Combining direct methods (PIT tags and radio-telemetry) with an indirect method (mtDNA) to measure movement and dispersal at different scales in North American tarantulas (*Aphonopelma* spp.).

by

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ABSTRACT

Despite their conspicuousness and importance as predators in arid and semi-arid ecosystems, almost nothing is known about the behavioral ecology and genetic structure of populations of North American tarantulas (Araneae, Theraphosidae). Additionally, two members of this genus are threatened and listed on CITES Appendix II. We used three methods of measuring movement and dispersal in these animals--the direct methods of radio-telemetry and passive integrated transponders, and the indirect method of mitochondrial DNA analysis.

We radio-telemetered 18 adult female tarantulas, *Aphonopelma hollyi*, in order to gather information regarding their time budgets, the effect of environmental factors on behavior, interactions between colonial females, and individuality in behavior between tarantulas. Date and cloud cover were the only environmental factors measured that affected activity levels in the tarantulas. Equilibrium behavioral frequencies indicated time budgets that indicate the spiders spent more than 96% of their time inside the burrow, even in their peak activity season. We introduce a novel method for measuring the individuality of behavior between animals by comparing individual equilibrium behavioral frequencies with the frequencies estimated for the entire study group. We found no indications of individuality in behavior in the tarantulas monitored.

We used Passive Integrated Transponders (PIT tags) as permanent, internal markers in order to monitor burrow fidelity and movement within a colony of the tarantula *Aphonopelma hollyi* over the span of 14 months. We used two data sets of weather variables in the analyses, one collected at the study site and the other collected 9.75 km from the study site. All the variables were statistically the same between the

data sets except for days of measurable precipitation per month. To determine how typical the period of the study was in relation to the previous ten years, we performed an ANOVA on data that included the past 10 years and there was no significantly different year found in the eleven years tested. Over the span of the 14 months of the study, all 16 tagged females abandoned their burrows. Using logistic regression we determined that days of measurable precipitation was the only weather variable to make a significant contribution in explaining the percentage of PIT-tagged females remaining in their burrow at each monthly time interval.

Because North American tarantulas are relatively unique in their natural history when compared to other spiders, even member of the same family, diversity studies previously published are not generally applicable to members of the genus *Aphonoplema.* Here we present the first data (16S) that can be used to evaluate specimens within the genus *Aphonopelma* for hierarchical structure in genetic diversity in order understand the long-term patterns of movement and dispersal in these animals. We were able to isolate contiguous 16S sequences from 27 animals that were 430 base pairs in length. We were able identify a total of 9 haplotyes present in the 27 animals analyzed and there were 28 polymorphic nucleotides found in the 27 16S sequences analyzed (6.4%). The only significant F_{ST} values were found at the generic level of analysis ($F_{ST} = 0.546$). There was no apparent relationship between geographic and genetic distance within the genus, although there are three clear divisions apparent in the neighbor-joining network, which we believe to represent natural species divisions. Our analyses show a pattern of divergence that is consistent with the presence of three species: *A. hentzi*, *A. hollyi*, and

an unidentified species *A.* sp. There was an average of 4.42% difference between species. We found no evidence of regionally selective pressures on the 16S gene.

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CHAPTER 1

Tarantulas (Araneae: Theraphosidae) are unique creatures in almost every aspect of their natural history. They live longer than any other family of spiders and take longer to mature sexually than any other spider. Although they are not socially interactive with each other, they choose to live in clustered arrangements of burrows that are informally called colonies. They molt their exoskelelton yearly, and the females continue to molt yearly after they reach sexual maturity. These post-maturity molts are another unique feature of the tarantula family. They are not only able to regenerate lost body parts and repair damage but because the females shed the sperm-storing spermathecae with the rest of the exo-skeleton, they are newly virginal each year.

As a family they are exclusively carnivores, although almost nothing else is known about their feeding behavior. They have only extremely limited maternal care and unlike most other spiders, they disperse completely on foot.

Unfortunately the state of the taxonomy in the family Theraphosidae is muddled and confusing and in a state of constant flux. Raven's revision of the infraorder Mygalomorphae (1985) established the basis for much of the current taxonomy; however, it addresses these issues at the level of the infraorder, which spans 15 families. Within the family Theraphosidae there are 13 subfamilies, including Theraphosinae. This subfamily includes 27 genera, one of which is *Aphonopelma*. Within *Aphonopelma* there are approximately 50 species. So structuring at the level of infraorder is not directly helpful at the level of population analyses. Some recent efforts (Longhorn et al. 2007) have addressed the phylogenetic structure within a sister genus, *Brachypelma*, but no such work has been completed for the genus *Aphonopelma*. Although several species within this genus are well-described (Baerg 1958; Prentice 1992, 1997), the majority of the species within *Aphonopelma* are poorly described or the descriptions are based on few to single samples (Smith 1994).

They are regarded as fearsome by many but are also prized possessions for some, as the booming pet trade will attest. Despite their conspicuous appearance and key carnivore position in some ecosystems, very little work has been done to elucidate the mechanisms and methods behind their natural history. This dissertation is focused on examining the movement and dispersal of North American tarantulas belonging to the

genus *Aphonopelma* (the sole genus within the United States). Each of the following chapters has been written as a journal publication so each has its own sections (Abstract, Introduction, Materials and Methods, Results, Discussion, and Literature Cited), and each is formatted according to each journal's specifications.

In Chapter 2, I describe an experiment in which passive integrated transponders were implanted in colonial females to monitor burrow fidelity. The females were monitored for more than a year, and burrow fidelity over that year was evaluated in regards to weather variables at the site such as precipitation, air temperature, and humidity. This chapter will be submitted to the Journal of Arachnology.

In Chapter 3, I present the results of a radio-telemetric effort to monitor nightly movements and activity of colonial females. Radio transmitters were attached to a group of females that were monitored for six complete time periods that include their most active periods. Again, the behavior of the animals was evaluated in respect to weather variables. This chapter will be submitted to Animal Behaviour and so is formatted with British English.

In Chapter 4, I evaluated the genetic diversity at hierarchical levels of organization within the genus using the mitochondrial gene 16S. I tested male migrating groups, colonial females, one confirmed species, and three apparent species within *Aphonopelma* for genetic structure and haplotype diversity. After expanding the dataset, this chapter will be submitted to Molecular Ecology.

The final chapter, 5, is a synthesis of the work accomplished. Additionally I discuss the implications of my work, failed experiments, and potential future directions.

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CHAPTER 2

To be submitted to the Journal of Arachnology

Using implanted passive integrated transponders to monitor long-tem burrow fidelity in a theraphosid spider, *Aphonopelma hollyi*.

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Abstract

Burrows serve a variety of purposes, ranging from providing environmental stability to safety from predators to a secluded place for recuperation or reproduction. Site choice and burrow fidelity are critical parameters to evaluate, as they determine the energy cost of burrowing to an animal in its lifetime. We used Passive Integrated Transponders (PIT tags) as permanent, internal markers to monitor burrow fidelity in the tarantula *Aphonopelma hollyi* over the span of 14 months. During this time span, all 16 tagged females abandoned their burrows. We analyzed how typical the period of the study was in terms of climate in relation to the previous ten years statistically, but we failed to reveal any significant differences in terms of precipitation or air temperature. Using logistic regression we determined that days of precipitation per month was the only significant variable explaining the percentage of PIT-tagged females remaining in their burrow at each monthly time interval ($p = 0.039$). It may be that tarantulas in this environment respond to instability in the soil substrate caused by intermittent heavy rainfall by abandoning their burrows.

Key words: PIT tags, behavior, tarantula, burrow abandonment, weather

Introduction

Burrowing is one of the most primitive characters found in the terrestrial ancestors of Class Arachnida (Decae 1984) and is still found in the most primitive members of that class. Especially in more arid regions, a safe, environmentally controlled burrow can be a critical component to survival (Crawford 1981). Burrows serve a variety of purposes, ranging from providing environmental stability to safety from predators to a secluded place for recuperation or reproduction. Although burrowing has been studied extensively in vertebrates (for a review see Kinlaw 1999), less attention has been given to burrowing in invertebrates.

Given that a burrow can play a crucial role in survival and/or reproduction, it is important to understand how burrow locations are selected and defended. Site choice and burrow fidelity are critical parameters to evaluate, as they determine the energy cost of burrowing to an animal in its lifetime. Burrowing arachnids typically manipulate soil using their chelicerae (Foelix 1996) or front walking legs (Wharton 1987), and the number of burrows dug in an animal's lifetime represents a significant portion of its energy budget; therefore, a burrow is highly likely to be placed with care and defended with vigor.

Tarantulas in the genus *Aphonopelma* burrow as juveniles and adults, and although some individuals may opportunistically utilize rock piles as refuge sites (Baerg 1958), subterranean burrowing is a significant component to their life history. Adult female tarantulas are found in colonies that represent clusters of burrows. Although there are no obvious interactions between females within a colony, spatial analyses of the dispersion of burrows (Reichling 2000, Janowski-Bell 2001) have shown that burrows

within colonies are under- or over-dispersed, indicating that burrows are located non-randomly.

Although some fossorial spiders are known to have fidelity to a single burrow for life, enlarging them as the spider grows (Miller and Miller 1987), this has not been studied extensively in tarantulas. We present here the first long-term study of tarantula burrowing behavior since Baerg's seminal work in 1958. We used Passive Integrated Transponders (PIT tags) as permanent, internal markers in order to monitor burrow fidelity in the tarantula *Aphonopelma hollyi* over the span of 14 months. In so doing, we also present here the first use of this marking technology in a wild population of arachnids.

PIT tags are small (< 0.2 g in mass, 12 mm in length), inert transponders about the size of a grain of rice. When the transponder is activated by a signal emitted by a hand-held reader, it transmits a unique 10-character identification code to the reader. PIT tags have been used in the commercial poultry (Jamison et al. 1999) and fisheries (Bruyndoncx et al. 2002) industries as well as in studies of wild animals (e.g. Eggert 2002, McCormick and Smith 2004). Once implanted, they are permanent because the life span of the transponder is virtually limitless. Because they are internal, they have minimal effects on animal behavior, and their small size makes them amenable to applications with small-bodied animals such as invertebrates.

Studies of tarantulas and other arachnids have been hampered by the lack of a unique marking technique that could be applied to a small-bodied animal and yet not be easily molted. PIT tags are small enough to be implanted into a large-bodied spider, such as a tarantula. Although a method was developed in 2001 (Reichling and Tabaka) to

insert PIT tags into tarantulas in order to permanently and uniquely identify them, our study represents the first application of the procedure in natural populations of arachnids.

Methods and Materials

Our study site was on a privately owned cattle ranch in Briscoe Co., Texas. The tarantula colony was located next to a playa, a flat-bottomed depression that held water for the duration of the study. The total area of the colony approached 2 km^2, although the tarantulas examined in this study were in burrows within a smaller subset of this area (~0.5 km^2) (Figure 1).

In July and August 2006, female tarantulas were collected from their burrows and taken to the lab. While the females were in the lab, their burrows were plugged with tissue paper to prevent them from being co-opted by others. The females used in this study weighed between 4.39 - 8.32 g (average = 5.94 g). We did not use any females weighing < 4 g, as that was determined to be the lower weight limit for safe implantation of a PIT tag (Reichling and Tabaka 2001). The PIT tags represented ~1.6% of a female's average body weight.

In the lab, the PIT tags were implanted into the female tarantulas following the procedure described in Reichling and Tabaka (2001). Of 29 attempted implantations (including trial study data not included in our statistical analyses), 27 were successful. The two unsuccessful implantations resulted in excessive fluid loss that could not be staunched, and so the animals were euthanized. After implantation, the tarantulas were monitored for a day and then fed one commercially bred cricket. Two to three days after implantation, the marked spiders were returned to their burrows. Thereafter, the burrows

were monitored one time per month for 14 months to determine if the marked spider was still in residence. If a tarantula was found to not be in the burrow, the immediate area was searched using the hand-held PIT tag reader. The hand-held reader was able to read the tags when the spider was in the burrow only if the spider was directly under it, so if the spider was too far from the entrance to be visible, we used a long piece of grass to tease the spider up to the entrance. If the spider was not located in its burrow, an area of approximately 10 square meters was searched for the missing spider.

We hypothesized that the predominant influences on fidelity to burrows would be one or more weather variable. We used two sets of weather data to test this hypothesis. One set was obtained from a NOAA weather monitoring station located in Silverton, TX, 9.75 km from the study site. Air temperature (C°) and wind velocity (km h^{-1}) were measured every five minutes and precipitation was recorded daily, compiled into monthly totals. Additionally, the total number of days per month that measurable precipitation occurred were compiled. In addition to data collected during the time of the study, we also retrieved yearly summary data for the year of the study and for the preceding decade, annual summaries that were unavailable in the other data set from a Mesonet weather station located in the playa directly beside our study site (Table 1). Air temperature (C°), wind speed (km h^{-1}) and relative humidity (%) were measured every five minutes and precipitation was recorded daily; as with the NOAA data, data were compiled into monthly totals and also included total number of days per month with precipitation.

Since not all the variables were normally distributed, analysis of the possible discrepancies between the two weather data sets was done using a Kruskal-Wallis nonparametric one-way analysis of variance. Data collected for the year of the study and

the previous nine years were analyzed using an ANOVA test to determine if the weather experienced during the course of the study was unusual. The effect of the weather variables on the spiders' fidelity to their burrows was investigated using logistic regression analysis that included backwards stepwise elimination of variables. Weather variables were analyzed using both monthly increments and divided into seasons. All analyses were done with the InStat 1.12 program for Macintosh (GraphPad Software) except for the logistic regression, which was completed using the program R (R Development Core Team 2007).

Results

We used both data sets in our analyses to evaluate different aspects of the relationship between weather and the rate of burrow fidelity in tagged females. However, we also wanted to determine how closely matched the two data sets were, as one was from a Mesonet station on the study site itself and the other was from a NOAA weather station 9.75 km away (NOAA 2008). In terms of maximum air temperature, minimum air temperature, relative humidity, cumulative precipitation and days of measurable precipitation per month, both data sets were statistically the same as determined by the nonparametric ANOVA performed with the Kruskal-Wallis test ($p = 0.992$).

To determine how typical the period of the study was in relation to the previous ten years, we performed an ANOVA test on data retrieved from the NOAA weather station nearby. There was no significantly different year found in the 11 years tested ($F = 1.59$, $df = 49$, $p = 0.154$).

Over the span of the 14 months of the study, all 16 tagged females abandoned their burrows (Figure 2). To determine the effect of the weather variables on rates of burrow fidelity, we used a backward elimination logistic regression analysis, and to correct for muliticollinearity with maximum air temperature, we removed minimum air temperature and relative humidity. The logistic regression model that included maximum air temperature, cumulative monthly precipitation and days of precipitation per month was optimal in predicting the number of PIT-tagged females abandoning their burrow at each monthly time interval ($p = 0.054$). Days of measurable precipitation (Figure 3) was the only variable that was found to make a significant contribution ($p = 0.039$) in explaining burrow occupancy.

Discussion

As other behavioral studies have found, it is much more difficult to tease apart the combined effects of localized factors such as environment and nearest-neighbor distance on individuals within a population than it is to evaluate the average behavior of that population (Gordon 1997). The non-random placement of burrows within a colony suggests that burrow location is driven by some deterministic factor, such as selection for certain environmental traits. Attempts to characterize the variables responsible for habitat selection have been successful in other invertebrates (Wharton 1987, Sinclair et al. 2001); however, the one attempt to discern optimal environmental variables, such as vegetation, prey density or soil characteristics, for the genus *Aphonopelma* failed to reveal any traits that were associated with the location of colonies (Janowski-Bell 2001).

We were fortunate to have two weather data sets available, as they were both useful in different ways. The NOAA data set collected 9.75 km from the study site allowed us to compare the yearly averages for maximum air temperature, minimum air temperature, precipitation and days of precipitation. By comparing the averages for the years that included our study with the previous ten years, we were able to determine that the weather conditions during our 14-month study were not unusual. We can therefore assume that the abandonment rates for the burrows we included in our study were typical based on the influences of weather.

Although we were able to use the NOAA data set to eliminate the possibility that the time of our study experienced unusual weather, we determined that this data set was not completely reliable in reporting conditions at the study site. When we compared the NOAA data set with the Mesonet data set collected at the study site itself, we found that the two differed statistically in number of days per month with measurable precipitation and cumulative totals of precipitation. Evidently air temperature is a parameter that is likely to be the same across a distance as far as 9.75 km, whereas rainfall is much more localized and specific to a site. This is not surprising, given that most of the precipitation in this area comes as localized convective thunderstorms from April-September. And although we were unable to compare measurements of wind velocity (since these data were not available from the NOAA site), we hypothesize that this parameter is also too variable to be estimated accurately from data collected away from the actual study location. Numerous studies have found that scale of the physical environment is a critical factor in any mark-recapture study and can significantly affect the results (Bell et al. 2001, Schneider 2003). Clearly, this effect of scale extends to measures of weather

variables. Our results emphasize the importance of on-site measurements whenever possible in studies attempting to evaluate animal behavior at this fine of a scale.

Based on previous observations of the long-term fidelity of tarantulas to their burrows, we were unprepared for the high rate of burrow abandonment observed in this species (100%). In 2003 and 2004 we completed a pilot study in which we implanted 11 adult tarantulas and marked the locations of their burrows. Upon our return to the study site one year later, all 11 had abandoned their burrows and were not found in a localized search; thus the present study was designed on a smaller temporal scale (monthly observations). Even so, burrow abandonment was commonplace and may have been due to perceived stress from being implanted with a PIT tag. Stress may force a spider to abandon its burrow for more secure quarters.

The only weather variable found to be a significant predictor of burrow abandonment in our study was days of precipitation per month. This was true both when we organized the data by month and also when we organized the data by season. This colony of *Aphonopelma hollyi* is established adjacent to a playa, which collects precipitation runoff in the area. It may be that frequency of precipitation events and subsequent runoff has an influence on the structural integrity of the burrows and subsequently on the rate of burrow abandonment by the tarantulas. Occasionally, we found burrows that had completely collapsed. Additionally, the colony we used in our pilot study was directly adjacent to an area that remained flooded for more than 50% of the study period. It may be that the relatively high rates of burrow abandonment observed here are not a behavior typical of *Aphonopelma hollyi* but simply a response to environmental instability due to a water-soaked substrate.

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Figure 1

The locations of burrows within the study colony. The PIT-tagged animals are represented by triangles; non-study burrows are represented by circles. The position of the Mesonet weather station is indicated by the asterisk

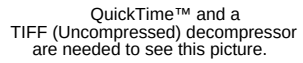

Figure 2

The rate of attrition from the burrows over time. The X-axis represents the number of tagged females that remained in their burrows on each of the study site visits

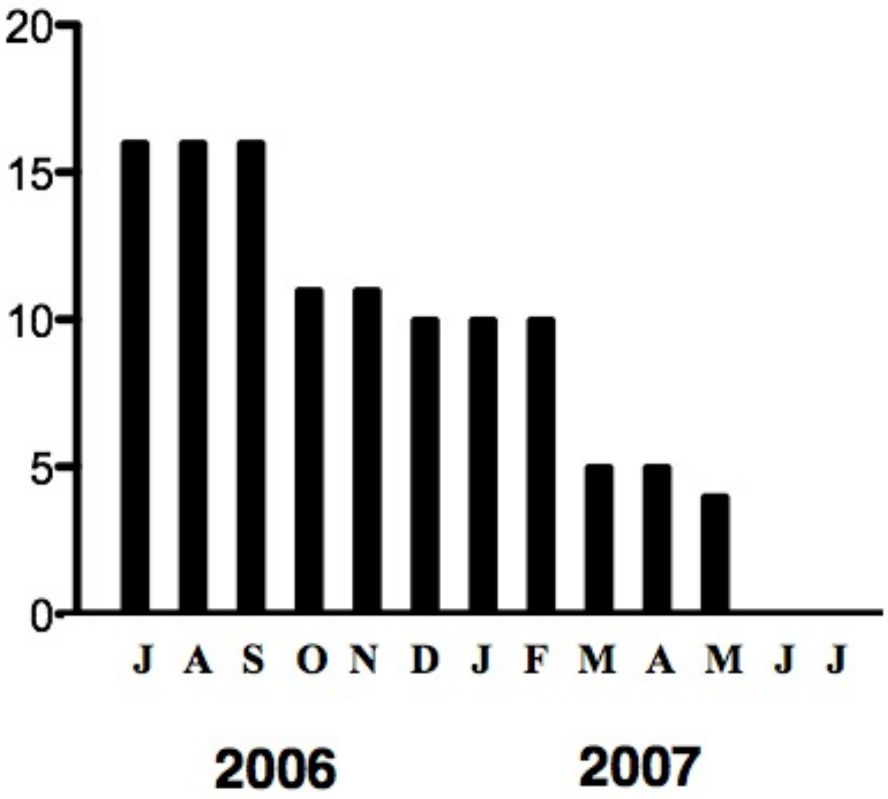

Figure 3 Histogram of the precipitation from the Mesonet station located at our study site

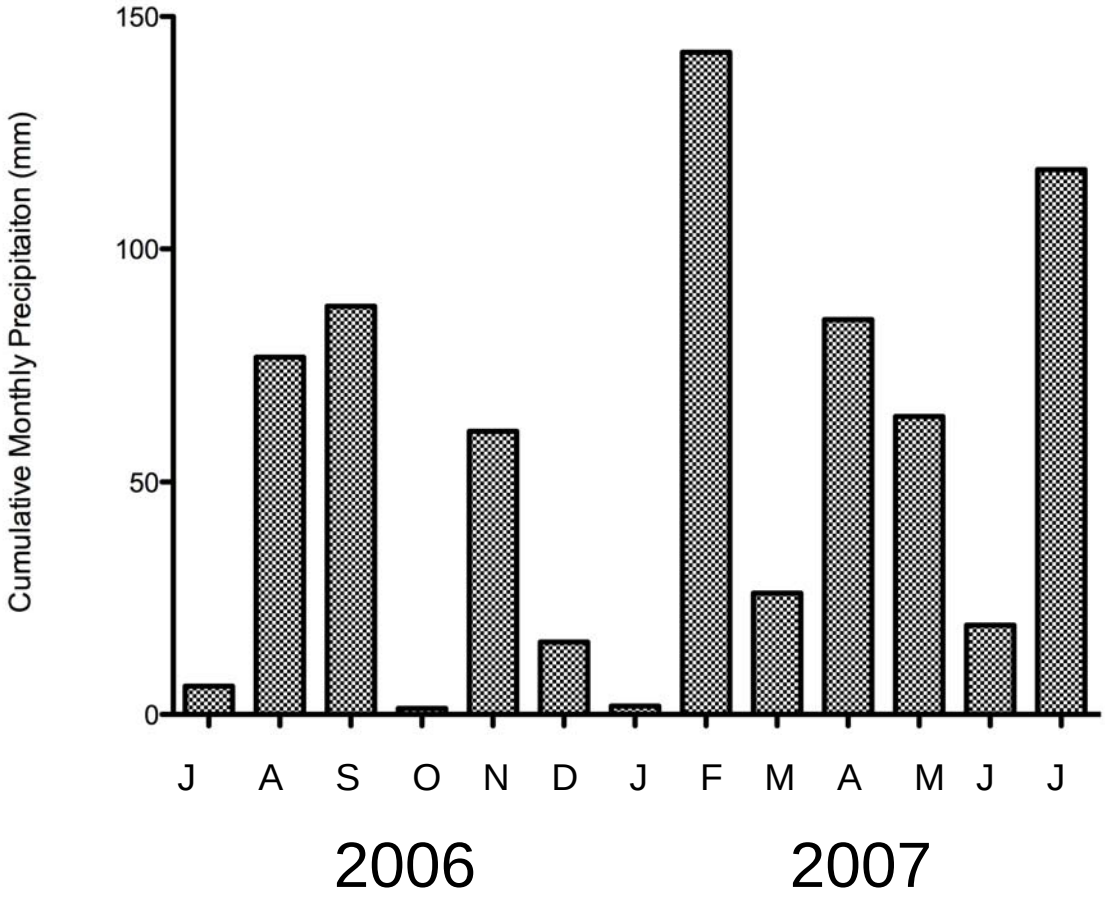

Table 1

Summary of seasonal weather data from the Mesonet station at the study site (Summer 2006 through Summer 2007)

Variable	Summer Mean ± S.D.	Fall Mean ± S.D.	Winter Mean ± S.D.	Spring Mean ± S.D.	Summer Mean ± S.D.
Wind speed (km h^{-1})	12.6 ± 4.0	13.3 ± 5.0	14.0 ± 6.7	16.6 ± 9.3	13.8 ± 4.7
Relative humidity (%)	54.0 ± 16.9	62.9 ± 17.5	70.4 ± 20.0	68.0 ± 19.7	67.0 ± 14.1
Maximum air temperature (C°)	33.4 ± 2.9	22.3 ± 5.9	13.8 ± 8.2	19.2 ± 6.9	29.6 ± 2.7
Minimum air temperature (C°)	18.7 ± 2.2	6.9 ± 6.5	-4.4 ± 4.3	7.5 ± 6.0	16.9 ± 1.8

CHAPTER 3

To be submitted to the journal Animal Behaviour

Using radio telemetry and transition matrix modeling to study the behavioural ecology of cryptic animals: An example with North American tarantulas, *Aphonopelma hollyi* (Araneae, Theraphosidae).

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Abstract

A cryptic lifestyle size poses challenges to the study of animal behaviour. For example, despite their conspicuousness and importance as predators in arid and semi-arid ecosystems, almost nothing is known about the behavioural ecology of North American tarantulas (Araneae, Theraphosidae). We used radio-telemetry to track 18 adult female tarantulas, *Aphonopelma hollyi*, in order to gather information regarding their time budgets, the effect of environmental factors on behaviour, interactions between colonial females, and individuality in behaviour between tarantulas. We introduce a novel method for measuring the individuality of behaviour between animals by comparing individual equilibrium behavioural frequencies with the frequencies estimated for the entire study group. Only 42 out of 1096 observations recorded behaviours outside the burrow, and no interactions between colonial females were observed. Date and cloud cover were the only environmental factors measured that affected activity levels. Equilibrium behavioural frequencies of time budgets indicated that the spiders spent more than 96% of their time inside the burrow, even during their peak activity season (summer). This high level of difference between time spent in the burrow and all other behaviours severely reduced the power in our analyses, so we found no indications of individuality in behaviour among the tarantulas we observed.

Keywords: behavioural matrices, remote sensing, spider, invertebrate

Introduction

The behavioural ecology of most animals is incompletely understood, particularly for cryptic species, and especially for arthropods. This has been due in large part to our inability to locate and distinguish individuals from a distance that is far enough away to avoid changing their natural behaviours. For example, despite their conspicuousness and importance as predators in arid and semi-arid ecosystems, almost nothing is known about the behavioural ecology of North American tarantulas (Araneae, Theraphosidae). These long-lived invertebrates live in fossorial groups, informally called colonies (clusters of burrows), that are known to consist of females and immature males (Baerg 1958) and are thought to consist of a female and her female relatives and progeny. Because adult females are sit-and-wait predators that are thought to rarely leave the confines of their burrows, it is generally believed that members of a colony do not interact. However, studies have shown that burrows within a colony are not randomly distributed, instead being either under-dispersed (Kotzman 1990; Bradley 1996; Reichling 2001) or over-dispersed (Janowski-Bell 2001), both of which would imply some effect of neighbours on burrowing females. Given these patterns, we hypothesized that adult female tarantulas may be more interactive than previously thought.

In order to study such potential interactions, it is necessary to be able to identify animals as individuals. Traditional mark-and-release techniques are limited in their value with tarantulas because spiders can molt off standard external markings. Additionally, pilot work completed using fluorescent powdering to track the movements of individual tarantulas was unsuccessful: perhaps due to the structure of the urticating hairs covering

the abdomen, the powder did not provide a trail of more than 10 meters in any of 14 trials.

Use of internal markers that cannot be shed (e.g. Passive Integrated Transponders, or PIT tags) is possible, but these markers cannot be used to locate, follow and monitor animals from a distance. Moreover, because tarantulas are not visible when in their burrows and are often secretive and cryptic when outside the burrow, an electronic method of monitoring position is required. Harmonic radar has been used with success on some arthropods (Lovei et al. 1997; Capaldi et al. 2000) but presents problems such as the inability to identify specific individuals because of a lack of individual frequencies, and limited detection distances (Lovei et al. 1997). In contrast, radio telemetry can be used to both uniquely mark and follow animals. Until recently, however, this technology was simply too large to be used on small-bodied animals.

In the past decade, radio telemetry of arthropods has become possible with the ongoing miniaturisation of transmitters. Prior to this recent miniaturisation, radio transmitters were too large to be placed on small animals without significantly affecting their behaviour. Now, however, there are transmitters that weigh <0.5 g. Even though this may still represent an enormous percentage of the weight of some very small animals, arthropods are well-known for their ability to carry burdens that would stagger vertebrates. For example, Lorch et al. (2005) compared movements of outbreak and non-outbreak katydids and determined that carrying a radio transmitter had no apparent effect on short-term movement behaviours such as speed, distance traveled and turning, even though their study was conducted with animals carrying a transmitter that was approximately 50% of their body weight. Other studies have similarly shown that the

weight of radio transmitters did not affect dispersal distances in beetles (Riecken and Raths 1996; Hedin and Ranius 2002). As a result of these technological advances, radio telemetry has been used in several recent studies of arthropods to monitor the migration of endangered beetles (Rink and Sinsch 2007) as well as to quantify flight distances of beetles that cause damage to commercial ventures (Beaudoin-Olliver et al. 2003).

Radio telemetry can provide data on the habits and activity budgets of tarantulas that are critical to our understanding and conservation of these animals. Two theraphosid genera and 22 species are threatened and listed on Appendix II of CITES, primarily due to heavy collection for the pet trade. These two genera are the only araenids listed on any of the CITES Appendices and include two species in the genus *Aphonopelma*: *Aphonopelma albiceps* and *Aphonopelma pallidum*. The tarantula pet trade is booming, and it is highly likely that other species within this genus and other tarantula genera will be similarly listed in the future. Therefore, measuring and understanding individuality and the suite of individual behaviours present in a population could allow us to make predictions regarding higher-level interactions between populations and between species (Real 1994). To date, there are no studies that we are aware of that address individuality in the behaviours of these animals.

Radio telemetry has been used to follow another species of the genus *Aphonopelma*: sexually dispersing males in the species *Aphonopelma hentzi* were tagged with radio transmitters to evaluate directedness and distance of travel (Janowski-Bell and Horner 1999). These males could be relocated after 24 hours, even when they had moved significant distances (e.g. 1300 m). Our study, in contrast, focused on behaviors of adult females of *Aphonopelma hollyi*. We used radio telemetry to measure nightly movements

of adult female tarantulas established in colonies to gain not only baseline information about their nocturnal behaviours and potential interactions but also to assess independence in activity budgets among individuals.

Regardless of the technology used to study animal behaviours, our analyses (for all species and not merely cryptic ones) are compromised if we fail to account for temporal autocorrelation in activity: because an animal performs a series of behaviours, the transition from one behaviour to another may not be independent. Therefore, we also adapted behavioural transition matrix modeling to the analysis of behavioural suites in tarantulas.

Methods and Materials

Our study site was on a privately owned cattle ranch in Briscoe Co., Texas, USA. The tarantula colony was located next to a playa, a source of water for the duration of the study. The total area of the colony was ~2 km^2, although the tarantulas used for this study were in burrows within a smaller subset of this area (~0.5 km^2) so as to better sample individuals that could potentially interact with each other (Figure 3).

We used Advanced Telemetry Systems (Isanti, MN, USA) transmitters (model # A2415) that were configured at unique frequencies ranging from 150.000 to 151.999 MHz. Battery life for these transmitters was approximately 21 days. We used Advanced Telemetry Systems R2000 receivers and 3-element Yagi antennas to determine the animals' positions.

A trial run was performed with five animals in early June 2007 in order to test the lifespan of the transmitters and to make sure the signal would transmit from within the

burrow. Female tarantulas were collected from their burrows and weighed before transmitters were attached. The females used in this study weighed between 4.52 and 10.42 g (average = 7.38 g) and the transmitters weighed between 0.47 and 0.51g (average = 0.49 g), making the transmitter, on average, an increase of 6.6% of a female's body weight. We attached the transmitters by manually restraining the tarantula and using gel formula cyanoacrylate instant adhesive (Superglue©) to affix the transmitter to the right posterior portion of the cephalothorax (Figure 4). We tagged 18 adult female tarantulas with transmitters, after which they were returned to their burrows and allowed two days to equilibrate before monitoring began.

All 18 study subjects were monitored at 45-min intervals during each observational period. The periods of observation were divided into two shifts: 8 p.m. to 2 a.m., and 2 a.m. to 8 a.m. All 18 spiders were monitored each shift, so every tarantula was observed from 8 p.m. to 8 a.m. within a 48-hr span. This was repeated 6 times from 10-28 July 2007, resulting in 1296 potential data points (72 readings for each tarantula). Due to lightning and tornadic storms, however, monitoring was interrupted or delayed on occasion, so we were able to take 1206 readings during the 19-day study. We recorded the distance of each tarantula from its burrow as well as whether it was engaged in one of four mutually exclusive behaviours: in the burrow, walking, sitting or hunting. "In the burrow" (B) was defined as when the animal was inside the burrow: only in evaluating the effect of environmental factors did we differentiate positioning in the mouth of the burrow and in the chamber of the burrow. "Walking" (W) was defined as when the animal was outside the burrow and walking. "Sitting" (S) was defined as when the animal was outside the burrow but not moving. "Hunting" (H) was defined as when the

animal was outside the burrow and actively handling prey as opposed to simply walking or sitting.

Behavioural transitions (i.e., the number of times a spider followed one particular behavior sequentially by another) were compiled from all observations into a transition matrix to establish the overall time budgets and the mean transition probabilities for the eighteen animals in the study as well as the populations studied as a whole. These were estimated and evaluated using the Matlab functions SpiderBehaviorAnalysis, SequencetoTransition, OverallTransitionsProbs, and TimeBudgetDiffs (Strauss 2008). These distances were then visualized using a neighbour-joining tree.

Every 45 minutes, air temperature, humidity and wind speed were recorded using a Kestrel 3000 Weather Meter (Boothwyn, PA, USA). To gain an approximation of available light during each behavioural reading, we visually estimated cloud cover (0-24%, 25-49%, 50-74%, and 75-100%) and phase of the moon for each period of observation. The effects of environmental factors on spider behaviour were analysed using multiple regression and ANOVA with SAS software (SAS 2004).

Results

Only 42 instances of movements outside the burrow were observed. Most of these were short (mean = 6.43 cm, max = 40 cm). Four of the 18 females were never observed outside of the burrow.

Using the behavioural transition probabilities (Table 2), we estimated the equilibrium frequencies for the four behaviours to be: B 96.5%, H 0.2%, S 2.8% and W

0.05%. The behavioural transition matrix was used to construct an ethogram representing the time budgets of these animals (Figure 3).

The estimated transition probabilities were tested against the null hypothesis that a single sequence of behavioural states is consistent with multinomial expectations using a first-order Markov process. We found that our data were consistent with the multinomial probabilities expected due to randomness ($p = 0.99$ with a mean square error of 0.00175). Due to the small number of animals exhibiting behaviour away from the burrows, we found no significant pattern in the behavioural transitions observed during this study (Figure 5).

To assess for variation in behaviour among the individual tarantulas, we first calculated the equilibrium frequencies for each spider and then compared those frequencies to the equilibrium frequencies calculated for the population as a whole. We estimated a distance matrix based on mean squared differences in individual equilibrium frequencies relative to those calculated for the population as a whole. Although five spiders (ID #s 1, 9, 20, 18 and 12) could be differentiated from the rest in the neighbor-joining analysis, the differences were found to be due to levels of activity during the study rather than to differences in behavioural transitions (individuals 1, 9, 20, 18 and 12 exhibited more unique behaviours, chiefly hunting, and more unique behavioural transitions than the members of the other "clade" in Figure 6. The preponderance of behaviours being categorised as B for all spiders, however, meant that the two "clades" could not be statistically differentiated.

MANOVA revealed that Julian date ($p = 0.002$) and cloud cover ($p = 0.029$) significantly affected behaviour. Follow-up ANOVAs showed that the date influenced

time spent in the mouth of the burrow and walking, whereas cloud cover influenced walking (Table 3). Tukey's post-hoc tests showed that the females spent more time in the mouth of the burrow during the latter portion of the study, and more time was spent walking when it was cloudier, although no walking was observed when cloud cover was >75%.

Discussion

North American tarantulas are inactive during the winter months and gradually become more active as summer approaches. The increase in activity in the later part of our study is most likely due to the fact that late summer and early fall is the peak breeding season for many species of tarantula in north Texas and may reflect the females' increased availability to dispersing males. It may also be that as autumn approaches, these animals increase their activity levels in order to take advantage of higher prey availability during the summer in preparation for colder weather.

The percentage of cloud cover had a significant effect on behaviour, likely due to these ectothermic poilkilotherms being sensitive to weather conditions that could adversely affect them. Desiccation is a significant danger to all animals living in arid and semi-arid regions, and terrestrial arthropods are especially susceptible due to a relatively high surface area to body mass ratio. Increased cloud cover would provide increased protection from the desiccating action of the sun and predators such as skunks and hawks whereas cloud cover greater than 75% could indicate on-coming storms, presumably detected by the tarsal organs of the spiders that are sensitive to relative air humidity (Foelix 1996).

Unfortunately, the overwhelming number of observations of the tarantulas in the burrow in relation to observations of a tarantula engaged in any activity outside the burrow greatly reduced the statistical power of our analysis. More than 96% of all observations found the tarantula in the burrow, so we were unable to reject the null hypothesis that the data pooled from all 18 tarantulas was the result of randomness in their behavioural transitions. Behavioural matrices have been used in studies of behaviour ranging from fruit flies (Meffert and Bryant 1992; Chen et al. 2002) to primates (Silk 1999) and they are an excellent method to measure and evaluate animal behaviour. However, to be an effective tool with these animals, we would have to increase the behavioural categories as well as the number of animals, the number of observations in each 12-hour period and the length of the study.

These factors highlight the intrinsic limitations to these kinds of studies with fossorial animals such as tarantulas. Using radio telemetry, we were able to locate the tarantulas when they were not visible in their burrows, but we were not able to record their actual behaviour underground since we could not see what they were doing. The cost of transmitters is decreasing along with the size; however, investigators are still limited by the amount of time required to locate each individual versus the number of investigators. Insufficient sample size was also a limiting factor for Janowski-Bell and Horner (1999), who were unable to make any significant conclusions regarding the directedness of travel in dispersing males in this same genus. We feel that, unless endowed with unlimited resources, researchers may be more successful in evaluating most aspects of the behavioural ecology of these animals using lab studies. Although lab-based studies are inherently limited by their artificial nature, the natural history and

secretive nature of theraphosid spiders will continue to restrict the value of field-based studies. However, further studies using both radio-telemetry and matrix modeling behavioural analysis have the potential to shed light on many uninvestigated aspects of these animals' natural history.

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Table 2

Equilibrium transition probabilities for the entire sample with 95% confidence intervals

	Burrow	Hunting	Sitting	Walking
Burrow	0.9773 ±0.0043	0.0025±0.0015	0.0185±0.0039	0.0017±0.0012
Hunting	1.0000	0	0	0
Sitting	0.5888±0.0852	0	0.3235±0.0802	0.1176±0.0553
Walking	0.8333±0.1521	0	0.1667±0.1521	0

Table 3

Significant results of the ANOVA analysis of the effect of weather variables on the behavior of the study animals

Behavior	Independent variable	df	F	P-value
At burrow mouth	Date	4	3.17	0.023
Walking	Date	4	4.04	0.007
Walking	Cloud cover	3	3.98	0.014

Figure 4

Each symbol represents a tarantula burrow within the colony; star symbols represent the burrows of telemetered individuals

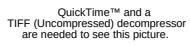


Figure 4

Adult female tarantula with transmitter attached

Figure 6

Ethogram representing the equilibrium behavioural transitions among all spiders observed: in the burrow (B), sitting (S), walking (W) and hunting (H). The size of the circles represent the frequency of the tarantula continuing the same behaviour from one observation to the next. The numbers next to the circles and arrows indicate the percent of the time that the animals made a particular behavioral transition, and the direction of the arrow indicates the direction of the transition

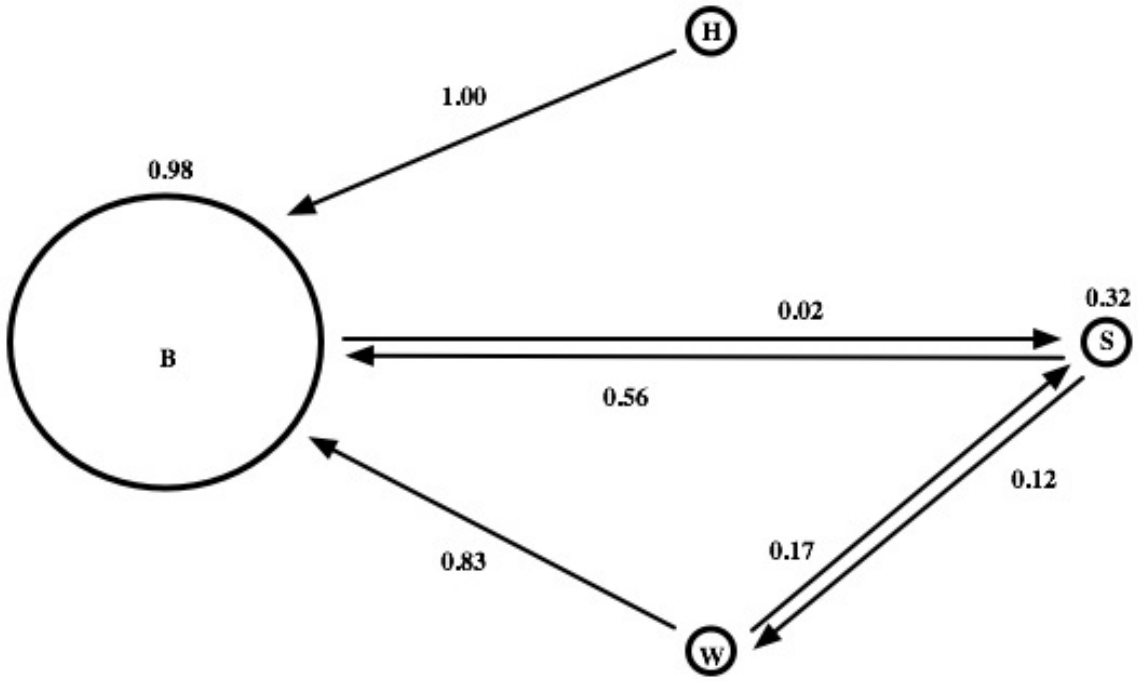
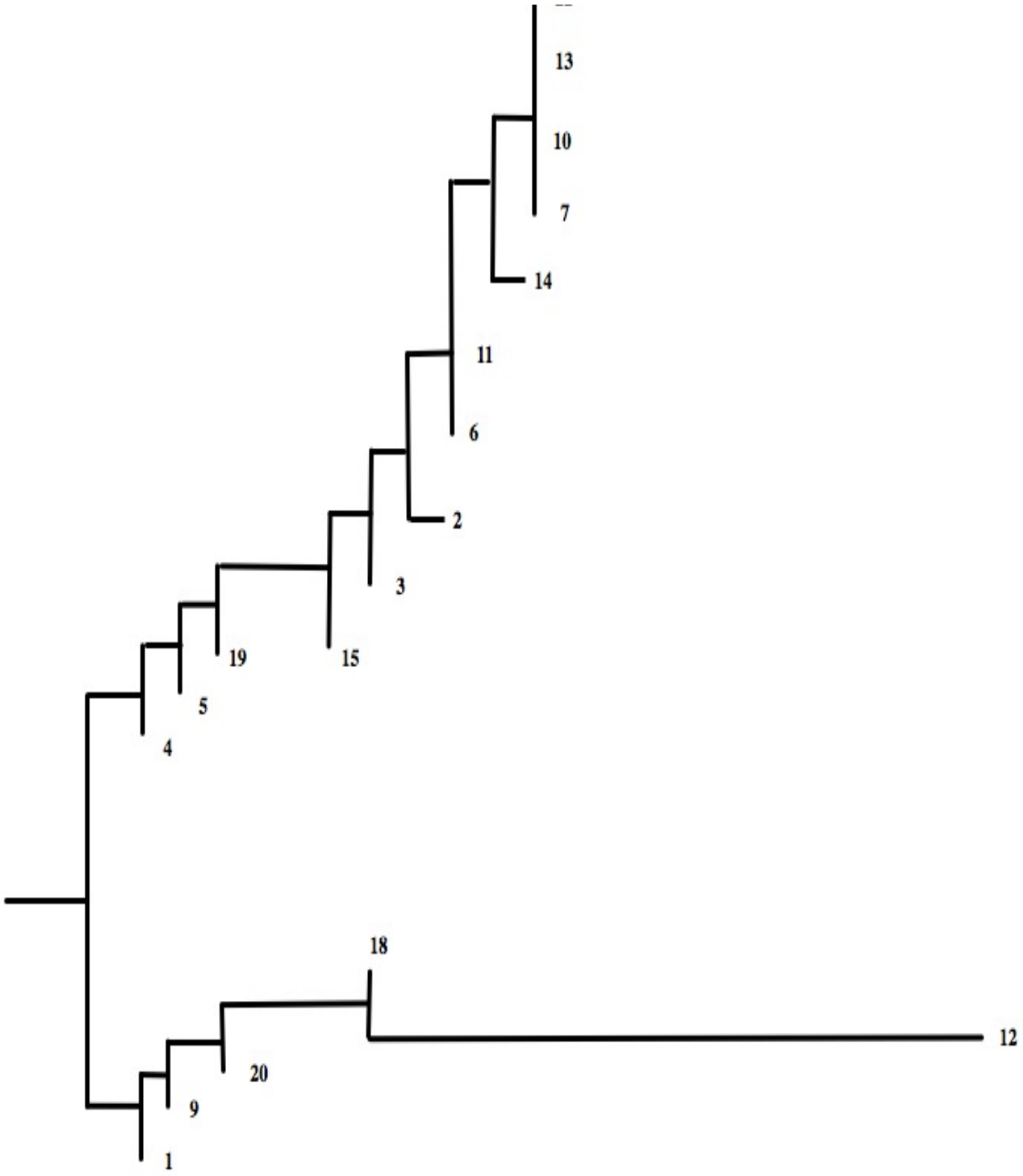


Figure 7

Neighbour-joining tree representing the distances calculated between an individual's behavioural frequencies compared with overall population frequencies. Each number represents an individual animal



CHAPTER 4

This chapter will be submitted to the journal Molecular Ecology

A hierarchical study of genetic diversity in the genus *Aphonopelma* (Araneae) at the colonial, species and genus level using the mitochondrial large subunit rRNA gene (16S).

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Abstract

Because North American tarantulas are relatively unique in their natural history when compared to other spiders, even member of the same family, studies previously published are not generally applicable to members of the genus *Aphonopelma.* Here we present the first molecular data that can be used to evaluate specimens within the genus *Aphonopelma* for hierarchical structure in genetic diversity in order understand the long-term patterns of movement and dispersal in these animals. We were able to isolate contiguous mitochondrial rRNA 16S sequences from 27 animals that were 430 base pairs in length. We were able identify a total of 17 haplotyes present in the 27 animals analyzed and there were 28 polymorphic nucleotides found in the 27 16S sequences analyzed (6.4%). The only significant F_{ST} values were found at the generic level of analysis (F_{ST} = 0.546). There was no apparent relationship between geographic and genetic distance within the genus; although there are three clear divisions apparent in the neighbor-joining network, which we believe to represent natural species divisions. Our analyses show a pattern of divergence that is consistent with the presence of three species: *A. hentzi*, *A. hollyi* and an unidentified species *A. sp* with an average of 4.42% difference between species. We found no evidence of regionally selective pressures on the 16S gene.

Keywords: sympatry, haplotype, tarantula

Introduction

Tarantulas belong to one of the most easily recognized but paradoxically least-studied families of spiders in North America. The common name of tarantula is applied in North America to members of the family Theraphosidae, genus *Aphonopelma*. Theraphosidae is regarded as a primitive, morphologically conserved family of spiders belonging to the suborder Mygalomorphae.

Both sexes of these spiders take at least 7 years to mature, and after maturation the females may live 13 more years; the males typically die after a single year of maturity (McCook 1887, Baerg 1958, 1962). They molt annually, allowing for repair to damaged body parts. Females and immature males construct subterranean burrows. Both sexes are considered to be opportunistic sit-and-wait predators and generally do not range far from the burrow while it is being inhabited.

When males mature, they leave their burrows in search of mates. Individual migrating males can be seen on country roads from June to November, and in some species mass migrations of as many as one hundred males have been reported (Baerg 1958). Despite efforts to track them using radio-telemetry (Janowski-Bell and Horner 1999), however, it is still unknown how far the males are able to travel to find suitable mates. Unlike most other spiders, tarantulas do not employ ballooning for dispersal; instead, they travel over land completely on foot.

Tarantulas live in groups that are informally called colonies (clusters of burrows) that are thought to consist of a female and her female relatives and progeny. Colonies are known to consist of females and immature males (Baerg 1958). However, the idea that tarantulas live in true, genetic colonies has not been confirmed by scientific analysis. In a

recent study of Texas tarantulas, it was determined that the burrows were over-dispersed, which may be an indication of aversion between individuals (Janowski-Bell 2001).

The past two decades have produced many analyses of spider populations ranging from phylogenetic analyses (e.g.: Hedin 1997; 2001, Vink et al. 2002) to more fine-scale population analyses (e.g.: Roeloffs and Reichert 1988, Schaefer et al. 2001). Most studies have been completed on spiders belonging to the infraorder Araneomorphae and since members of this suborder are able to balloon (release strands of silk to catch wind currents and float to new locations) the resulting genetic structure is not especially comparable to tarantulas, which are completely non-volant and disperse solely on land. Additionally, although tarantulas frequently live in aggregated collections of burrows (informally called colonies) they are almost completely non-social, another difference in life history that will affect the resulting genetic structure of a population: therefore most of these studies have little relevance to this family. Although there have been several studies focusing on this infraorder, Mygalomorphae (Bond 2004; Bond et al. 2001, 2006; Woodman et al. 2005), the differences between these studied mygalomorphs and the tarantula family Theraphosidae in aspects of natural history (i.e., relatively long-distance migration by males and extended time to maturity and total life span) make these studies limited in applicability as well. For example, Bond et al. (2006) combined geospatial and genetic data for the mygalomorph trapdoor spider genus *Apostastus* and were able to statistically define a relationship between geographic distance and genetic distance, indicating an isolation-by-distance model of gene flow. However, they inferred that the limited dispersal capability of females was a significant factor in being able to predict gene flow by geographic proximity. This model of dispersal does not necessarily apply

to members of the genus *Aphonopelma*, as they have 5-7 years between hatching and sexually maturity in which they could disperse significant distances before contributing to the gene pool.

The only published study to date to focus on genetic diversity within the tarantula family, Theraphosidae, is that of Longhorn et al. (2007), which focused on the Mesoamerican *Brachypelma vaganss.* However, even within Theraphosidae, there are significant differences in natural history, which may confound comparisons; the most significant problem concerns juvenile group dispersal. There are two reports of group dispersal in tropical theraphosids. Magnusson (1985) described a group of 89 mature males moving in coordinated and directed movement in Brazil. Reichling (2000) observed groups of juvenile tarantulas in Belize dispersing in a similar way. It is unknown how common group dispersal is in *Brachypelma vaganss*; however, it is not known to happen in North American tarantulas in the genus *Aphonopelma.*

Because North American tarantulas are relatively unique in their natural history when compared to other spiders (even members of the same family) it is necessary to evaluate specimens within *Aphonopelma* for hierarchical structure in genetic diversity in order understand the long-term patterns of movement and dispersal in these animals. Additionally, increased data on the variability in the mygalomorph 16S region will contribute to efforts in phylogeny in higher taxonomic levels (Smith and Bond 2003). The mitochondrial gene 16S has been identified as an ideal marker for these types of analyses in spiders since it is “super variable” (Smith and Bond 2003), it is a faster evolving gene that is embedded in very conserved regions (and thus more resistant to transposition) and rRNAs are relatively abundant in cells (Tautz et al. 2003).

Methods and Materials

Samples were collected in one of two ways. The samples collected from Missouri and from Wilbarger County, Texas (Table 4) were collected between 1998 and 2001. These animals were teased to the entrance of their burrows using stalks of grass or crickets tied to a short piece of string. They were collected from the burrow and taken to the laboratory where they were fed 1-2 crickets and maintained for 1-2 weeks in small plastic containers with ad lib water. Single legs were collected in a manner similar to that described in Longhorn et al. (2007) with several differences. We encouraged the autopasy of the rearmost left leg instead of a medial limb and we utilized CO_2 as an anesthetic. CO_2 gas was introduced into the animal's container by way of a small tube from the gas canister to the container. When the spider began to show slow movements, the CO_2 was discontinued and the spider encouraged to autonomize the leg. The leg samples were immediately frozen at -80°C and the spiders were again fed 1-2 crickets and maintained in the laboratory for an additional week to ensure their continued health. Representatives of these populations were sent to Rick West (formerly of the Royal B.C. Museum in Canada) for identification.

All other samples were collected between 2004 and 2007 as whole animals. Female burrowing tarantulas were teased to the entrance as previously described and collected as whole animals. Upon return to the lab, they were immediately frozen at -80°. The two groups of migrating males were both collected along roads and each group was found within a distance of 2 km and a time period of less than 3 weeks. Males collected were either frozen at -80°C or preserved in 100% ethanol (Figure 7). Collections were identified as follows: WRC – specimens collected near White River Lake in Crosby

county, TX; BRC – specimens collected near Silverton in Briscoe county, TX; DRC – specimens collected near Ransom Canyon in Lubbock County, TX; WLB – specimens collected on the Waggoner Ranch in Wilbarger county, TX; and MOS – specimens collected near Columbia in Breene County, MO.

Using sterile technique, muscle tissue was extracted from the leg of each animal and incubated at 65°C in a 1% Proteinase K solution. DNA was extracted using Qiagen (Valencia, CA) DNA extraction kits and stored at -80°C. The 16S region of the mitochondrial genome was amplified using the primers LR-N-12945 (CGACCTCGATGTTGAATTAA) (Hedin and Maddsion 2001) and NI-J-12261 (TXXTAAGAATTATTTGA) (Hedin 1997) to yield sequences of approximately 430 base pairs. Fragments were amplified using one of two thermal cycles: 95°C (for 120s), 5 cycles of 95°C (30s), 46°C (30s), 72°C (120), then 35 cycles of 95°C (30s), 48°C (30s), 72°C (120s), and then 72°C for 10 minutes: or 95°C (120s) then 35 cycles of 95°C (30s), 46°C (60s), 72°C (60s) and a final extension of 72°C for 7 minutes. Amplification was achieved using 1-2 μl of template DNA and a master mix of 20 μl of buffer (0.3 M Tris, 0.0175M Mg Cl_2, 0.075 M Ammonium Sulfate at pH 8.5), 0.5 to 1.0 μl of each primer, 0.5 μl of dNTP's (0.040 M each), and 0.25 μl of Promega *Taq* polymerase (Promega Corp.) with the total volume increased to 50 μl with ddH_2O. In some amplifications, DMSO was added at a concentration of 3% to address possible complications introduced by the presence of significant secondary structure (Smith and Bond 2003). PCR products were purified using Qiagen clean up spin columns (Valencia, CA) and cycle sequenced in both directions with Big Dye 3.0. Sequences were obtained using an ABI Prism 310 automated sequencer and edited and analyzed using MacVector 9.5 with Assembler 1.0.

Results

We were able to isolate contiguous 16S sequences from 27 animals that were 430 base pairs in length. Using MacVector 9.5 and Assembler 1.0 to align sequences and identify base pair changes we were able identify a total of 17 haplotyes present in the 27 animals analyzed. The haplotypes were named with respect to the species designation indicated by our analysis (HE-X indicates *A. hentz*i, HE-X indicates *A. hollyi*, and SP-X indicates *A.* sp.). In all levels of analysis we used the ClustalW portion of MacVector 9.5 to create distance matrices and neighbor joining techniques to analyze the haplotype diversity present (see Table 2). There were 28 polymorphic nucleotides found in the 27 16S sequences analyzed (6.4%). The mean nucleotide composition of these fragments was A:0.365%, C:0.163%, G:0.101% and T:0.361%.

Within groups of migrating males

We analyzed two groups of migrating males collected, DRC and WRL, and found 2 haplotypes in each population. In the DRC population, the two haplotypes were separated by 6 sequence differences and one haplotype was represented by one individual (haplotypes HO-1 and HO-2). In the WRL population, the two haplotypes were each found in two individuals and were separated by only 3 base changes (SP-1 and SP-2).

Within groups of colonial females

We analyzed collections of burrowing females from three colonies: BRC, WLB-1 and WLB-2. The BRC females included 3 haplotypes, 2 of them only separated by 3 base changes (HO-3 and one previously identified as HO-2) and the third haplotype was an additional 5 base changes in difference (HO-4). WLB-1 and WLB-2 both showed remarkably higher levels of difference in base pair composition, resulting in two major

"clades" that were each present in each colony. Because the two colonies were geographically close to each other we then combined these samples (WLB) and analyzed the result. The WLB represented 7 haplotypes: 3 were separated by 2-6 base changes (HO-2, HO-3) and the other four were identical (HE-1) However, there was a major division between these two "clades" of haplotypes that was not associated with geography or the putative colony from which animals were collected.

Within the species *Aphonpelma hentzi*

The animals collected from WLB and MOS were both identified to be *Aphonopelma hentzi* and so were combined to evaluate diversity within this species. We found 9 haplotypes present, the 7 observed within the WLB collections and an additional two haplotypes were found in the MOS sample, each found in a single individual (HE-2 and HE-3).

Within the genus *Aphonopelma*

When all 27 sequences were combined to evaluate diversity within the genus, we were able to identify 9 different haplotypes (Figure 8). There was no apparent relationship between geographic and genetic distance within the genus, although there are three clear divisions apparent in the neighbor-joining network, which may represent natural species divisions. The only significant F_{ST} values were found at this level of analysis ($F_{ST} = 0.546$).

Discussion

Overall, the only level of analysis with significant diversity and a significant F_{ST} value was within the genus. Males of the genus *Aphonpelma* in North America have been described as migrating in a group (Baerg 1958). However, our analyses were

unable to support or refute the idea that mature males from a colony migrate together. The level of haplotype diversity was not significantly different from the level seen within a colony or within the species *A. hentzi*. Although an increase in sample size may help to elucidate the relationship among males seemingly migrating together, it is unlikely that we will be able to resolve this using the 16S mitochondrial marker.

In the analysis of colonies, we found a significant division within both of the colonies sampled in Wilbarger County, Texas (WLB-1 and WLB-2). Because these two colonies are geographically close (approximately 5 km apart) we then combined the samples together for further analysis. In total, these samples were comprised of 7 haplotypes that fell into two groups (WLB-A and WLB-B), which were separated by 11 base pair changes. We believe that these two groups represent two species, *Aphonopelma hentz*i and what has been putatively identified as *A. hollyi* (Smith 1995), living in sympatry. Additionally WLB-B grouped in the neighbor-joining analysis with the *A. hollyi* collected as the WRC sample. Although species within this genus are known to live in sympatry (Prentice 1997), this is the first example of sympatry in tarantulas found in a Texas population.

When we tested haplotype diversity within the two populations of *A. hentzi* (MOS and WLB-A), we found a level of diversity similar to that found in the colonial and male group samples (1-2%). We were, however, able to detect two unique haplotypes in MOS, each collected from a different glade vicinity of Columbia, Missouri. Glade habitats in Missouri are semi-xeric, grassland "oases" surrounded by forested areas and have historically featured limestone soils and no hardwood plants. The glades are remnants of the last glacial period in North America (Janowski-Bell 2001) and due to the isolated

nature of glades in Missouri (Martin and Houf 1993), these unique haplotypes were expected and with further sampling, we would expect to find additional unique haplotypes from these relic habitats.

The samples were pooled and tested as a whole for diversity within the genus *Aphonopelma*. The only significant F_{ST} value was found at this level (F_{ST} = 0.546) and indicates that the majority of the diversity present in this genus is found at the species level. Our analyses show a pattern of divergence that is consistent with the presence of three species: *A. hentzi* (WLB-A and MOS), *A. hollyi* (DRC and BRC) and an unidentified species *A.* sp. (WRL) (Figure 9). There was an average of 5.42% difference between species. We found no evidence of regionally selective pressures on the 16S gene. Presumably, however, we will need to increase our sample sizes across all the levels of investigation in order to avoid the confoundments of low statistical power and vicariance in our conclusions.

And, although our analyses showed a level of polymorphisms in the 16S gene that was comparable with that found in the genus *Brachypelma*, we are unable to be definitive in our conclusions and would require relatively large samples (+10 per population) to robustly estimate gene flow (Slatkin 1989). Although Longhorn et al. (2007) postulate that direct tracking methods such as implanted tags (Chapter 2) and radio-telemetry (Chapter 3) may be preferable methods of estimating or measuring gene flow, we feel that molecular analyses such as this one are invaluable in creating an over-arching picture of the dispersal and movement patterns of animals within this genus.

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Table 4

Collection IDs, locations and additional data for specimens collected

ID and Collection Site	Sex	(N)	Centralized GPS coordinates
WRL White River Lake Crosby County, TX	Male	4	33° 28’ 08.68 N 101° 05’21.97 W
DRC Ransom Canyon Lubbock County, TX	Male	5	33° 31’ 03.17 N 101° 39’56.09 S
BRC Briscoe County, TX	Female	4	34° 27’ 12.71 N 101° 19’50.29 W
WLB-1 Waggoner Ranch Wilbarger County, TX	Female	6	33° 34’44.49 N 98° 32’41.98W
WLB-2 Waggoner Ranch Wilbarger County, TX	Female	4	33° 34’44.49 N 98° 32’41.98W
MOS Columbia Breene County, MO	Female	3	38° 39’13.60 N 92° 22’51.52 W

Figure 7

Geographic representation of collection sites

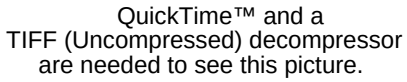


Table 5

Genetic diversity of *Aphonopelma.* N indicated the number of animals sampled, h indicates the number of haplotypes present, and π is a measure of nucleotide sequence diversity between two random samples within the sub-sample tested

Location	Sex	(N)	H	π
WRL	Male	4	2	0.889
DRC	Male	5	2	0.786
BRC	Female	4	3	0.243
WLB-1	Female	6	6	2.653
WLB-2	Female	4	5	2.019
JUN	Female	1	1	0
MOG	Female	3	2	0.245

Figure 8

Neighbor-joining network of all 27 sequences and 16 haplotypes found in the genus *Aphonopelma.* Each circle represents a haplotype and the number inside the circle indicates the number of specimens found to have that haplotype. The numbers next to each line represent the number of base pair changes between two haplotypes. The black squares indicate a hypothetical ancestral state. The red circle indicated the haplotypes in *Aphonopelma* sp., the blue circle indicates *A. hentzi* and the green circle indicates *A. hollyi*

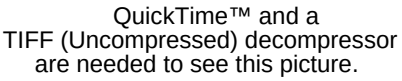
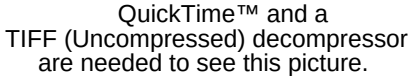


Figure 9

Geographic representation of the species identified. The stars indicate *Aphonopelma hentzi*, the cross indicates *A.* sp. and the circles indicate *A. hollyi*



CHAPTER 5

Conclusions and Future Directions

The work represented here has offered new insights into the movements of tarantulas, both in real time and in longer, genetically significant periods of time. Each of the three experiments had false starts or failed attempts and at least part of what was learned was what not to do. But as a whole, this dissertation gives the first comprehensive look at movement and dispersal in North American tarantulas.

The results of the PIT tag study were a surprise, as I expected the animals to have a high level of fidelity to a burrow once it was established. Digging a burrow is a significant expenditure of energy and although other genera of tarantulas are regularly found inhabiting rock piles and the hollows of tree trunks, *Aphonopelma* are entirely fossorial. In the pilot study completed in 2004, I released 11 tagged females to their burrows and returned one year later to document their burrow fidelity. When all 11 had disappeared, I assumed it was an aberration and not representative of the behavior of this genus. The follow-up study in 2006-2007, however, confirmed these results. Several times, I found the burrow to be collapsed entirely. It is unclear whether the tarantulas died or simply abandoned burrows that were, for some reason, no longer suitable. It is likely to be a combination of the two.

Although the PIT tag implantation procedure has been shown to not affect the animals' behavior or mortality in the lab, my study was the first field test of the technique. It may be that the stress of implantation combined with normal field conditions can adversely affect the longevity of the tarantulas. In retrospect, it would have been ideal to monitor a control group of animals. The control group would consist

of animals that were removed from their burrows, taken to the lab, been exposed to the same surgical procedure but without implantation, and then returned to their burrows. In this way, we would some measure of burrow fidelity without the trauma of PIT tag implantation. The weakness to this control is that the animals would not have been uniquely identified and the only data we would have been able to collect would be occupancy, not fidelity.

At some point I plan to attempt a PIT tag study under more controlled field conditions. As the field site used in these studies were both a significant distance away, I was only able to make monthly visits to take readings. Hopefully in the future I will be able to either discover or create a colony at a permanent study site that could be monitored much more closely.

The results of the PIT tag study implied that burrowing tarantulas were much more aware of environmental factors outside the burrow and more versatile in their responses than expected. To monitor activity levels I first attempted to use fluorescent powder tracking in two different ways. I first placed powder in a ring around the mouth of several burrows and then returned the following evening with a UV light, hoping to find the powder displaced and perhaps be able to record the distances traveled from the burrows. This experiment failed, as I never found the powder displaced in any significant way and no tracks were ever discovered. A second attempt to use fluorescent powder to directly track movement addressed the movements of migrating males, collected along roads at night. The males were coated with powder and released at the site of collection. Upon my return 30-60 minutes later, I used the UV light to identify the path of the tarantula as shown by the shed powder. In every case, the trail disappeared within 10

meters. I experimented with additives to enhance the tracking properties of the fluorescent powder, cornstarch and corn meal. I was never able to make the powder any more effective. It may be that because the urticating hairs covering the abdomen are barbed, the powder was held to the body of the tarantulas. Or perhaps they crawled under a tuft of grass and immediately began grooming; as I was unable to track them I have no way of knowing what actions the tarantulas took.

The application of radio-telemetry to animals of this size was only recently possible and in the pilot study I was able to verify that we could locate the animals using telemetry, that the transmitters would stay fixed, and to verify the lifespan of the transmitters. The study itself was completed in late July and early August and as this is during the mating season for these animals as well as prime hunting season, I expected to find fairly high levels of activity. Additionally, the PIT tag data implied that the females were capable of adjusting their behavior to cope with their environment (evidenced by a high degree of burrow abandonment). So the extremely low levels of activity we found in this study was disappointing. I feel the sample size and observation schedule were adequate to record any significant levels of activity, and so one of the conclusions of this work is that field studies may be of limited value in documenting behavior in these animals. My next study of tarantula behavior will be a lab study. As we found no interaction between burrowing females in the same aggregation and tarantulas are not prey-limited, it is unclear why they choose to aggregate into non-social colonies. I have, therefore, designed a study testing whether tarantulas are able to utilize public information in making their choice of burrow sites. I will test whether or not the presence of other tarantulas can be used as an indicator of site quality.

Another disappointment was our failure to document individuality in tarantula behaviors. Based on testimonials from the pet trade collectors, I was expecting to find some indication of individuality in these animals. In fact, we were only able to conclude that some animals are more active than others. It may be that this too will require lab studies to be documented.

My genetic analysis of the diversity in mitochondrial DNA found at different levels yielded some interesting results and pointed to the next steps in determining the patterns of long-term movements of tarantulas. I was fortunate to "inherit" some of the Texas samples and all of the Missouri samples from a colleague and so was able to establish the continuity of the species *Aphonopelma hentzi* across a significant geographic distance. It would have been ideal to have samples of *Aphonopelma* from the intervening range and I did attempt to collect those. I spent two weeks in August of 2006 making a transect collection across the panhandle of Texas, through Oklahoma, and into Kansas. I was entirely unable to find tarantula colonies on public land and although I drove country roads for hours each night, I was also unable to collect migrating males. I have plans to repeat this transect in shorter, more concentrated efforts in the future as it would be exciting to be able to delineate the entire range of the species. Additionally, I have plans to collect more extensively in south Texas and across the southwestern United States. Our results at some of the levels of analysis were inconclusive and especially at the generic level, more extensive sampling will allow a clearer picture to emerge.

The second approach in answering some of my questions regarding relatedness of females in a colony and males in a migrating group is to use AFLPs, a set of nuclear markers that should be able to describe genetic diversity at a much finer scale. I have

hypothesized that females are unrelated and not choosing burrow sites based on familial ties, and the information that can be gained using AFLPs will allow me to make some conclusions as to whether or not this hypothesis is supported.

When I began this dissertation, I was surprised at how little work had been done with these animals in the areas of movement and activity and genetic dispersal. However, I now understand how challenging these studies are due to the natural history of tarantulas. By their very nature they are difficult to study. However, the information gathered in this dissertation has provided the foundations for years of future work.