press, New York, NY.

Gantenbein, B., V. Fet, and A. V. Gromov. 2003. The First DNA Phylogeny of Four Species of *Mesobuthus* (Scorpiones, Buthidae) from Eurasia. Journal of Arachnology **31**:412-420.

Gantenbein, B., V. F. Fet, C. R. Largiader, and A. Scholl. 1999. First DNA phylogeny of Euscorpius Thorell, 1876 (Scorpiones, Euscorpiidae) and its bearing on taxonomy and biogeography of this genus. Biogeographica **75(2)**:49-65.

Gaskett, A. C. 2007. Spider sex pheromones: emission, reception, structures, and functions. Biological Reviews **82**:27-48.

Gertsch, W. J. and M. Soleglad. 1966. The Scorpions of the *Vejovis boreus* Group (Subgenus *Paruroctonus*) in North America (Scorpionida, Vejovidae). American Museum Novitates:1-54.

Hadley, N. F. 1990. Environmenatl Physiology. Pages 321-340 *in* G. A. Polis, editor. The Biology of Scorpions. Stanford University Press, Stanford, Ca.

Hadley, N. F. and S. C. Williams. 1968. Surface Activities of Some North American Scorpions in Relation to Feeding. Ecological Society of America **49**:726-734.

Haradon, R. M. 1983. *Smeringurus*, a new subgenus of *Paruroctonus*, Werner (Scorpiones, Vaejovidae). Journal of Arachnology **11**:251-270.

Haradon, R. M. 1984a. New and redefined species belonging to the Paruroctonus baergi group (Scorpiones, Vaejovidae). Journal of Arachnology **12**:205-221.

Haradon, R. M. 1984b. New and redefined species belonging to the Paruroctonus borregoensis group (Scorpiones, Vaejovidae). . Journal of Arachnology **12**:317-339.

Haradon, R. M. 1985. New Groups and Species Belonging to the Nominate Subgenus Paruroctonus (Scorpiones, Vaejovisae). Journal of Arachnology **13**:19-42.

Hjelle, J. T. 1990. Anatomy and Morphology. Pages 9-63 *in* G. A. Polis, editor. The Biology of Scorpions. Stanford University Press, Stanford, Ca.

Huelsenbeck, J. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics **17**:754-755.

Kaissling, K. E. 1997. Pheromone-controlled anemotaxis in moths *in* M. Lehrer, editor. Orientation and Communication in Arthropods. Birkhauser Verlag, Boston, Ma.

Kjellesvig-Waering, E. N. 1966. Silurian Scorpions of New York. Journal of Paleontology **40**:359-375.

Krapf, D. 1986. Contact Chemoreceptioon of Prey in Hunting Scorpions (Arachnida: Scorpiones). Zoologischer Anzeiger **217**:119-129.

Locket, A. 2001. Eyes and Vision. Pages 79-106 *in* P. H. Brownell and G. A. Polis, editors. Scorpion Biology and Research. Oxford University Press, New York, Ny.

Melville, J. M., S. K. Tallarovic, and P. H. Brownell. 2003. Evidence of Mate Trailing in the Giant Hairy Desert Scorpion, *Hadrurus arizonensis* (Scorpionida, Iuridae). Journal of Insect Behavior **16**:97-115.

Myers, C. 2001. Evolution of Geographic Variation in Body Size and Life-History Traits. Pages 317-333 *in* P. Brownell and G. A. Polis, editors. Scorpion Biology and Research. Oxford University Press, New York, Ny.

Nylander, J. 2004. MrModeltest (Program distributed by the author). Evolutionary Biology Centre, Eppsala University.

Parmakelis, A., I. Stathi, M. Chatzaki, S. Simaiakis, L. Spanos, and C. Louis. 2006. Evolution of *Mesobuthus gibbosus* (Brulle, 1832)(Scorpiones: Buthidae) in the northeastern Mediterranean region. Molecular Ecology **15**:2883-2894.

Persons, M. H., S. E. Walker, A. L. Rypstra, and S. D. Marshall. 2001. Wolf spider predator avoidamce tactics and survival in the presence of diet-associated predator cues (Aranae: Lycosidae). Animal Behavior **61**:43-51.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling **190**:231-259.

Polis, G. A. 1980a. Seasonal Patterns and Age-Specific Variation in the surface activity of a Population of Desert Scorpions in Relation to Environmental Factors. The Journal of Animal Ecology **49**:1-18.

Polis, G. A. 1980b. The Effect of Cannibalism on the Demography and Activity of a Natural Population od Desert Scorpions. Behavioral Ecology and Sociobiology **7**:25-35.

Polis, G. A. 1981. The Evolution and Dynamics of Intraspecific Predation. Annual Reviews of Ecology and Systematics **12**:225-251.

Polis, G. A. 1990a. Ecology. Pages 247-293 *in* G. A. Polis, editor. The Biology of Scorpions. Stanford University Press, Stanford, Ca.

Polis, G. A. 1990b. Introduction. Pages 1-9 *in* G. A. Polis, editor. The Biology of Scorpions. Stanford University Press, Stanford,Ca.

Polis, G. A. 2001. Population and Community Ecology of Desert Scorpions. Pages 302-316 *in* P. Brownell and G. A. Polis, editors. Scorpion Biology and Research. Oxford University Press, New York, Ny.

Polis, G. A. and R. D. Farley. 1979. Behavior and Ecology of Mating in the Cannibalistic Scorpion, *Paruroctonus mesaensis* Stahnke (Scorpionida: Vaejovidae). Journal of Arachnology **7**:33-46.

Polis, G. A. and W. D. Sissom. 1990. Life History. Pages 161-223 *in* G. A. Polis, editor. The Biology of Scorpions. Stanford University Press, Stanford, CA.

Posada, D. and K. Crandall. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics **14**:817-818.

Riddle, B. R. and R. L. Honeycutt. 1990. Historical Biogeography in North American Arid Regions: An Approach Using Mitochondrial-DNA Phylogeny in Grasshopper Mice (Genus onychomys). Evolution **44**:1-15.

Roberts, J. A. and G. W. Uetz. 2004. Chemical Signaling in a Wolf Spider: A Test of Ethospecies Discrimination. Journal of Chemical Ecology **30**:1271-1284.

Ronquist, F. and J. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**:1572-1574.

Shachak, M. and S. Brand. 1983. The relationship between sit and wait foraging strategy and dispersal in the desert scorpion, *Scorpio maurus palmatus*. Oecologia **60**:371-377.

Sissom, W. D. 1990. Systematics, Biogeography, and Paleontology. Pages 64-160 *in* G. A. Polis, editor. The Biology of Scorpions. Stanford University Press, Stanford, Ca.

Sissom, W. D. 2000. Family Vaejovidae. Pages 503-553 *in* V. Fet, W. D. Sissom, G. Lowe, and B. M. E., editors. Catalog of the Scorpions of the World (1758-1998). The New York Entomological Society, New York.

Sissom, W. D. and V. Fet. 2000. Family Iuridae Thorell, 1876. Pages 409-420 *in* V. Fet, W. D. Sissom, G. Lowe, and M. E. Braunwalder, editors. Catalog of the Scorpions of the World (1758-1998). New York Entomological Society, New York, Ny.

Sissom, W. D. and O. F. Francke. 1981. Scorpions of the Genus *Paruroctonus* from New Mexico and Texas (Scorpiones, Vaejovidae). Journal of Arachnology **9**:93-108.

Sissom, W. D. and W. R. Lourenco. 1987. The Genus Centruroides in South America (Scorpiones, Buthidae). Journal of Arachnology **15**:11-28.

Sokal, R. R. and F. J. Rohlf. 1995. Assumptions of Analysis of Variance. Pages 440-451 *in* R. R. Sokal and F. J. Rohlf, editors. Biometry. W. H. Freeman and Company, New York, NY.

Sonenshine, D. E. 1985. Pheromones and Other Semiochemicals of the Acari. Annual Review of Entomology **30**:1-28.

Stahnke, H. L. 1972. UV Light, A Useful Field Tool. BioScience **22**:604-607.

Steinmetz, S. B., K. C. Bost, and D. D. Gaffin. 2004. Response of male *Centruroides vittatus* (Scorpiones, Buthidae) to aerial and substrate-borne chemical signals. Ecuscorpius:1-6.

Stockwell, S. A. 1992. Systematic Observations on North American Scorpionida with a Key and Checklist of the Families and Genera. Journal of Medical Entomology **29**:407-422.

Swofford, D. 1999. PAUP*: Phylogenetic analyses using parsimony (* and other methods). Sinauer Associates, Sunderland.

Swoveland, M. L. 1978. External Morphology of Scorpion Pectines. San Francisco State University, San Francisco, Ca.

Tallarovic, S. K., J. M. Melville, and P. H. Brownell. 2000. Courtship and Mating in the Giant Hairy Desert Scorpion, *Hadrurus arizonensis* (Scorpionida, Iuridae). Journal of Insect Behavior **13**:827-838.

Tourtlotte, G. I. 1974. Studies on the biology and ecology of the northern scorpion *Paruroctonus boreus* (Girard). . The Great Basin Naturalist **34**:167-179.

Uetz, G. W. and G. Denterlein. 1979. Courtship Behavior, Habitat, and Reproductive Isolation in *Schizocosa rovneri* Uetz and Dondale (Araneae: Lycosidae). Journal of Arachnology **7**:121-128.

Vandermeer, J. H. 1972. Niche Theory. Annual Reviews of Ecology and Systematics **3**:107-132.

Vink, C. J., S. M. Thomas, P. Paquin, C. Y. Hayashi, and M. Hedin. 2005. The effects of preservatives and temperatures on arachnid DNA. Invertebrate Systematics **19**:99-104.

Warburg, M. R. and G. A. Polis. 1990. Behavioral responses, rhythms, and activity patterns. Pages 225-246 *in* G. A. Polis, editor. The Biology of Scorpions. Stanford University Press, Stanford, Ca.

Wyatt, T. D. 2003. Pheromones and Animal Behavior. Cambridge University Press, New York, Ny.

Yamashita, T. and V. Fet. 2001. Molecular Approaches to Biogeography. Pages 347-369 *in* P. Brownell and G. A. Polis, editors. Scorpion Biology and Research. Oxford University Press, New York, Ny.

Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. University of Texas, Austin.

BIOGRAPHICAL INFORMATION

Abraham Miller graduated with his BS in Biology from West Texas A&M University in 2001. He then attended the University of Tasmania in Australia where he received his Honors Degree in Zoology in 2002. Then he attended the University of Texas at Arlington where he received his PhD in Quantitative Biology in 2008. He will be joining the faculty of the University of Tampa, where he will teach Anatomy and Physiology. He hopes to continue to teach at the university level and pursue research on the behavioral ecology and geographical variation in scorpions.