INTRA-SPECIFIC VARIATION ACROSS A SMALL TEMPERATURE DIFFERENCE IN THE SPIDER *RABIDOSA RABIDA* (ARANEAE: LYCOSIDAE) FROM THE MOUNTIANS IN ARKANSAS

by

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November 3, 2010

ABSTRACT

INTRA-SPECIFIC VARIATION ACROSS A SMALL TEMPERATURE DIFFERENCE IN THE SPIDER *RABIDOSA RABIDA* (ARANEAE: LYCOSIDAE) FROM THE MOUNTIANS IN ARKANSAS

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Temperature affects all levels of biological organization and ultimately affects multiple aspects of ecological performance and fitness. Generalist arthropod predators are ectothermic and are strongly affected by temperature in ways that are not fully understood. Descriptions of thermal ecology of important generalist arthropod predators are, therefore, essential pieces of information for studying ecology in changing thermal environments. *Rabidosa rabida* is a wolf spider that inhabits much of the eastern United States and plays an important role as a generalist predator of large herbivorous arthropods. This study makes the first description of the thermal ecology of *R. rabida*. A description of intra-specific variation in this spider and applications of this data to habitat selection for this generalist arthropod predator are also made.

Descriptions of thermal preference, critical thermal maxima, and locomotor performance were made for spiders collected at four locations in the mountains of Arkansas which differed by less than three degrees. Comparisons of thermal ecology and other ecologically significant thermal performance measures, such as predation and immunity, were made between these populations to determine if intra-specific variation could be described and if small temperature

differences would result in measureable differences in thermal response. Estimates of heritability were also made for size, sprint speed, and immunity measures to determine if variation found could be attributed to genetic factors or if environmental factors such as temperature could explain any variation observed.

Carapace length differed between spiders from different mountains with spiders from Mt. Magazine having a greater CL. Body mass also differed between sexes and between spiders from different elevations with females and spiders from low elevation showing the greater body mass. *Rabidosa rabida* was found to have an acute thermal preference of 32.0 ± 0.011°C. Thermal preference differed between spiders from different mountains. The critical thermal maximum was estimated to be 43.52 ± 0.105°C. Thermal limits did not differ between mountain or elevation groups. Sprint speed was significantly affected by temperature and differed between spiders from different elevations with spiders from higher elevations showing a greater mean sprint speed. Comparisons with characteristics of insects using various thermal strategies showed that *R. rabida* is a thermal conformer and thermal generalist with moderate thermal sensitivity. Thermal sensitivity, which did not significantly differ between 5° intervals, was greatest at the thermal extremes and between 25° and 30°C for all but male spiders. Measures of prey capture showed an apparent increase in prey capture as temperature increased but the ability for statistical interpretation was limited. Immunity showed limited variation between spiders and no significant differences between spiders from different elevations or mountains. Heritability estimates for size, sprint speed, and immunity measures were low and not significantly different from zero.

This first description of the thermal ecology and thermal performance in this large non web building spider gives insights into the thermal habitat selection of this animal. This spider showed a thermal preference suggesting that it can detect temperature differences and actively chooses to occupy one temperature over another. Thermal performance tests show that as temperature increases performance also increases. Survival may be negatively influenced by

warmer temperatures especially during early instars and molting. The thermal sensitivity of this spider showed an apparent increase in the temperatures between 25° and 30°C which would suggest that if all other factors were the same then this spider should choose to inhabit temperatures in the low thirties similar to its thermal preference. In the field these spiders are found actively hunting in the low vegetation at night when temperatures are dropping from the 30°'s into the 20°'s and in an area where wind would have negative effects on body temperature and hydration. This suggests that other factors such as inter-specific interactions influence habitat choice in this spider.

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CHAPTER 1

INTRODUCTION

1.1 Temperature

Body temperature affects an animal's behavior, physiology, and development (Stevenson 1985). The relationship between body temperature and physiology is of interest because physiological processes affect all aspects of an organism's ecology. In most cases physiological processes are optimized within a fairly narrow range of body temperatures. If an organism maintains its body temperature within this range it would be expected that the organism would have higher fitness (Blouin-Demers & Nadeau 2005). In ectotherms, body temperature range often follows ambient temperature range closely. Choosing a habitat that has beneficial thermal characteristics can be important for ectotherm ecology and fitness.

Habitat choice has been a popular subject of ecological study because of its influence on animal growth, survival, and reproduction (Shear 2004). Habitat structure (Kronk & Riechert 1979), spatial avoidance behavior (Van de Meutter et al. 2005), predation pressure (Miner & Stein 1996), food availability (Kronk & Riechert 1979), and temperature (Anderson et al. 2010) have all been used in studies of habitat choice. In general fish and other aquatic animals have received the most attention concerning thermal habitat choice due to the convenience of being able to control water temperatures more easily than air temperatures (Neill et al. 1972). Terrestrial animals have had their habitat choice examined but often temperature is not emphasized. Instead it is only mentioned in discussions of prey availability and activity time (Patrick et al. 2008). Greater understanding of thermal habitat choice and how much of a temperature difference is needed to represent two thermal habitats for an animal to choose between is needed.

Naturalists and ecologists have long recognized that temperature influences not only the survival (Miquel et al. 1993; Li & McClane 2006) and distribution (Brich 1953; Portner 2001; Bryant et al. 2002) but also the behavior (Hutchison & Maness 1979; Prange & Hamilton 1992; Van Dyck & Matthysen 1998), fitness (Bennett et al. 1992; Leroi et al. 1994; Gilchrist & Huey 2001), and evolution (Van Damme et al. 1990; Bennett et al. 1992; Crill et al. 1996) of animals. Thermal ecology is the study of the effects of temperature on the interactions of an organism and its environment. Temperature affects animals by altering the physical structure of the animal's internal environment and by influencing the chemical reactions that allow that animal to move, survive, grow, and reproduce (Johnston & Bennett 1996; Angilletta jr. et al. 2002).

1.2 Thermal Ecology Questions

The specific questions most often addressed in thermal ecology can be grouped into several general groups. The first group of questions has to do with whether or not animals regulate their body temperature or conform to the environmental temperature. These thermal regulation questions are often addressed when the thermal ecology of an organism is first being described. In many cases, studies looking at thermal regulation lead to questions about why one strategy is used instead of another. A general comprehensive answer to why one thermal strategy is used over another has not been agreed upon but several theories exist (Huey & Stevenson 1979).

A second group of questions has to do with the range of temperatures over which an animal can successfully perform important processes over or the sensitivity to temperature of the animal's performance (Wilson 2001). Some organisms only perform well at a very narrow range of temperatures while others perform well over a wider range of body temperatures. There is not a universal range of temperatures that make an animal a specialist or a generalist (Heinrich 1993). Researchers must be clear in defining their use of these labels. Studies of thermal sensitivity are often followed by questions regarding how animals end up using one

thermal strategy instead of another and what are the costs and benefits of either strategy in a given environment (Huey & Hertz 1984; Gilchrist 1995).

A third group of questions that often follows studies of thermal regulation or thermal specialization strategy involve asking what adaptations animals use to maintain their thermal strategy. Animals use adaptations in behavior (Hertz et al. 1983), morphology (Forsman 2000), life history (Can Dyck & Wiklund 2002), physiology (Chown & Gastron 1999), and cell and molecular structure (Andersen 1979) to help regulate and maintain body temperatures (Heinrich 1996). Adaptations that help regulate and maintain body temperature sometimes develop or start with other uses making them evolutionarily interesting in many animal groups (Gould & Vrba 1982).

A fourth group of common thermal ecology questions relate to the effects of a changing climate on animals and ecosystems, particularly the effects of global warming (Logan et al. 2003; Dwon et al. 2010; Laws & Belovsky 2010). Our limited understanding of the effects of temperature on many organisms is among the factors which prevent us from understanding effects of temperatures change. Over the last century mean global temperatures have increased approximately 0.7°C and are expected to increase at a greater rate over the next century (Wang 2009).

The final group of questions discussed here does not apply to thermal ecology or thermal physiology alone. This question asks what the variation in thermal reaction is within a species over space and time. This question can be applied to behavior (Van Dyck & Matthysen 1998), morphology (Sweeney & Vannote 1978), genetics (Merila 1996), physiology (Schmid-Hempel 2005), and whole animal performance (Miles 2004). Few studies have focused on performance variables of any kind despite the belief that natural selection is more likely to act directly on higher performance capacities such as sprint speed (Irschick et al. 2005). Addressing the presence and amount of intra-specific variation involves looking at portions of many of the other questions discussed above. This question concerning variation within a

species is a starting place that can lead to interesting evolutionary conclusions and many more ecological questions. Before addressing the aims of this study further background on the general thermal ecology questions and some theories and methods used to address them will be discussed.

1.3 Thermal Regulation of Conformity

The benefits of thermoregulation have been studied a great deal in insects and lizards and several hypotheses have emerged to explain what causes an organism to become a thermal regulator or a thermal conformer. Huey and Slatkin (1976) suggested that the relationship between the cost of thermoregulation and the benefit of maintaining a preferred body temperature would determine which strategy an organism would use. This hypothesis has been tested in numerous animals with mixed results (Blouin-Demers & Nadeau 2005; Hereczeg et al. 2006) yet remains a commonly cited idea (Boyles et al. 2011; Hadamova & Gvozdik 2011).

The question of which strategy is used and how an animal evolved that strategy has received a lot of attention in vertebrate ectotherms such as lizards (Huey & Slatkin 1976). These studies have begun to address the evolution of endothermy in vertebrates (Bennett & Ruben 1979; Bennett et al. 2000). Invertebrates have not been ignored in studies of body temperature regulation strategies. The invertebrate studies, using mainly insects, have focused more on the general influences that have influenced the evolution of thermoregulatory behaviors, structures, and physiology (Heinrich 1996). One factor that is important for both vertebrates and invertebrates is body size (Wilson 1975; Reeve et al. 2000). The smaller the body size the greater the surface area to volume ratio and the faster the heat loss. If heat can be produced but not maintained that raises the cost of thermoregulation for the organism as it would have to constantly produce heat.

1.4 Thermal Specialist or Generalist

Thermal specialization refers to how wide a range of temperatures that support high levels of performance. There are two general classifications referring to how broad or narrow this range is. Thermal specialists perform at some high levels over a small range of temperatures. Thermal generalists perform at a high level over a wider range of temperatures (Gilchrist 1995). These classifications are ideal extremes and do not cover all cases that occur in reality. There is no set range of temperatures that qualify an animal as a specialist or a generalist (Heinrich 1996). Thus, these terms represent relative comparisons, and must be specifically defined in context when used (Heinrich 1993).

A general assumption often used in studies of thermal specialization is that "a jack of all trades is a master of none." In other words the ability to perform across a wide range of temperatures would require physiological costs that would potentially take away from maximal performance (Huey & Hertz 1984). This tradeoff assumption is considered a corollary of the principle of allocation which states that resources are limited and organisms must allocate limited resources to the processes that will maximize fitness (Rollo 1986).

1.5 Intra-Specific Variation

Variation within a species has always been a promising subject of study. Climate change, habitat alteration, biological invasion, over-exploitation and pollution have led to a renewed interest in physiological diversity and the mechanisms underlying it at all levels of biology including within species (Chown & Gastron 2008). Studies of variation in physiological traits over geographical and temporal scales are providing new understanding of fundamental biological patterns, the processes that underlie these patterns, and their effects on mankind (Denlinger & Lee 2010). Intra-specific variation will remain a productive area of study because it is this intra-specific variation that provides the basis for biogeography (Fujii et al. 1999), evolution (Freeman & Herron 2004), speciation (Graves 1985), and the diversity that makes biology interesting.

1.6 Co-adaptation of Physiology and Performance

Co-adaptation is the co-evolution of traits within a population via natural selection. Co-adaptation is a unifying principle in evolutionary thermal ecology. In the past half a century thermal biology has moved its focus from the strictly physiological to more of a whole animal ecological focus (Angilletta jr et al. 2006). One way to look at the effect of temperature on an organism is to look at its performance across a range of naturally encountered temperatures. Choosing an appropriate performance variable is an important first step when looking at the effects of temperature on performance. A good performance variable will tell you something about the biology of the organism. Actions associated with movement (Swoap et al. 1993), feeding (Schmalhofer & Casey 1999), and mating (Wilmer 1991) are often popular choices for types of performance to be measured.

One of the most common performance variable used is sprint speed. Sprint speed has been used as a representation of physiology, general well being, and fitness on numerous terrestrial animals (Crowley 1985; Jayne & Bennett 1990; Irschick et al. 2005). Tests of performance or morphology relating to fitness were first proposed by Arnold (1983). He suggested that some aspects of physiology should correlate well with or be co-adapted with an ecologically relevant measure such as sprint speed in what some call the "performance paradigm"(Arnold 1983; Irschick 2001). Studies using sprint speed have been criticized over whether this variable really tells anything about the biology and fitness of the organism (Irschick 2001). There is evidence from studies of wolf spiders from the genus *Pardosa* that sprint speed has a direct affect on spider survivorship in both the laboratory and the field (Formanowicz in process)

The ability to make comparisons to past research and the difficulty in directly testing fitness are often given as explanations for the use of this variable without testing the assumption that a given performance is correlated with fitness (Irschick et al. 2005; Husak et al. 2006). Any

performance variable that can be measured across a range of temperatures encountered in nature can be used in a thermal ecology test of performance.

1.7 Heritability

Many general thermal ecology questions lead to questions about the adaptations and selective pressures that have led an animal to the given thermal strategy or characteristic. Heritability is a measure of the amount of phenotypic variance in a population that can be explained by genetic causes. Variance that is not explained by genetics is assumed to be the result of various environmental factors. Environmentality is an estimate of the amount of phenotypic variance that can be attributed to environmental factors such as temperature (Rushton et al. 2007). There are two commonly used ways of estimating heritability. Narrow sense heritability is the fraction of total phenotypic variation that results from the additive effects of genes. Broad sense heritability is an estimate of the fraction of total phenotypic variation that results from all types of genetic effects (Freeman & Herron 2004). Heritability estimates can give an idea of the amount of heritable phenotypic variance that is present in a population and heritable variation is required for evolution to take place (Simons & Roff 1994).

1.7.1 Heritability of Performance

Often the traits being examined, in estimates of heritability, are physical traits such as color, size, or the presence or absence of a structure (Evans et al. 2009). The ability to perform a given task or the level of performance can also be heritable traits. Livestock animals have been the subject of many heritability studies involving levels of performance of speed and endurance in show and racing horses (Hintz 1980), milk production and offspring survival in cows (Brinks et al. 1962), and even growth and fat production in pigs (Kennedy et al. 1985). Arthropods (Prout & Barker 1989; Simons & Roff 1994; Shaffer & Formanowicz Jr. 2000; Cotter & Wilson 2002), reptiles (Garland jr. et al. 1990), small mammals (Reed 1988), plants (Ruttencutter et al. 1979; Ashraf 1986; Soleri & Smith 2002), and even humans (Jensen 1967; Stins et al. 2004) have all been subjects of tests looking at heritability of performance.

Heritability estimates for arthropods are available for many measures (Shuster et al. 2006) but estimates for arachnids are rare.

1.8 Study Organism

Thermal ecology has used a great variety of study organisms during its history. Mammals were and still are a common interest because of their connection to human biology (Bonaccorso et al. 1992; Ashton & Feldman 2003). Ectotherms have been common subjects for thermal ecology studies because of the fact that their body temperature, which often stays close to the ambient, is convenient for laboratory study (Heinrich 1996). Including the majority of life on earth there are a large number of ectotherms to choose from. Fish (Wells 1935; Le Morvan et al. 1998), reptiles (Christian & Weavers 1996; Xiang et al. 1996), amphibians (Brattstrom 1963; Hadamova & Gvozdik 2011), and insects (Heinrich 1995; Laws & Belovsky 2010) have all been popular subjects for thermal ecology.

Insects are arthropods, many of which are ectothermic, that have been around for hundreds of millions of years. Insects produce large numbers of offspring and have generation times that in some cases can be as short as a few days. Much like the insects, spiders are ectothermic arthropods that have a relatively short generation time (Foelix 1996) and a high fecundity (Li & Jackson 1996). Spiders probably appeared in the Devonian period around 400 million years ago. The arachnids, spiders included, were likely at their peak of diversity during the later periods of the Paleozoic era while insects were still early in their evolution and their ability to fly, which has been an important factor in their thermal ecology, had not developed (Foelix 1996). The spiders are less well understood and far less studied than the insects in most areas including thermal physiology and thermal ecology despite their long evolutionary history and their ecological importance.

In both the reptiles and insects a progression of technique and methodology has occurred starting with basic descriptive lab tests followed by field temperature observation and progressing to more advanced combinations of lab tests with field observations to address

deeper questions of thermal physiology and ecology (Heinrich 1993). Spiders, having so little thermal biology information available, are still in the early stages of this methodological progression. Basic thermal tests done in the lab are still needed to describe basic characteristics. Field observations will be needed in the future to fill in the many gaps in understanding of thermal ecology for this ecologically important group of arthropod predators. Because of the need for a better understanding of arachnid thermal ecology a large widespread cursorial spider, which has been used in numerous general spider biology and ecology studies, was chosen for this study. The organism used is a spider, from the family Lycosidae, added to the genus *Rabidosa* in 1994 (Brady & Mckinley 1994). Older publications refer to this species as belonging to the genus *Lycosa (Rovner 1968; Tietjen 1978)*.

1.8.1 The Family Lycosidae

The Lycosidae is a large family of cursorial spiders with more than 2,200 different species found across the world. They vary greatly in size from 4mm to greater than 20mm in body length and occupy a wide range of ecological habitats (Foelix 1996). Thermal ecology studies of spiders have focused mainly on web building spiders due to the ease of working with a more or less stationary organism (Turnbull 1973; Riechert 1975). In recent years non web building spiders have received some limited attention focusing on a few species of cursorial spiders and mygalomorphs (Schmalhofer 1999; Hanna & Cobb 2006). Lycosids are good subjects for ecological studies because they are found in large numbers and are important generalist predators in most terrestrial habitats. They are also relatively easy to capture and work with. However, very little work has been done specifically on Lycosid thermal ecology. The earliest Lycosid thermal ecology work was done by Humphreys looking at a burrowing Geolycosid from Australia (Humphreys 1974; Humphreys 1978). Following Humphrey's work few studies directly addressed the effects of temperature on the behavior and ecology of this diverse and species rich family of spiders.

1.8.2 The Genus Rabidosa

Rabidosa is a genus of spiders in North America that has members which range in size from medium to very large for a wolf spider (body length mean ± SE of (*R. hentzi*) 11.33 ± 0.58 – (*R. rabida*) 21.22 ± 1.24mm for females). The only Lycosid genus in North America with any species reaching a larger size is the genus *Hogna*. These two groups can be easily distinguished by their stockiness and habitat choice. *Rabidosa* is more slender than *Hogna* and *Rabidosa* is generally found above the ground in the low vegetation when active while *Hogna* remains largely on the ground. *Rabidosa* also has distinctive striped patterns individual for each species (Brady & Mckinley 1994)(Figure 1 & 2).

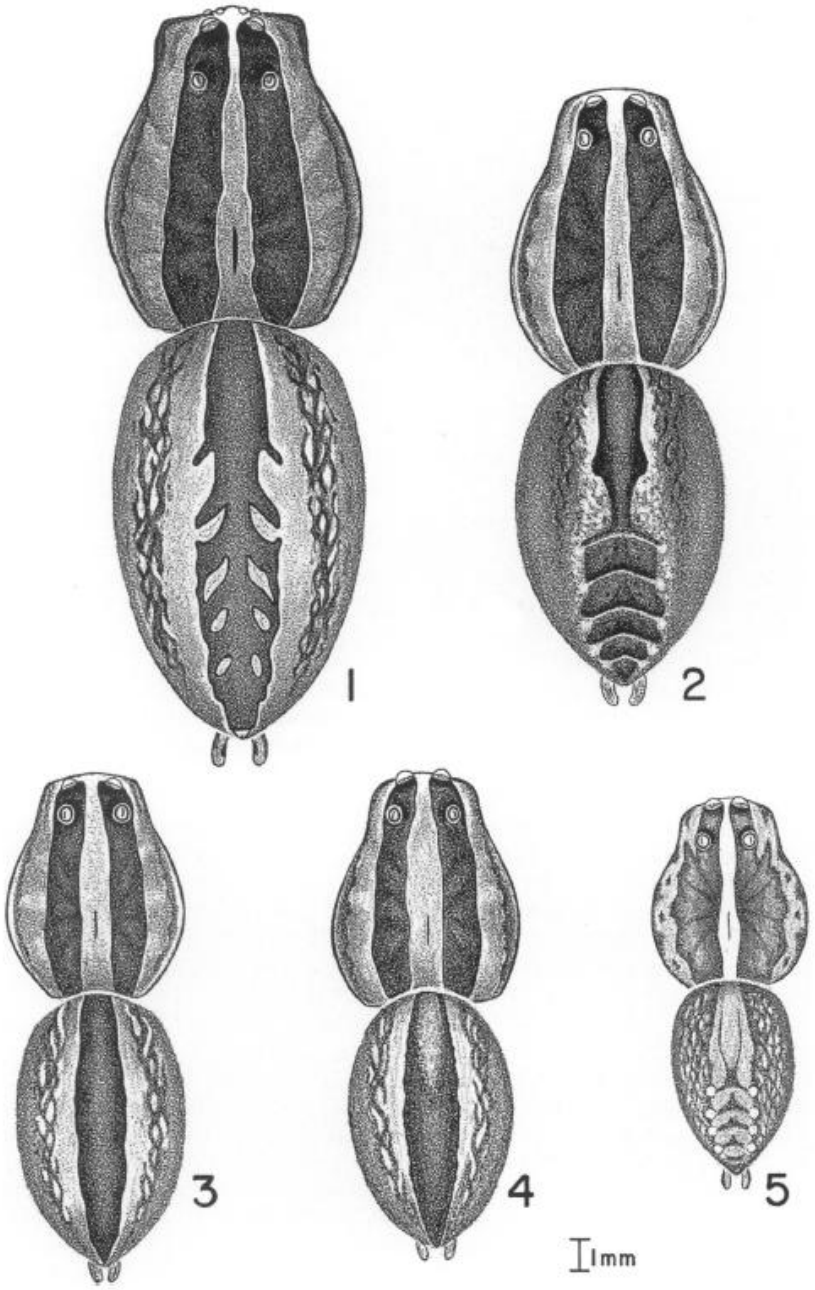
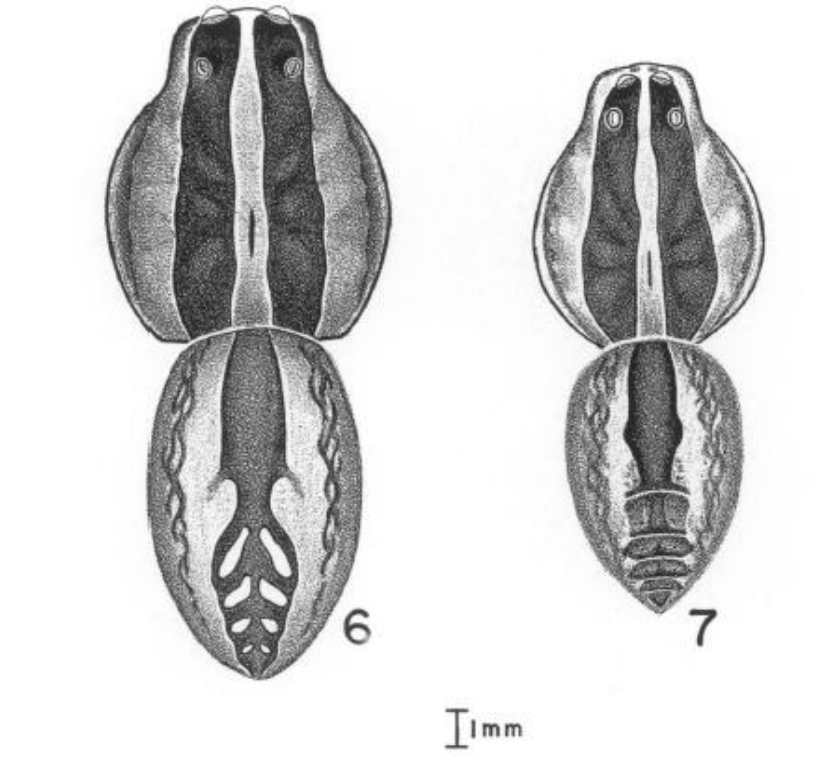


Figure 1: Dorsal View of *Rabidosa spp*. Females
1. *Rabidosa rabida* 2. *R. santrita* 3. *R. punctulata* 4. *R. carrana*
5. *R. hentzi* from Brady & McKinley (1994).



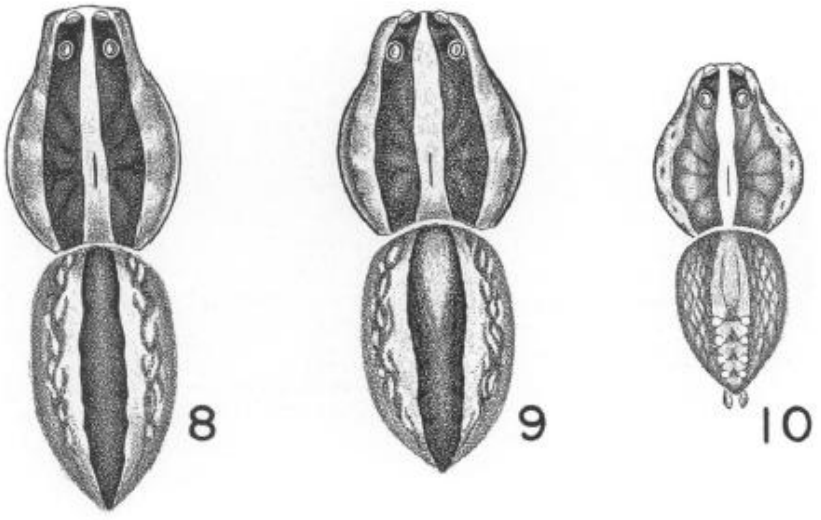


Figure 2: Dorsal View of *Rabidosa spp*. Males
1. *Rabidosa rabida* 2. *R. santrita* 3. *R. punctulata* 4. *R. carrana*
5. *R. hentzi* from Brady & McKinley (1994).

Rabidosa rabida is a large wolf spider reaching a body length of 27mm and a width of 9.1mm sometimes weighing more than a gram. Females are slightly larger than males, though with less sexual dimorphism than is found in many other spiders. Lycosids, such as *R. rabida*, possess long legs which allow them to move quickly across the substrate to capture prey without the aid of a web. *Rabidosa rabida*, like most Lycosids, is a generalist predator (Riechert et al. 1999). Predators of these spiders are mostly other arthropods including spiders and wasps (Kuenzler 1958; Redborg 1982). Birds and other animals which often prey on other spiders are avoided by nocturnal activity, camouflage, and the speed of escape from threats when moving down plants into the safe tangle of roots and stems (Gunnarsson 2007/2008).

1.8.3 Range

Rabidosa rabida is found across the eastern half of North America (Figure 3) (Brady & Mckinley 1994). This wide range encompasses a large variety of geography, flora, fauna, and thermal environments. Given problems associated with spider taxonomy (Coddington & Levi 1991), there are some understandable questions about whether the spiders found across the large range are truly one species. Despite this, no characteristics have yet been described across its distribution which justify breaking this species into multiple species (Brady & Mckinley 1994).

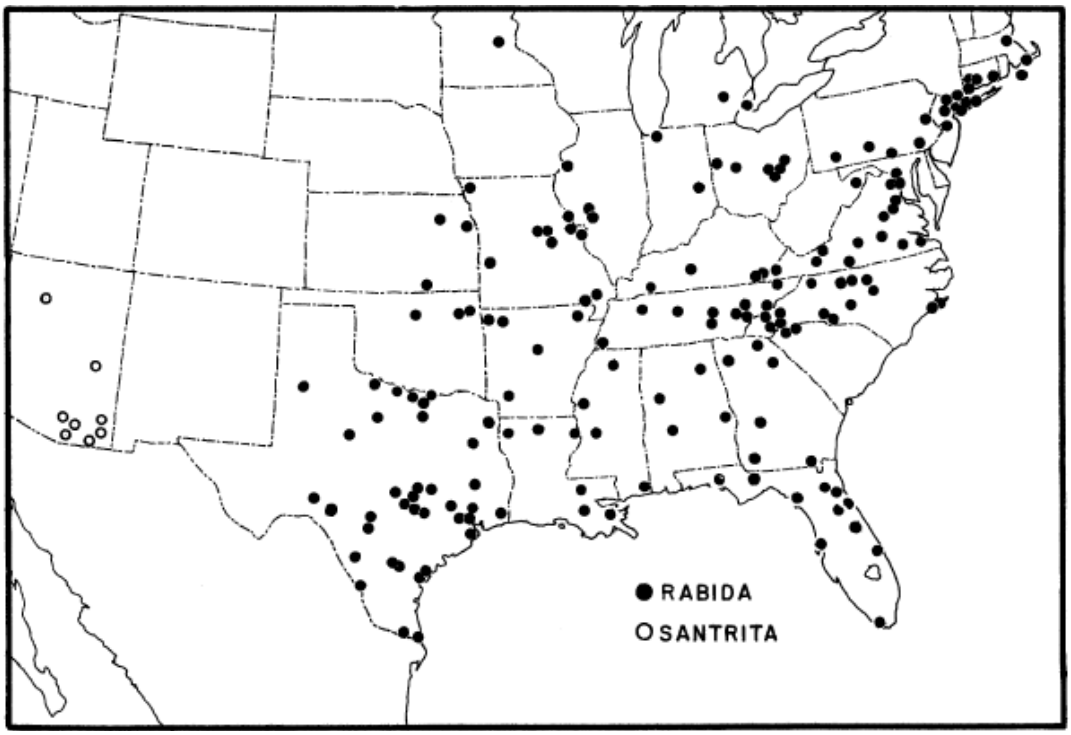


Figure 3: Geographic Range of *Rabidosa rabida* and *R. santrita*.
Taken from Brady and McKinley (1994)

1.8.4 Habitat

Rabidosa rabida is found in low vegetation and tall grasses. They hunt nocturnally by climbing to the top of the vegetation and ambushing prey (Brady & Mckinley 1994). They must choose vegetation which can support their relatively large size while still attracting prey items for capture. It has been observed that these spiders do sometimes hunt near flowers or nectar sources which attract arthropod prey but the preference of certain types of vegetation over others has not been explored (personal observation).

1.8.5 Ecological Importance

Rabidosa rabida is a generalist predator which will capture any organism that it can overpower. They have been observed taking prey slightly larger than themselves, which along with their large size makes them important predators of larger herbivorous arthropods. *R. rabida* is often found in high densities. Because of their numbers and ability to handle larger sized and large amounts of prey they have been found to exert top down benefits on the plants

where they live making them ecologically important and also the subject of many biological control studies (Riechert 1999; Sokol-Hessner & Schmitz 2002; Schmitz 2003).

As large, generalist predators *R. rabida* have great influences on the populations of many of the herbivorous arthropods on which they prey and on the community ecology of any system in which they are present (Sokol-Hessner & Schmitz 2002; Hlivko & Rypstra 2003). In addition to being influential predators, this spider is ecologically important as a prey item. Since this spider is nocturnal and well camouflaged and protected in the vegetation, birds and small mammals may not be as influential as arthropod predators such as parasitoid wasps.

1.9 Specific Aims

Using intra-specific variation in physiology between four populations of spiders from the mountains of Arkansas, this study aimed to determine how relatively small temperature differences of 1-3°C affects the ecology of this temperate arthropod predator. The specific aims of this study are as follows:

1.9.1 Thermal Ecology (Chapter 3)

There are few descriptions of arachnid thermal ecology and none for this species. This section aimed to describe the basic thermal biology of this ecologically important arthropod predator and make comparisons between groups of spiders using thermal ecology measures. Laboratory tests of temperature preference and critical temperature were made. A measure of locomotor capacity was also made across five ecologically relevant temperatures and comparisons between groups were made for all of these measures. A review of characteristics of organisms categorized into different thermal strategies was also made and compared to the characteristics of *Rabidosa rabida*. It was hypothesized that this spider would be found to be a thermal conformer based on its basic biology and comparisons with other animals. It was also hypothesized that this spider would be found to be a thermal generalist by examining its performance across temperature and thermal sensitivity. These data provide a basic

description of thermal biology and thermal strategies that help identify the potential effects of small temperature differences on temperate arthropod predators.

1.9.2 Evidence of Intra-Specific Variation (Chapters 2, 3,& 4)

Intra-specific variation is the basis for speciation, biogeography, and evolution. This section aimed to determine if there is measurable intra-specific variation across a relatively small temperature difference and in which measures this variation can be found. Studies looking at small temperature differences can be meaningful in that animals are more likely to encounter small temperature differences and must choose between these rather than larger temperature differences often used in laboratory tests.

This section makes a general description of the environmental conditions at each of the four locations where spiders were collected for this study. To describe variation between these populations, thermal performance and physical measurements were made and compared between groups from different elevation and temperatures that differed by only 1° to 3°C. This temperature difference is closer to the differences that might be experienced by this nocturnal predator than the temperatures used in the laboratory. I hypothesized that variance would be seen in some but not all measures even across this small environmental difference. These performance differences suggesting differences in physiology provide the variation that, if heritable, shows potential for evolution in this ecologically important arthropod predator.

1.9.3 Is this variation heritable and thus subject to selective pressures? (Chapter 5)

Not all intra-specific variation is heritable. Only heritable traits can evolve when acted on by selective pressures. Some variation is the result of phenotypic plasticity caused by environmental differences. If a natural population shows a heritability near zero then this would diminish the response to selection by a factor such as temperature (Prout & Barker 1989). This section aimed to determine if the intra-specific variation described in the previous sections is heritable and can evolve in response to selective pressures. To accomplish this, estimates of heritability were made using parent offspring and sibling-sibling comparisons using both

graphical and variance partitioning methods. Physical measurements of body size and leg segment lengths along with a measure of the innate immunity enzyme PPO and the performance variable sprint speed were used for these heritability estimates. Estimates of the environmentality were also made for some of the same variables. I hypothesized that there would be low estimates of heritability for all of the measures tested as has been described for similar measures in other ectotherms. I also hypothesized high estimates for environmentality as would be expected from a low heritability.

1.9.4 How does a small temperature difference affect temperate arthropod predators? (Chapter 6)

In this section the data gathered in chapters 2-5 were synthesized in order to draw conclusions regarding the thermal biology of *Rabidosa rabida* and if and how this predator's habitat choice and performance might be affected by small temperature differences. Finally the importance of using arachnids in addition to insects in future studies of evolutionary thermal ecology is addressed along with future research directions.

CHAPTER 2

POPULATION AND COLLECTION LOCATION DESCRIPTIONS

2.1 Introduction

Before looking for variation in different ecologically important measures, a comparison of the thermal environments at each collection location needed to be quantified. Using modeled data, comparisons of four collection locations were made regarding temperature. In addition to a description of thermal environments where these spiders were captured several measures, which are ecologically important, were taken including body size, growth rate, and survival at temperature.

Body size and growth rate can have numerous influential ecological effects (Stevenson 1985; Warren & Lawton 1987; Turner & Williams 2005; Shepherd et al. 2008). Juvenile body size and mass give insight into the growth rate in that spiders born at the same time and beginning at the same size and mass show different sizes and masses when maintained at different temperatures. These tests cannot comment on adult size as multiple years would be required to raise the spiders to sexual maturity and that was not available for this test. It was predicted that carapace length and body mass would differ between populations of spiders from different thermal environments including elevation groups and mountain locations showing a physiological or life history difference between these populations. It was also predicted that juveniles maintained at different temperatures would show differences in size, mass, and survival.

2.2 Spider Collection and Care

Adult spiders were collected from Rich Mountain in the Ouachita National Forest in Arkansas and from Mount Magazine in the Ozark National Forest in Arkansas (Table 1).

Spiders were collected after dark using headlamps and plastic cups were used to capture spiders that were hunting up in the vegetation at one of two collection sites on each mountain. Spiders collected above 609m were labeled high elevation group and spiders collected below 365m were labeled the low elevation group.

Table 1: Mountain locations showing highest elevation and GPS coordinates.

Mountain	National Forest	Highest Elevation	GPS coordinates
Rich Mountain	Ouachita, AR	817.2m	N 34.7, W -94.3
Mount Magazine	Ozark, AR	839.1m	N 35.2, W -93.6

Once captured, spiders were stored in 50mL centrifuge vials kept in a temperature controlled insulated bag to prevent overheating during transport. After collection, spiders were transported to the University of Texas at Arlington for testing. Spiders were then placed individually into 10x7.5x19cm clear plastic boxes. Each box had sand covering the bottom and a small 1.5x4.5cm shell vial filled with water and stopped with cotton to provide water as needed. Boxes were kept in a closed room at 25.6˚C with a reverse 14:10hr light to dark cycle.

Spiders were sorted into five groups for tests on successive days of the week containing similar numbers of spiders from each mountain, elevation, and sex. After acclimation for one week, each group had its hunger standardized with a single feeding of four large crickets on the assigned day of the week. Any remaining live crickets were removed after 24 hours. Following hunger standardization, spiders were offered two large crickets once a week on the assigned day and provided water as needed. Spiders were not fed for one week prior to being run in a test.

2.3 Thermal Differences between Collection Locations

For this study spiders were captured on two mountains in Arkansas, Mount Magazine and Rich Mountain. On each of these mountains spiders were collected from the low vegetation outside of the tree line at high elevation (>609m) and at low elevation (<365m). Mount Magazine is located in the Ozark mountain range in Arkansas and Rich Mountain is located in

the Ouachita mountain range (Table 1). The mountains where spiders were collected are separated by less than 85km. All four collection locations, two at each mountain, have similar vegetation and climate. Temperature data was modeled using the DIVA-GIS program and the worldclim database (Hijmans et al. 2005).

Temperatures at these mountains can range from the upper 30's to below 0°C depending on the season and time of day (Figure 4). There is a slight but consistent temperature difference between the high and low elevation collection locations of 1-2°C produced in part due to differences in elevation, humidity, and wind between the elevations. There are also difference in mean temperature between the mountains were spiders were collected. These differences are found mostly in the colder months when *R. rabida* was not active and at the beginning and end of the active season with the greater and more numerous differences found at the low elevations (Tables 2, 3).

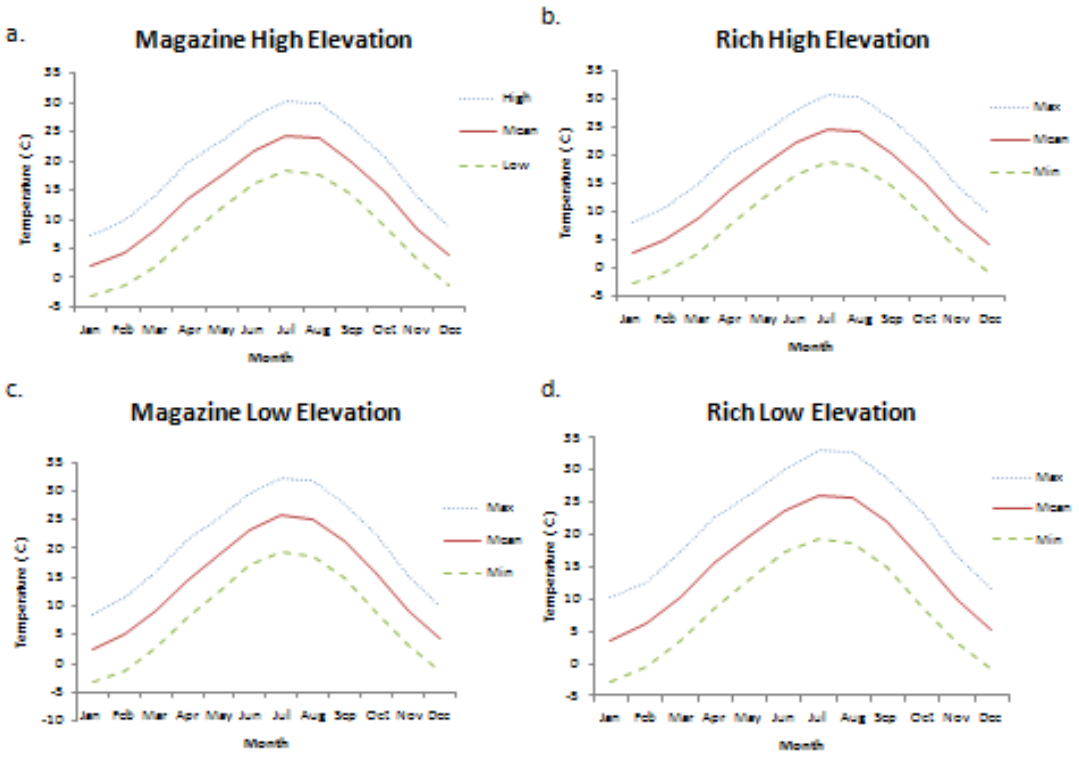


Figure 4: Maximum, mean, and minimum temperatures at high and low collection locations at each mountain as modeled by DIVA-GIS using worldclim data..

Table 2: Monthly mean temperatures as modeled by DIVA-GIS using worldclim data for comparison between collection locations.

	Mean Temperature			
Month	**Magazine High Elevation**	**Magazine Low Elevation**	**Rich High Elevation**	**Rich Low Elevation**
Jan	2	3	3	4
Feb	4	5	5	6
Mar	8	9	9	10
Apr	14	15	14	16
May	18	19	18	20
Jun	22	23	22	24
Jul	24	26	25	26
Aug	24	25	24	26
Sep	20	21	20	22
Oct	15	16	15	16
Nov	8	9	9	10
Dec	4	4	4	5

Table 3: Differences in modeled mean temperatures between elevations and mountains

Month	Elevation Difference Magazine	Elevation Difference Rich	Mountain Difference High	Mountain Difference Low
Jan	1	1	1	1
Feb	1	1	1	1
Mar	1	2	1	1
Apr	1	2	0	1
May	1	2	0	1
Jun	1	2	0	0
Jul	1	1	0	0
Aug	1	2	0	1
Sep	1	2	0	1
Oct	1	1	1	0
Nov	1	1	1	1
Dec	1	1	1	1

2.4 Size Methods

Carapace length measures from adult spiders used in the sprint speed test were used to look for differences in body size between groups. One-hundred and seventy two spiders were used (Table 4). Homogeneity of variances were tested using Bartlett's and Levine's tests and distribution assumptions were tested using a probability plot with a linear smoother and a Shapiro-Wilk test of normality. An analysis of variance was run using carapace length as the dependent variable and using test groups, sex, elevation groups, and mountains as main factors. Means and standard errors for each group were calculated and graphed for comparison.

Table 4: Sample size for carapace length comparisons

	N
Mt. Rich	83
High Elevation	43
Male	24
Female	19
Low Elevation	40
Male	17
Female	23
Mt. Magazine	89
High Elevation	43
Male	20
Female	23
Low Elevation	46
Male	12
Female	34
All Spiders	172

Body mass data was taken from spiders used in the immunity enzyme assay in section 4.7. Thirty five spiders were used (Table 5). Parametric assumptions were tested as described above. An analysis of covariance was run without interaction terms due to degrees of freedom. The dependent variable used was body mass and the main factors were elevation, sex, and mountain location. The covariate used was carapace length. Mean and standard errors were calculated for each group and graphed for comparison.

Table 5: Sample size for body mass comparisons

	N
Mt. Rich	25
High Elevation	14
Male	7
Female	7
Low Elevation	11
Male	8
Female	3
Mt. Magazine	10
High Elevation	5
Male	1
Female	4
Low Elevation	5
Male	0
Female	5
All Spiders	35

2.5 Size Results

There was no statistical difference in mean carapace length between sexes ($F_{1,132}$=0.064, p=0.80) or elevation groups ($F_{1,132}$=0.102, p=0.75). There was a mean difference found in carapace length between the spiders from different mountains ($F_{1,132}$=7.22, p=0.01). There was also a difference in carapace length between groups of spiders tested on different days or "Test Groups" ($F_{4,132}$=3.77, p=0.01). All interaction terms did not explain a significant portion of the variation ($F_{1\text{-}4,132}$<2.04, p>0.09) (Table 6). The sexes showed an apparent difference in mean carapace length with the females having a greater length. These means did not fall outside of two standard error units of each other. The mean carapace length of the mountain groups differed by greater than two standard errors with the spiders from Mount Magazine showing a greater length than those from Rich Mountain (Figure 5). The graph of carapace length of spiders from different mountains across elevations suggests an interaction effect of mountain and elevation (Figure 6). The graph of carapace length between the sexes across collection locations suggests and interaction term between these variables as well (Figure 7) (Table 7).

Table 6: Four-way ANOVA results comparing carapace lengths of test groups, sex, elevation, and mountains groups

Dep Var CL		**n=172**		**r^2=0.27854**	
Source	**SS**	**df**	**MS**	**F**	**p**
Test Groups	0.089	4	0.022	3.767	**0.01**
Sex	0.000	1	0.000	0.064	0.80
Elevation	0.001	1	0.001	0.102	0.75
Mountain	0.043	1	0.043	7.219	**0.01**
Group*Sex	0.018	4	0.005	0.781	0.54
Group*Elev	0.022	4	0.005	0.912	0.46
Group*Mtn	0.009	4	0.002	0.361	0.84
Sex*Elev	0.017	1	0.017	2.864	0.09
Sex*Mtn	0.000	1	0.000	0.003	0.96
Elev*Mtn	0.009	1	0.009	1.510	0.22
Group*Sex*Elev	0.048	4	0.012	2.034	0.09
Group*Sex*Mtn	0.010	4	0.002	0.405	0.81
Group*Elev*Mtn	0.016	4	0.004	0.668	0.62
Sex*Elev*Mtn	0.001	1	0.001	0.146	0.70
Group*Sex*Elev*Mtn	0.015	4	0.004	0.631	0.64
Error	0.779	132	0.006		

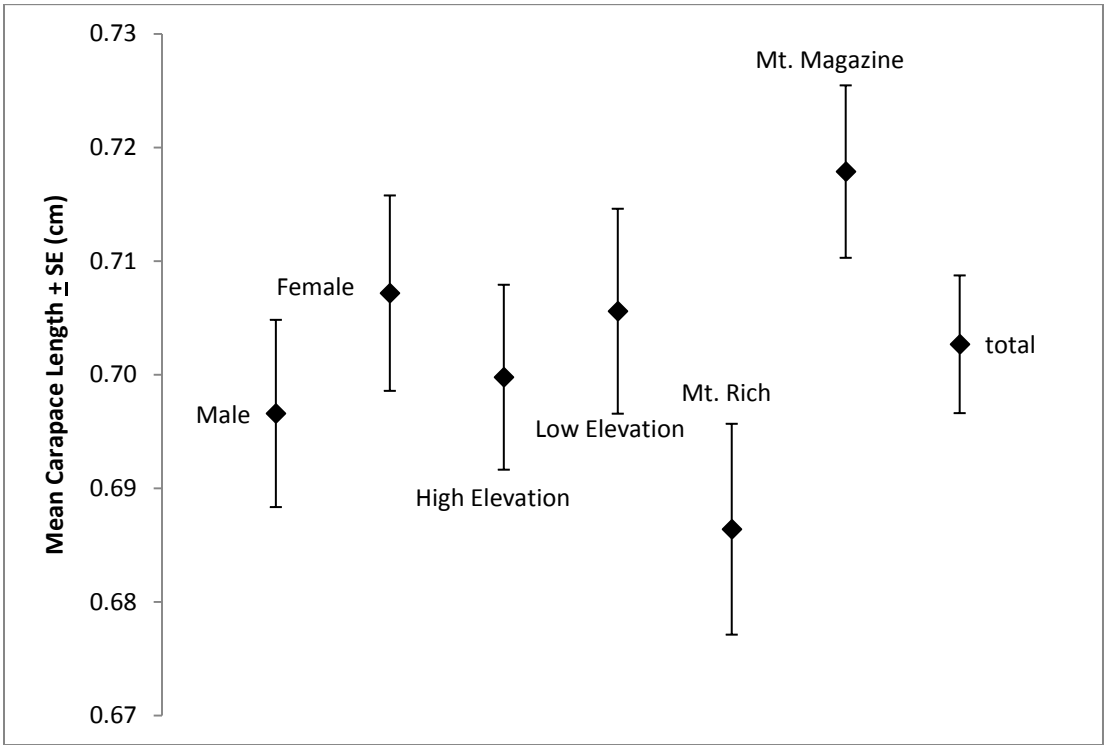


Figure 5: Mean carapace length (± SE) between sex, elevation, and location groups.

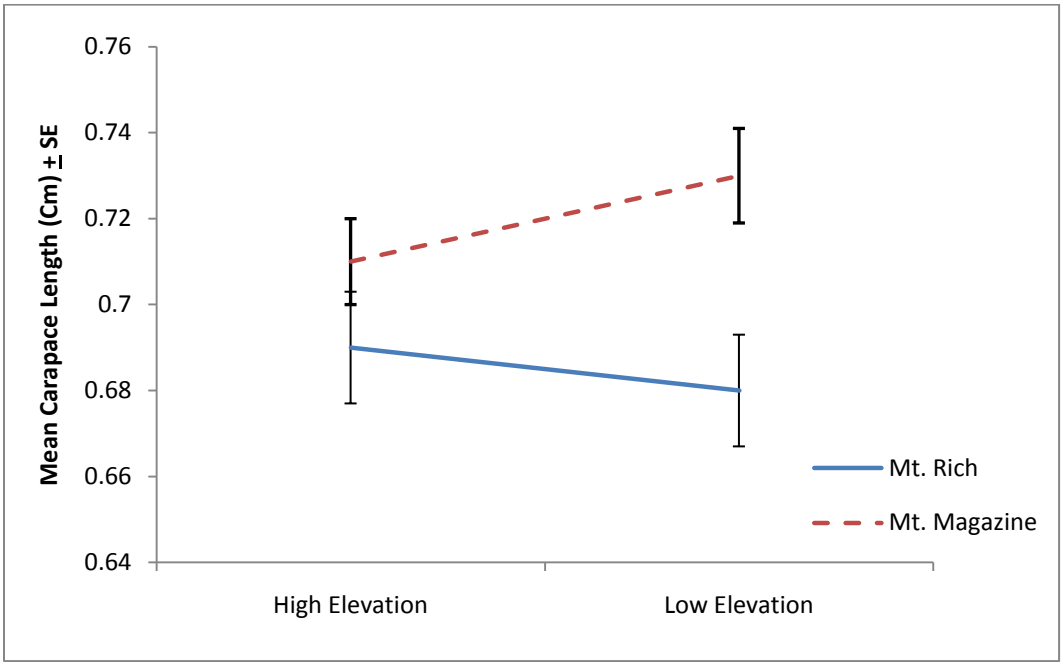


Figure 6: Mean carapace length (± SE) of mountain groups across elevation suggesting an interaction effect

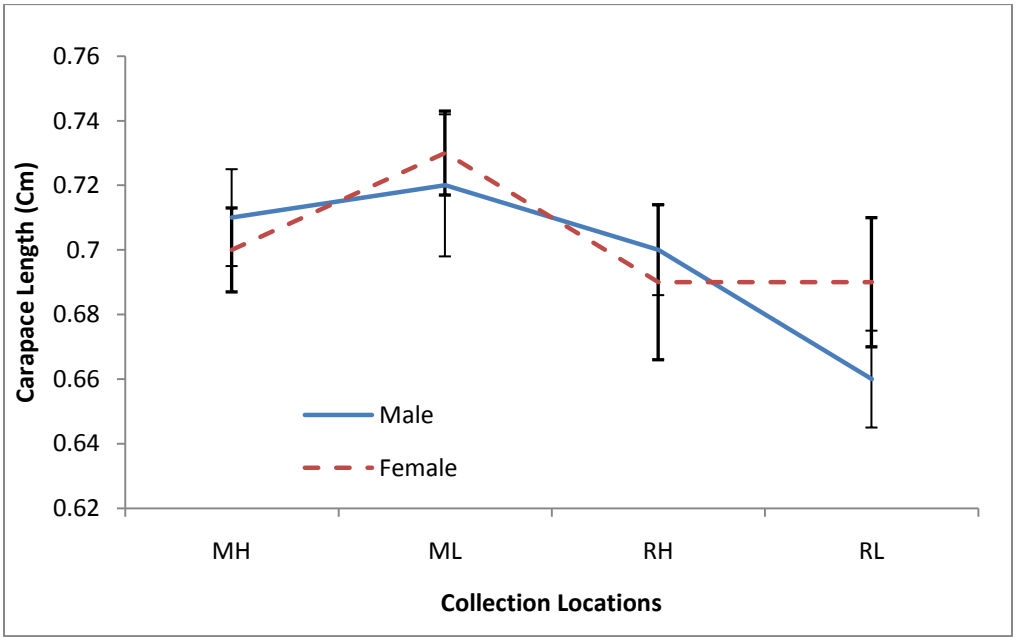


Figure 7: Mean carapace length (±SE) of sexes across collection locations suggesting an interaction effect

Table 7: Mean carapace length values for comparison between elevation and sex groups within each mountain

	Mean ± SE
Mt. Rich	0.69±0.009
High Elevation	0.69±0.013
Male	0.70±0.014
Female	0.69±0.024
Low Elevation	0.68±0.013
Male	0.66±0.015
Female	0.69±0.020
Mt. Magazine	0.72±0.008
High Elevation	0.71±0.010
Male	0.71±0.015
Female	0.70±0.013
Low Elevation	0.73±0.011
Male	0.72±0.022
Female	0.73±0.013
All Spiders	0.70±0.006

Mean body mass differed statistically between spiders from different elevations ($F_{1,30}$=7.46p=0.01) and from different sexes ($F_{1,30}$=25.13, p<0.0001). There was no significant difference in body mass between spiders from different mountains ($F_{1,30}$=0.086, p=0.77). The covariate carapace length also did not show any significant effect on body mass ($F_{1,30}$=2.32, p=0.14) (Table 8). The mean body mass of sexes differed by greater than two standard error units as did the mass of elevation groups and mountain groups. Female spiders showed a mean body mass greater than the males. Low elevation spiders showed a mean body mass greater than spiders from high elevations and spiders from Mount Magazine showed a greater mean body mass than spiders from Rich Mountain (Figure 8) (Table 9).

Table 8: Three-way ANCOVA results comparing body mass between elevation, sex, and mountain groups using carapace length as the covariate

ANOVA Dependent Variable Mass				**n=35**	**r^2=0.69**
Source	**SS**	**df**	**MS**	**F**	**p**
Elevation	0.060	1	0.060	7.457	**0.01**
Sex	0.203	1	0.203	25.129	**0.00**
Mountain	0.001	1	0.001	0.086	0.77
CL	0.019	1	0.019	2.324	0.14
Error	0.242	30	0.008		

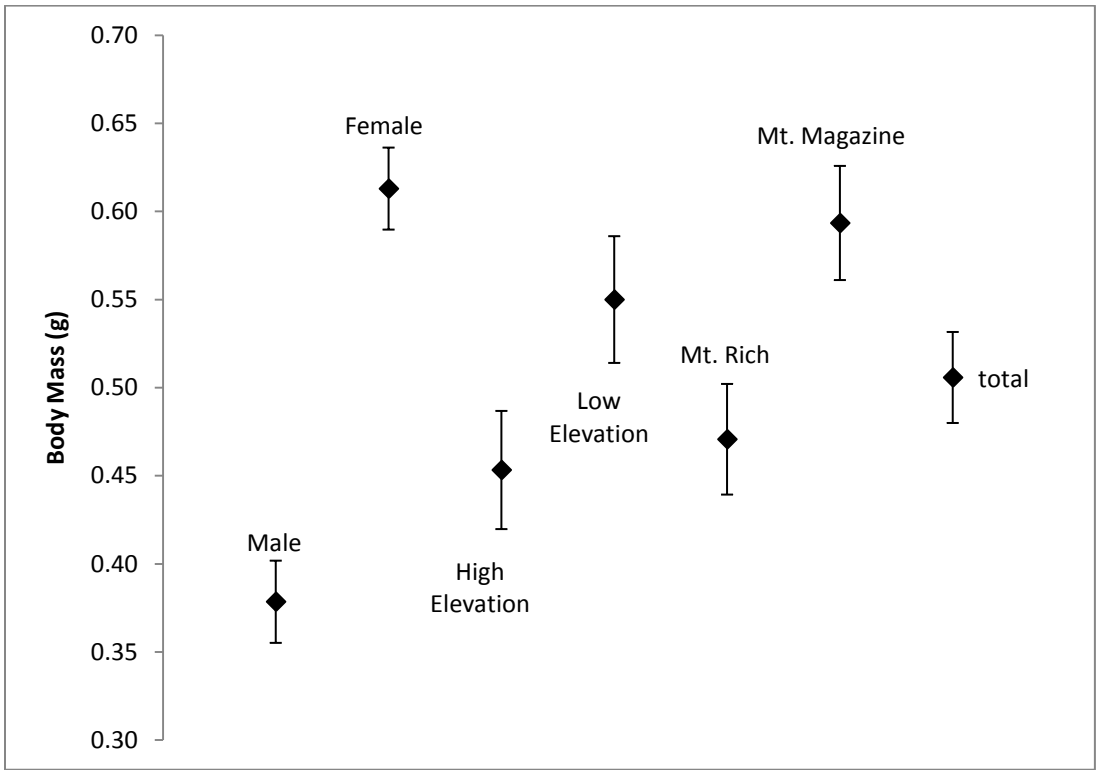


Figure 8: Mean body mass (±SE) between sex, elevation, and location groups

Table 9: Mean body mass values for comparisons between elevation and sex groups within each mountain

	Mean ± SE
Mt. Rich	0.47±0.031
High Elevation	0.53±0.044
Male	0.40±0.036
Female	0.66±0.041
Low Elevation	0.40±0.035
Male	0.35±0.032
Female	0.53±0..018
Mt. Magazine	0.59±0.032
High Elevation	0.61±0.052
Male	0.48
Female	0.65±0.052
Low Elevation	0.57±0.042
Male	N/A
Female	0.57±0.042
All Spiders	0.51±0.026

2.6 Juvenile Survival Methods

Females collected in August were set up in the lab in five feeding groups as described in section 2.2. Spiders which produced egg sacks were maintained on the same feeding regime until the eggs hatched at which time they were removed from their feeding group and set aside. Cultures of wingless *Drosophila melanogaster* were maintained in the lab for feeding the young. Once the young began to disperse, they were collected in 1.5oz or 2oz plastic condiment containers with moistened white aquarium sand covering the bottom and a clear plastic lid covering the opening. Each container was marked with the mother's number and a sequential offspring number. The young were maintained in these containers at lab temperature for approximately one month while other sibs were collected from their mothers. During this time all spiders set up in their own containers received a fruit fly for 24h and additional moisture as needed every three days. After this time they were grouped into cold, lab and hot temperature groups and placed in a location that maintained the temperature at 20°, 25.6°, or 28°C still on the same feeding cycle just increasing the number of flies and changing species of *Drosophila* provided each feeding as spider size increased. Offspring were maintained until they were used to conduct sprint speed, size, and immunity tests. A X^2 test was run comparing the number of spiders surviving in the cold and hot groups at each date to the number alive in the laboratory room temperature group. The original number of individuals in each group was multiplied by the proportion surviving in the laboratory room temperature group to obtain the expected value for the calculations. The X^2 statistic value was compared to critical values (Rohlf & Sokal 1995; Sokal & Rohlf 1995) using df=n-2 where n= the number of counts used in that comparison. One hundred and twenty juveniles were taken from each of 18 mothers and split randomly into the three temperature groups providing 2160 juveniles at the beginning of the trial. After the initial month in their containers, when juveniles were placed into their temperature treatment, 671 juveniles remained in each temperature group (Room group had one extra). The number killed and surviving were counted and recorded on each feeding day

for the last 2 months of the trial. The data was graphed showing the survival and final numbers in each group at the end of this trial. Measurements of Carapace length (CL), carapace width (CW), body length (BL), metatarsus length (Mt), and tibia length (Ti) were taken for spiders from the room and cold groups at the end of the trial. Mean and standard error for these measures were calculated and graphed for comparison. Homogeneity of variances was tested using Bartlett's and Levine's tests finding significant differences in variance between temperature groups. Mann-Whitney U tests were run using CL, CW, BL, Mt, and Ti measures for the dependent variables and temperature group (using only room and cold groups) as the grouping variable.

2.7 Juvenile Survival Results

On March 18 there were 493 spiders in the cold group, 486 in the room group, and 64 in the hot group (Table 11). On April 30th the number of spiders in the cold group did not significantly differ from the number of spiders in the room group (X^2=19.51, df=17, p>0.10) but the number in the hot group did differ (X^2=6158, df=17, p<0.0001). On May 16th, the final day counted, the number of spiders surviving in the hot group was significantly different from the number surviving in the room group (X^2=7526, df=25, p<0.001) as was the number surviving in the cold group (X^2=93.98, df=25, p<0.001) (Table 10). The room and cold group declined with a very similar slope only separating in the last month of the trial. The hot group had high mortality early and continued to die until only 15 remained. The cold group had the greatest percentage survive followed by the room group. The hot group had the highest mortality at over 97% (Figure 9) (Table 11).

Table 10: X^2 goodness of fit test results comparing survival of cold and hot groups to the room temperature group

	Cold		**Hot**		
Date	**X^2**	**p**	**X^2**	**p**	**df (n-2)**
30-Apr	**19.51**	**p>0.10**	**6157.92**	**p<0.001**	17
4-May	**29.51**	**P<0.05**	**6358.81**	**p<0.001**	18
16-May	**93.98**	**p<0.001**	**7526.15**	**p<0.001**	25

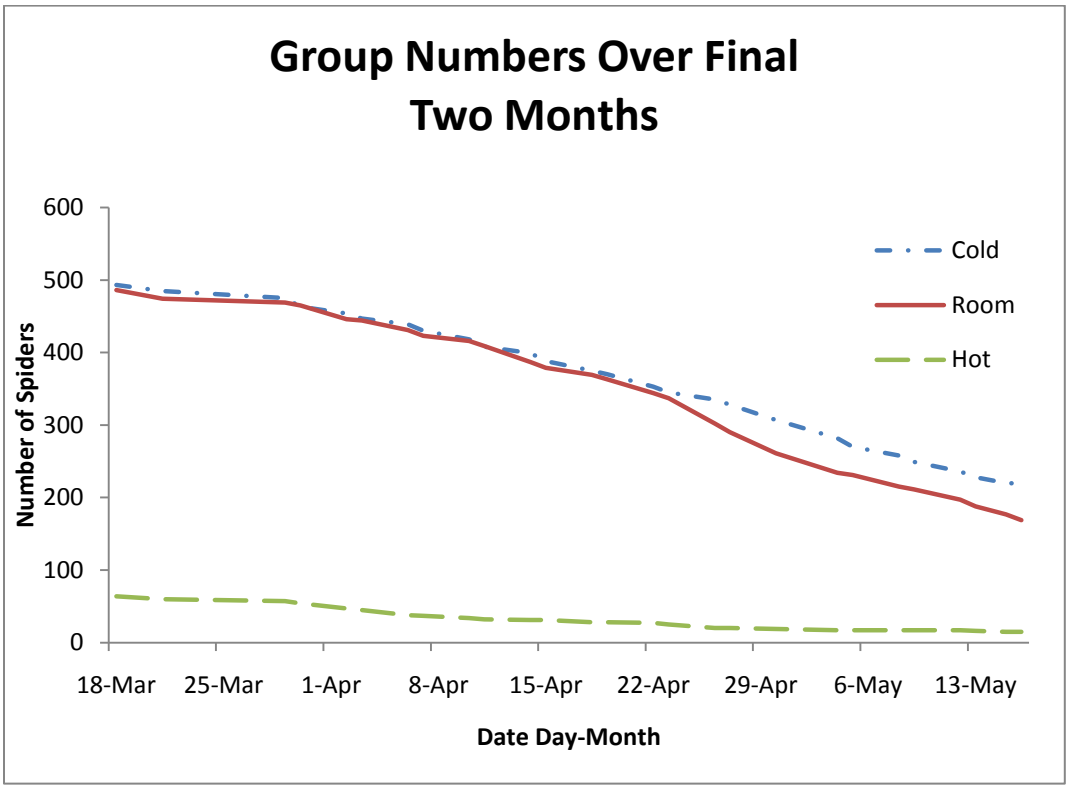


Figure 9: Decline in number of spiders in each temperature group over two months near the end of the test

Table 11: Group numbers and percent decrease between temperature groups near the end of the test.

	Cold	**Room**	**Hot**	**total**
Start	671	672	671	2014
Final Count	219	169	15	403
% Decrease	67.4%	74.9%	97.8%	80%

Spiders from the room temperature group were larger than spiders from the cold temperature group. All mean size measurements for the room group were larger than the means for the cold group by greater than two standard errors (Figure 10).

Table 12: Mann-Whitney U tests comparing juvenile size measurements between temperature groups

Grouping Variable	**TempGroup**	**TempGroup**	**TempGroup**	**TempGroup**	**TempGroup**
Dependent Variable	**CL**	**CW**	**BL**	**Mt**	**Ti**
Mann-Whitney U stat	443	1376.5	270	1587	697.5
p value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
n	221	221	221	221	221

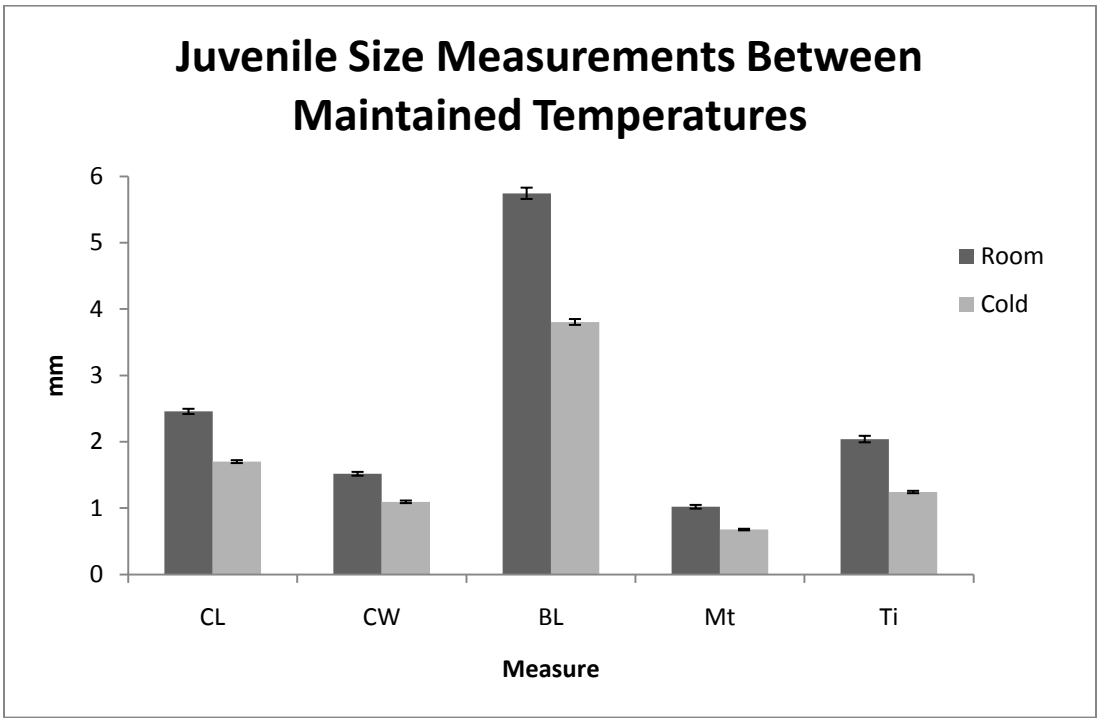


Figure 10: Mean size measurements (±SE) compared between temperature groups (CL=carapace length, CW=carapace width, BL=total body length, Mt=metatarsus length, and Ti=tibia length).

2.8 Population Comparisons Discussion

Basic descriptions of physical size were made for these groups of spiders using carapace length and body mass. Body size has been shown to be a significant factor in many ecological interactions (Dodson & Schwaab 2001; Turner & Williams 2005; Cordoba-Aguilar et al. 2009). Measurements of the carapace, the dorsal plate of the prosoma, are a basic measure of body size and developmental stage for spiders (Hagstrum 1971). This plate is useful in that it is hardened exoskeleton that does not stretch or shrink with hydration or feeding. Differences were found between mountain locations with spiders from Mount Magazine having a greater carapace length than spiders from Rich Mountain. This could be the result of the seasonal differences described between the mountains. If the warm active season is shorter on Mount Magazine than it might be expected that spiders would develop either a faster rate of growth and development to take advantage of the time they do have in favorable temperatures or the

ability to perform better at lower temperatures. Another possible explanation for these differences in body size are that the difference in season length has altered the growth rate of this spider causing it to overwinter at a larger instar than spiders that have longer warm seasons. There was also a difference in carapace length size between elevations at different mountains. The low elevation group from Mt. Rich was smaller than the high elevation group while the opposite trend was seen at Mt. Magazine. This could suggest some prey availability differences between the low elevation collection locations. Further field observations are needed to conclusively conclude which explanation fits best in this situation.

Body mass was also compared between groups and showed differences between the sex, and elevation groups. An apparent difference between spiders from different mountains was seen though this apparent difference in mass between mountains may be explained by differences in carapace length already described as this effect disappears when the covariate CL is used in the analysis. Females have been reported to be approximately 10% large than males (Walker & Rypstra 2001) and while the difference in carapace length was not great the difference in mass is noticeable probably showing a difference in fat stores reflecting a difference energy needs later on due to reproductive costs. In the elevation groups the greater body mass suggests a greater availability of food to build fat stores or a greater feeding rate.

Looking at juvenile survival at constant temperatures shows that the room and cold temperatures showed similar survival over the period from mid march until mid May when these observations were made. The hot temperature group showed a significantly decreased survivorship by mid March suggesting that this temperature is somehow detrimental to these juvenile spiders. The majority of deaths occurred during molting. This suggests that the availability of cooler and moist environments are needed to help spiders go through the molting process. The cold and room temperature groups showed similar survivorship until late April when the cold group began to show a greater survivorship than the room and hot groups. Despite having more spiders survive in the cold group the spider size in the two groups show

that the room group not only had moderate survival but also significantly larger growth. This suggests that a moderate temperature for growth is better than a hot or cold temperature though a temperature cycle between a warm temperature and a cool temperature for molting might be even better for survival and growth. Mortality in this test is not expected to follow natural rates as the conditions and food availability are not anything like what is expected in nature.

Some of the variation observed here could be explained at least in part by differences in temperature conditions at each collection location. The modeled differences found using this data showed a small difference of just 1-2°C between elevations. This is close to what was expected based on personal communication with the national forest rangers who work on these mountains. Rangers suggested there was a difference of 3-6°C between elevations (personal communication). The difference found between these two estimates could be partially explained by the resolution used by this program and database which is approximately $1km^2$ at its finest. The elevation collection locations on each mountain were not a full km apart and so the estimates of difference are probably slightly underestimated.

The differences reported between the mountain locations were smaller than the differences between elevations ranging from 0-1°C. The differences modeled between mountains were found mostly in the months at the limits of this spider's active season (May-August) and in the cooler months when the spider was not observed to be active in the field. These differences suggest a seasonal difference between the mountains. Seasonality has become a popular subject of research in thermal ecology (Danks 2004; Diaz et al. 2006; Kortet & Vainikka 2008). More work needs to be done with seasonality and fluctuating temperature regimes on arthropods including this spider to get a more complete view of thermal ecology and thermal performance for comparison between populations.

Spiders do not live in constant temperatures and fluctuating temperatures need to be explored with this spider much as they are being looked into in other arthropods (Buatois &

Croze 1991; Bryant et al. 1999; Gilchrist 2010). These results show differences in size suggesting differences in growth rate or life history due either to prey availability or temperature. Both prey availability (Greenstone 1984) and temperature (Ahnesjo & Forsman 2006) are linked and play recognized roles in habitat selection.

CHAPTER 3

COMPARATIVE THERMAL ECOLOGY

3.1 Thermal Ecology

Despite being a large, abundant, and commonly used ecological study organism, *Rabidosa rabida* has never had its basic thermal ecology explored. In looking at potential effects of a small temperature difference and thermal performance, some basic thermal ecology measures must be taken. In describing these basic thermal ecology measures for different groups of this spider there is potential to find intra-specific variation. There are three measures which have commonly been used to describe ectotherm thermal ecology. These are thermal preference, critical thermal limits, and locomotor performance (Huey & Stevenson 1979; Watson 2008).

Thermal preference represents a temperature value that the animal chooses to occupy if given a choice of temperatures (Light et al. 1966). There is doubt about the ecological significance of thermal preference measured in the lab due to the fact that temperature measurements in field active animals often differ from laboratory preferences. This is most likely because body temperature in field active animals results from a trade-off between physiology and ecology (Huey & Stevenson 1979). Critical thermal limits are temperatures, high and low, where the animal loses the ability to function normally yet can recover once its body temperature returns to within the critical range (Hutchison 1961). These critical thermal limits are rarely approached in the wild as thermal refuges are often available.

Locomotor performance is a measure of mobility across a range of temperatures. This measure can be used to describe the thermal optimum at a single point, or it can be used to describe the temperature range at which locomotor performance is above some defined level such as 80% of the optimum (Navas 1996). This measure has been used repeatedly and is

well documented in the literature (Hertz et al. 1983; Apontes & Brown 2005; Nelson & Formanowicz 2005). Locomotor performance includes more than just the sprint speed, or burst speed, of an animal it also includes measures of endurance and repeatability (Bennett & Huey 1990). Measures of endurance and repeatability of locomotor performance were not conducted in this study due to time limitations and the fact that spiders, due to their respiratory physiology, do not lend themselves to meaningful measures of endurance (Foelix 1996).

For this study a thermal generalist will be defined as a species having low thermal sensitivity over the range of temperatures measured. Thermal sensitivity can be measured using thermal performance across temperature. The factor by which metabolic processes change over a 10°C interval is referred to as the Q_{10} (Hegarty 1973). A higher Q_{10} shows greater temperature sensitivity for metabolic processes just as a greater slope of a thermal performance curve shows greater thermal sensitivity. A thermal specialist would be expected to have a greater Q_{10} than a thermal generalist. Because locomotor performance has been suggested to be closely linked to metabolic processes, looking at Q_{10} for locomotor performance can give an estimate of the thermal sensitivity of performance and metabolism. Because field body temperature measurements were greatly limited, thermal conformer or regulator status will be discussed by looking at examples of both in insects and comparing those traits to the known biology of this spider.

Insect thermoregulation has been extensively studied. Numerous examples of insects as thermal conformers have been described. Small bodies which prevent heat retention and which would increase the cost of maintaining a temperature different from the ambient are general characteristics shared by some of these insects. Another characteristics which is closely tied to whether an insect is a thermal conformer or regulator is flight (Heinrich 1995). Large flight muscles produce large amounts of heat. Without large flight muscles insects would not be able to produce metabolic heat and would not have evolved many of the adaptations that allow some insects to regulate their body temperature.

Insects that regulate their body temperature have received more attention than insects whose body temperatures conform to environmental temperatures. The mechanisms which allow for insects to regulate their temperature have in many cases been described. Flight muscles are important in producing internal heat. Adaptations which conserve heat either obtained metabolically or through behaviors such as basking are also important. Insulation, coloration, extremity length, body size, behaviors, and countercurrent exchanges have all been described in insects as mechanisms to either maintain or disperse heat in attempts to regulate body temperature within a desired range (Heinrich 1996). In general three of these characteristics have been identified as being most useful in predicting general body temperature characteristics in insects. These characteristics are body size, characteristics of flight, and insulation (Heinrich 1989). Spiders do not possess the ability to fly but they potentially possess insulating and body size characteristics which along with basking could allow them to regulate their body temperature apart from environmental temperatures.

Often adaptations which allow for thermal regulation are involved in tradeoffs with other aspects of ecology. For example a darker body color can help absorb heat from basking. A darker color may also attract predator attention. Similar tradeoffs can be described for many thermal regulatory adaptations. These tradeoffs greatly affect the cost to benefit relationship, which has been suggested as a potential way that insects “choose” which thermal strategy to take (Huey & Slatkin 1976; Huey & Hertz 1984).

Thermal ecology requires a connection to be made between results obtained in the laboratory and actual conditions in the field. As discussed earlier there are some difficulties in applying laboratory measurements to field ecology due to behavioral and microhabitat conditions that are difficult or impossible to reproduce in the laboratory. *Rabidosa rabida* is an excellent candidate for using laboratory obtained data to make real world applications. This spider spends its active time nocturnally up at the top of low vegetation hunting, where temperatures should more closely follow temperatures obtained from weather stations.

The goal of this study was to make a first description of basic thermal ecology for this ecologically important temperate arthropod predator and begin to look for differences between populations of this spider in the measures being made. Tests of thermal preference and critical thermal limits were made in the laboratory. Comparisons were made between examples of different thermal strategies found in insects described in the literature and the biology of this spider in an attempt to describe some of the basic thermal strategies used by this spider.

I hypothesized that there would be a difference between populations from different thermal environments in thermal preference and critical thermal limits due to suspected adaptation to local thermal habitats. I also hypothesized that *Rabidosa rabida* would be found to be a thermal conformer through comparisons with measures of basic thermal ecology and field temperatures along with comparisons with insect thermal conformer and thermal regulators in the literature. Finally I hypothesized that *Rabidosa rabida* would be found to be a thermal generalist because of its status as a thermal conformer and the fact that it lives in a temperate environment where temperatures span a relatively wide range between seasons and even between night and day.

3.2 Temperature Preference Methods

A thermal preference test was run in a thermal gradient as is customary in invertebrate thermal ecology (Dillon et al. 2009). The gradient established here was set up in a 30cm diameter 30cm depth stainless steel china cap. The apparatus was painted with brown acrylic paint which had sand pressed into its surface to provide a climbable surface. The 4cm vertical rim at the top of the apparatus was covered in petroleum jelly to prevent spider escape. A latex hose (0.95cm outer diameter 0.64cm inner diameter) was run around the top of the apparatus running from a hose in a container of ice water and emptying back into the same container. A 50w (120V, 60hz) heat bulb was placed at the apex of the apparatus just off of the lower apex. This apparatus provided a temperature gradient with the hottest point in the apex at the bottom

at around 50°C and the coolest area around the rim at 20°C. A red light was placed 30.5cm above the apparatus to provide the only illumination during trials.

Spiders were placed in the center of the apparatus (the hottest point) and allowed to move around for 30 minutes. After this time an infrared laser thermometer was used to determine the temperature where the spider came to rest by taking a measurement on the spiders back while still in the apparatus. The laser thermometer was aimed from the far side of the apparatus just above the rim. Spiders disturbed before measurement were removed from the analysis. Three identical apparati were used at the same time in the same room with one spider in each.

Carapace length, sex, elevation group, and collection location were all recorded along with the temperature where the spider came to rest. Over five days 122 spiders were tested (Table 13). Homogeneity of variances was tested using both Bartlett's and Levene's tests for each grouping variable. The distribution was tested using a probability plot with a linear smoother and a Shapiro-Wilk test for normality. A regression of carapace length on temperature preference was run and the residuals subtracted from the temperature preference to correct for body size. The regression was not significant ($F_{1,120}$=0.167, p=0.68) but was used here because body size is known to be influential in many aspects of ecology and can hide significance of biologically interesting factors if not corrected for (Walker & Rypstra 2001; Turner & Williams 2005; Huey & Pianka 2007). An analysis of variance was run using corrected temperature preference as the dependent variable. Elevation group, mountain group, and sex were used as main factors. Mean and standard error of the corrected thermal preference for each group was calculated and graphed for comparison.

Table 13: Sample size for thermal preference test

	n
Mt. Rich	55
High Elevation	25
Male	16
Female	9
Low Elevation	30
Male	14
Female	16
Mt. Magazine	67
High Elevation	33
Male	12
Female	21
Low Elevation	34
Male	11
Female	23
All Spiders	122

3.3 Temperature Preference Results

The mean preferred temperature was significantly different between mountain groups ($F_{1,114}$=9.62, p=0.002) with spiders from Mt. Rich showing the higher thermal preference than spiders from Mt. Magazine (Figure 11) (Table 15). Mean preferred temperatures did not significantly differ between sex ($F_{1,114}$=1.23, p=0.27) or elevation groups ($F_{1,114}$=0.001, p=0.97). All interaction terms showed no significant effect ($F_{1,114}$<3.1, p>0.08) (Table 14).

Table 14: Three-way ANOVA results comparing temperature preference corrected for body size between sex, mountain, and elevation groups

CorrectedPreference			**n=122**		**r^2=0.10**
Source	**SS**	**df**	**MS**	**F**	**p**
Sex	0.007	1	0.007	0.546	0.46
Mountain	0.124	1	0.124	9.616	**0.002**
Elevation	0.000	1	0.000	0.001	0.97
Sex*Mtn	0.001	1	0.001	0.093	0.76
Sex*Elev	0.039	1	0.039	3.051	0.08
Mtn*Elev	0.002	1	0.002	0.179	0.67
Sex*Mtn*Elev	0.001	1	0.001	0.100	0.75
Error	1.466	114	0.013		

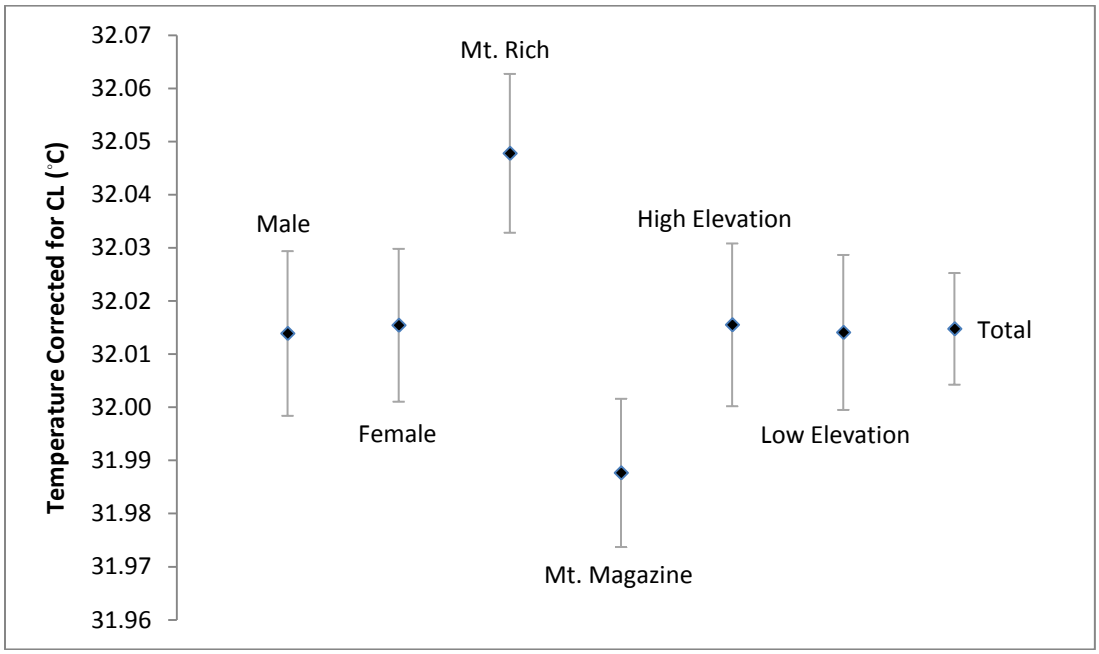


Figure 11: Mean thermal preference (± SE) between sex, location, and elevation groups

Table15: Mean thermal preference corrected for carapace length for comparisons between mountain, elevation, and sex groups

	Mean ± SE
Mt. Rich	32.05±0.015
High Elevation	32.04±0.024
Male	32.02±0.250
Female	32.07±0.051
Low Elevation	32.05±0.019
Male	32.07±0.026
Female	32.04±0.027
Mt. Magazine	32.00±0.014
High Elevation	32.00±0.019
Male	31.96±0.036
Female	32.02±0.022
Low Elevation	31.98±0.020
Male	31.99±0.033
Female	31.98±0.026
All Spiders	32.0±0.011

3.4 Critical Temperature Methods

Spiders were placed, one at a time, in a 50mL centrifuge tube with a thermocouple suspended inside of the tube. The tubes were then placed inside another container to slow the

rate of temperature change. In the critical high temperature test the tube was suspended in a glass bottle which was then placed over a heating bulb. For the critical low temperature test the tube was placed on a Styrofoam mat in a plastic box which was placed in a freezer. Spiders were either observed constantly in the critical maximum test or checked every degree of temperature change in the critical minimum test to determine when and at what temperature they lost the ability to move.

Spider mass, carapace length (CL), elevation group, mountain of collection and sex were recorded prior to exposing spiders to treatments. Homogeneity of variances was tested using both Bartlett's and Levene's tests for each grouping variable. The distribution was tested using a probability plot with a linear smoother and a Shapiro-Wilk test for normality. A regression of carapace length on critical thermal maximum was run and the residuals were subtracted from the critical thermal maximum measures to correct for spider body size. The regression was not significant ($F_{1,42}$=2.50, p=0.12) but was used here because body size is known to be influential in many aspects of ecology and can hide significance of biologically interesting factors if not corrected for (Walker & Rypstra 2001; Turner & Williams 2005; Huey & Pianka 2007). An analysis of variance was run using the corrected temperature at which the spider lost the ability to move as the dependent variable and sex, elevation, and location groups as main factors. Mean and standard error of the corrected critical high temperature estimates were calculated and graphed for comparison between groups. The critical cold temperature methods were only able to give a single estimate for all spiders tested due to mechanical limitations. Over a period of two weeks 44 spiders were used in a high temperature estimates and 37 were used in a low temperature estimates (Tables 16, 17).

Table 16: Sample size for Critical thermal maximum test

	n
Mt. Rich	26
High Elevation	14
Male	1
Female	13
Low Elevation	12
Male	4
Female	8
Mt. Magazine	18
High Elevation	6
Male	3
Female	3
Low Elevation	12
Male	1
Female	11
All Spiders	44

Table 17: Sample size for critical thermal minimum test

	n
Mt. Rich	24
High Elevation	15
Male	3
Female	12
Low Elevation	9
Male	1
Female	8
Mt. Magazine	13
High Elevation	4
Male	0
Female	4
Low Elevation	9
Male	1
Female	8
All Spiders	37

3.5 Critical Temperature Results

No statistical differences were found between sexes ($F_{1,36}$=2.04, p=0.16), elevation groups ($F_{1,36}$=3.33, p=0.08), or mountain groups ($F_{1,36}$=0.16, p=0.69). All interaction terms showed no significant affect on critical thermal maximum ($F_{1,36}$<0.53, p>0.09) (Table 18). When graphed both sexes and elevation groups fall greater than two standard error units apart with

the females and low elevation groups showing the greater critical thermal maximum (Figure 12). The difference between the sexes was only found at Mt. Rich while the difference between the elevation groups was only found at Mt. Magazine (Table 19). The critical thermal minimum test showed that all spiders were completely inactive below 0˚C.

Table 18: Three-way ANOVA results comparing critical thermal maximum corrected for carapace length compared between sex, elevation, and mountain groups

Corrected Critical Max		**n=44**		**r^2=0.29**	
Source	**SS**	**df**	**MS**	**F**	**p**
Sex	0.827	1	0.827	2.036	0.16
Elevation	1.352	1	1.352	3.328	0.08
Mountain	0.065	1	0.065	0.159	0.69
Sex*Elev	0.211	1	0.211	0.521	0.48
Sex*Mtn	0.001	1	0.001	0.002	0.96
Elev*Mtn	1.250	1	1.250	3.078	0.09
Sex*Elev*Mtn	0.160	1	0.160	0.394	0.53
Error	14.623	36	0.406		

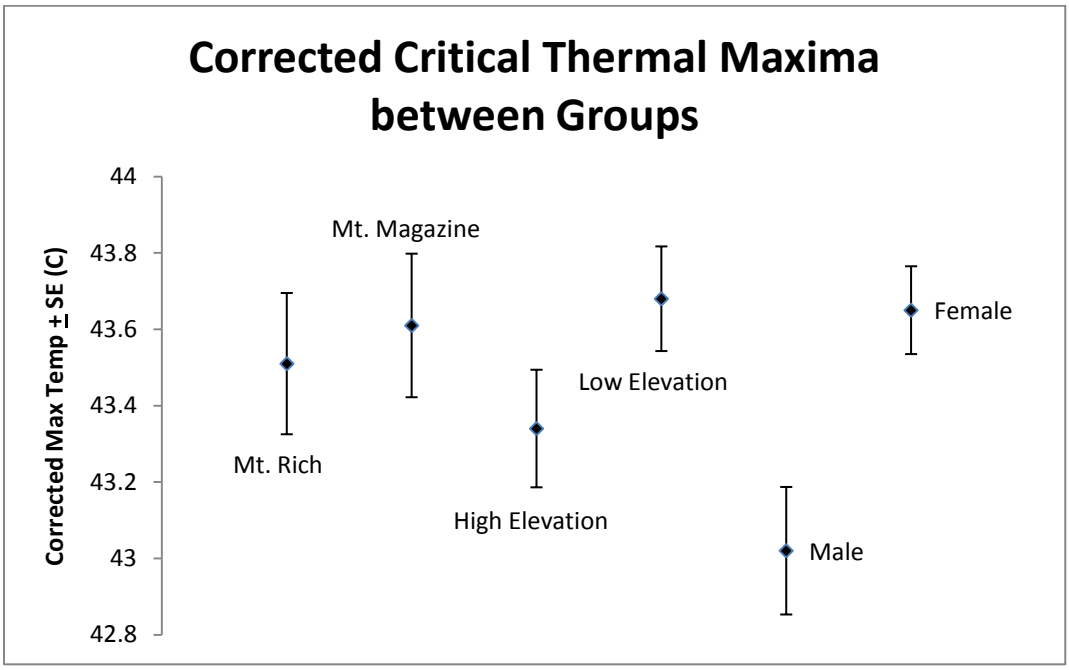


Figure 12: Mean (± SE) corrected critical thermal maximum compared between sex, location, and elevation groups

Table 19: Mean critical thermal maximum corrected for carapace length for comparisons between location, elevation and sex groups

	Mean ± SE
Mt. Rich	43.46 ± 0.121
High Elevation	43.51 ± 0.185
Male	43.09
Female	43.54 ± 0.197
Low Elevation	43.40 ± 0.157
Male	43.14 ± 0.166
Female	43.53 ± 0.212
Mt. Magazine	43.61 ± 0.188
High Elevation	42.93 ± 0.211
Male	42.54 ± 0.107
Female	43.32 ± 0.242
Low Elevation	43.96 ± 0.200
Male	43.95
Female	43.96 ± 0.219
All Spiders	43.52 ± 0.105

3.6 Sprint Speed Methods

Adult spiders were collected as described in section 2.2. After the period of not being fed, the Monday group was placed in a walk in incubator containing the sprint track and set to 20°C at 8am and allowed to acclimate for eight hours. A humidifier was also set up to prevent dehydration. After eight hours acclimation in the incubator trials were started. For Tuesday the same methods were used at 30°C, on Wednesday 15°C, on Thursday 35°C and on Friday 25°C.

In the incubator was a sprint speed track which had four lanes made from 5cm inner diameter clear plastic tubing cut in half and glued to a wooden base making a tunnel. The base was lifted off the ground approximately 30cm by four wooden legs to allow access to the underside. The tracks were marked with four half meter sections for timing purposes. A starting section was delimited by cuts into the tubing at the beginning of the track so that note card dividers could be inserted leaving a small 10cm space in which the spiders were placed and allowed to acclimate for five minutes. Four spiders at a time were loaded into the starting spaces and acclimated, each in a separate lane of the track. After five minutes, the note card divider facing the first track was removed and a 2.5cm plastic cap was used to chase the spider down the track. As one person operated the chasing cap, the other timed the splits run by the

spider using two stop watches starting the timer at the end of the first half meter and taking splits at the end of the last three half meter sections. A plastic cup affixed to the end of the track prevented the spider from escaping.

The same methods were used for all four tracks, one at a time from left to right, and then the whole process was repeated until all spiders had been run. In the case that results were not obtained, spiders were tested one extra time with the same methods. Adequate time for recovery was given for all spiders needing to be rerun. Spiders were run at one temperature only. The sprint speed in m/sec was calculated by dividing 0.5m by the fastest split time. The carapace length, carapace width, and body length were measured for each spider.

One hundred and seventy two spiders total were used in the analysis (table 20). Homogeneity of variances was tested using Bartlett's and Levine's tests and distribution was tested using a normal probability plot with a linear smoother and a Shapiro-Wilk test of normality. Following these tests, the sprint speed data was double square root transformed to more closely fit the normal distribution. An analysis of covariance was run using the double square root transformed sprint speed as the dependent variable and carapace length as the covariate. The mountain of capture, elevation group, sex, and test temperature were run as main factors. A Tukey post-hoc test was also run comparing sprint speeds among the temperatures.

Table 20: Sample size for adult sprint speed test

Temperature:	15°C	20°C	25°C	30°C	35°C
All Spiders	34	34	34	36	34
Mt. Rich	15	17	15	18	18
High Elevation	7	8	8	9	11
Male	4	4	5	4	7
Female	3	4	3	5	4
Low Elevation	8	9	7	9	7
Male	3	4	4	4	2
Female	5	5	3	5	5
Mt. Magazine	19	17	19	18	16
High Elevation	9	11	9	7	7
Male	4	5	4	3	4
Female	5	6	5	4	3
Low Elevation	10	6	10	11	9
Male	3	2	2	3	2
Female	7	4	8	8	7

Juvenile spiders were taken from mothers who had been collected at the same collection locations as the adults run in the sprint speed test. These juveniles were collected from the mother's container as soon as they dispersed and were placed in plastic condiment containers with moistened white aquarium sand covering the bottom of the container and a clear plastic lid covering the top. Each container held only one juvenile and was marked with the collection number of the mother and a collection number for the juvenile. The young were maintained in these containers at lab temperature (25.6°C) for one month while other juveniles were collected from their mothers. During this time all spiders set up in containers were offered a single fruit fly for 24 hours and additional moisture as needed every three days. All uneaten flies and fly parts were removed after the 24 hour period was up.

After a month sibling groups were split into three groups and each group was put into a cold temperature (20°C), a room temperature (25.6°C), or a hot temperature (28°C) location. The juveniles were kept on the same feeding cycle as the other groups. As juveniles increased in size they were offered more flies over the same 24 hour period every three days. Offspring were maintained at temperature for 10 months until they were used in a sprint speed test at

which time they were removed from temperature and had their CL measured under a dissecting microscope. The hot temperature group was removed from analysis due to high mortality.

For the juvenile sprint speed test a smaller track composed of clear plastic tubing with a diameter of 1cm and measuring 0.25m splits over a 1m track. All runs were conducted at lab temperature of 25.6°C. All other methods are the same as those used on the adults. An analysis of covariance was run using the square root of the arcsine square root transformed sprint speed as the dependent variable and the elevation group, mountain of mother's collection, and rearing temperature as main factors. The CL measure was used as the covariate. Two hundred and twenty-one juveniles were used in the analysis (table 21).

Table 21: Sample size for juvenile sprint speed test

	n
Mt. Rich	107
High Elevation	13
Cold	9
Room	4
Low Elevation	94
Cold	58
Room	36
Mt. Magazine	114
High Elevation	53
Cold	33
Room	20
Low Elevation	61
Cold	27
Room	34
All Spiders	221

3.7 Sprint Speed Results

The mountain ($F_{1,131}$=2.0, p=0.16) and sex ($F_{1,131}$=0.13, p=0.72) groups did not show significant differences in sprint speed. The carapace length body size measure also did not show a significant effect on sprint speed ($F_{1,131}$=0.01, p=0.91). This data did show a significant difference in mean adult spider sprint speed between temperature ($F_{1,131}$=36.6, p<0.00001) and elevation ($F_{1,131}$=4.0, p=0.049) groups (Table 22) (Figure 13). Tukey pair wise comparisons

between temperatures showed that the 15°C temperature differed from all other temperatures ($p<0.0001$). Sprint speed at 20°C was significantly slower than sprint speed at 30° and 35°C ($p<0.0001$). Sprint speeds at 25°C was significantly slower than sprint speeds at 30° and 35°C ($p<0.004$) (Table 23).

Table 22: Three-way ANCOVA results comparing adult sprint speed between elevation, location, and sex groups

ANCOVA		n=172	r^2=0.63	
Source	**SS**	**df**	**F-Ratio**	**p**
Temperature	0.90	4	36.6	**<0.00001**
Sex	0.00	1	0.13	0.72
Elevation	0.02	1	4.0	**0.049**
Mountain	0.01	1	2.0	0.16
Temp*Sex	0.03	4	1.4	0.24
Temp*Elev	0.02	4	0.69	0.60
Tem*Mtn	0.02	4	0.78	0.54
Sex*Elev	0.00	1	0.59	0.44
Sex*Mtn	0.00	1	0.49	0.48
Elev*Mtn	0.01	1	1.3	0.25
Temp*Sex*Elev	0.04	4	1.5	0.22
Temp*Sex*Mtn	0.04	4	1.7	0.16
Temp*Elev*Mtn	0.01	4	0.48	0.75
Sex*Elev*Mtn	0.01	1	2.0	0.16
Temp*Sex*Elev*Mtn	0.04	4	1.5	0.19
CL	0.00	1	0.01	0.91
Error	0.81	131		

Table 23: Post-hoc Tukey results comparing adult sprint speed between temperature groups

	15°	20°	25°	30°	35°
15°	1.00				
20°	<0.0001	1.00			
25°	<0.0001	0.52	1.00		
30°	<0.0001	<0.0001	0.003	1.00	
35°	<0.0001	<0.0001	<0.0001	0.31	1.00

Spiders collected at high elevation exhibited a mean sprint speed greater than the mean sprint speed of spiders collected at low elevation (Figure 13). Elevation groups differed

significantly only at the 25°C (Figure 14). The mean sprint speed of spider run at the two highest temperatures (30°, 35°C) were significantly faster than the mean sprint speeds measured in the two middle temperatures (20°, 25°C). The mean sprint speeds measured at the coldest temperature (15°C) differed significantly from all other temperatures (figure 14) (table 24).

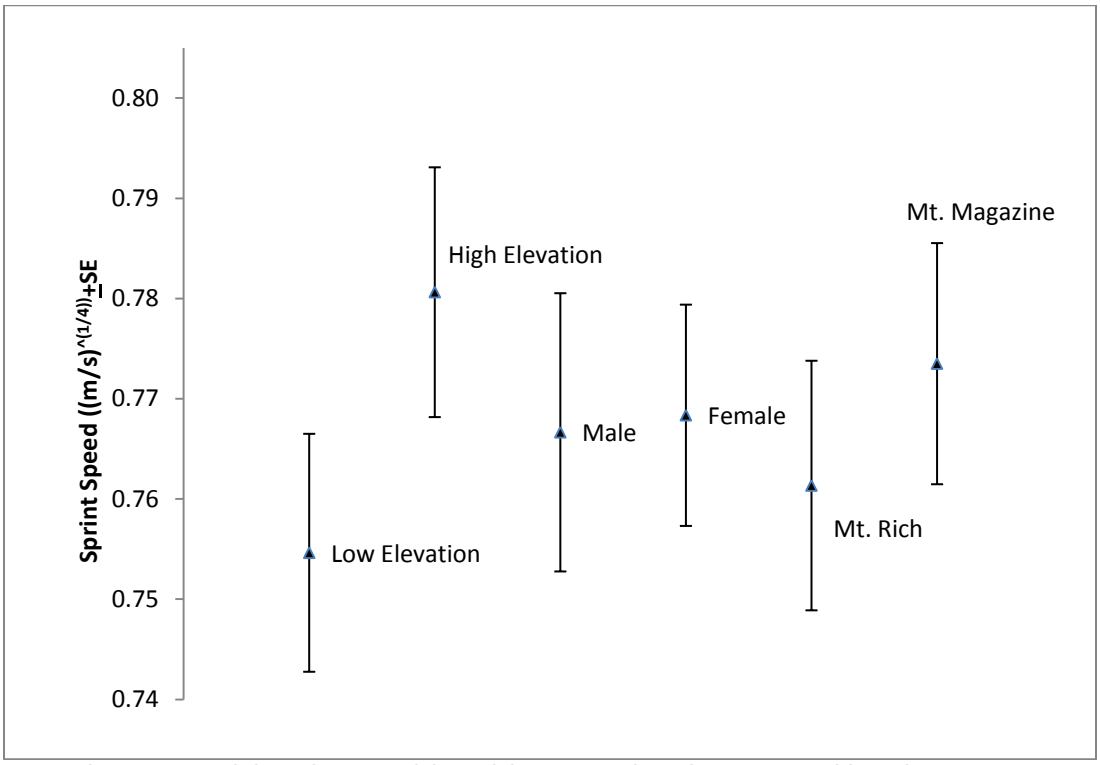


Figure 13: Adult sprint speed (± SE) between elevation, sex, and location groups

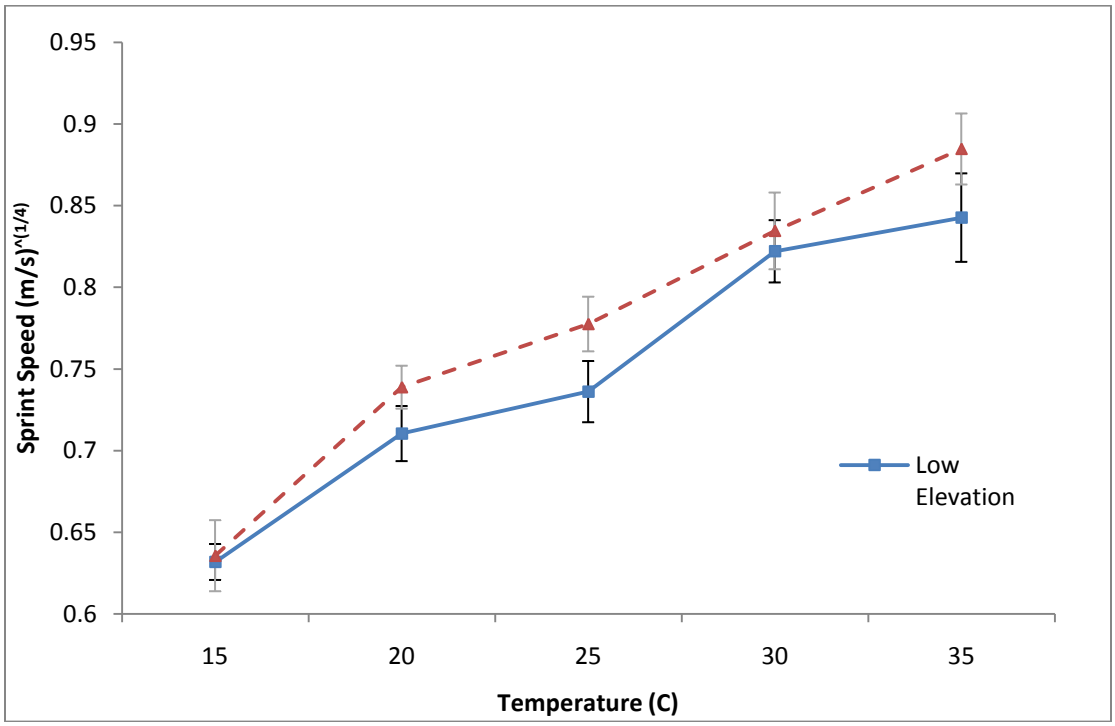


Figure 14: Adult sprint speed (± SE) across temperatures compared between elevation groups

Table 24: Mean adult sprint speed values for comparisons between elevation, location, and sex groups from each mountain within each temperature

m/s at temperature	15°C	20°C	25°C	30°C	35°C
All Spiders	0.17 ± 0.011	0.29 ± 0.17	0.34 ± 0.25	0.50 ± 0.035	0.60 ± 0.047
Mt. Rich	0.14 ± 0.013	0.28 ± 0.027	0.33 ± 0.027	0.51 ± 0.048	0.55 ± 0.052
High Elevation	0.13 ± 0.023	0.30 ± 0.040	0.36 ± 0.029	0.53 ± 0.082	0.59 ± 0.076
Male	0.11 ± 0.036	0.33 ± 0.038	0.38 ± 0.037	0.30 ± 0.051	0.66 ± 0.099
Female	0.15 ± 0.025	0.28 ± 0.075	0.33 ± 0.047	0.70 ± 0.070	0.48 ± 0.114
Low Elevation	0.156 ± 0.012	0.25 ± 0.036	0.30 ± 0.047	0.49 ± 0.053	0.47 ± 0.055
Male	0.15 ± 0.025	0.30 ± 0.076	0.32 ± 0.083	0.57 ± 0.093	0.57 ± 0.159
Female	0.16 ± 0.015	0.21 ± 0.018	0.28 ± 0.031	0.42 ± 0.051	0.429 ± 0.050
Mt. Magazine	0.19 ± 0.016	0.30 ± 0.020	0.36 ± 0.039	0.49 ± 0.053	0.67 ± 0.079
High Elevation	0.216 ± 0.024	0.31 ± 0.025	0.40 ± 0.065	0.51 ± 0.088	0.74 ± 0.107
Male	0.23 ± 0.041	0.29 ± 0.039	0.31 ± 0.060	0.49 ± 0.089	0.83 ± 0.155
Female	0.21 ± 0.033	0.33 ± 0.035	0.48 ± 0.098	0.53 ± 0.152	0.61 ± 0.135
Low Elevation	0.17 ± 0.020	0.29 ± 0.033	0.32 ± 0.047	0.48 ± 0.068	0.62 ± 0.115
Male	0.21 ± 0.059	0.37 ± 0.054	0.20 ± 0.035	0.41 ± 0.137	0.46 ± 0.284
Female	0.15 ± 0.013	0.25 ± 0.027	0.35 ± 0.053	0.51 ± 0.083	0.66 ± 0.132

The data comparing mean sprint speed at a single temperature of juveniles maintained at two different temperatures showed a significant difference between juveniles from mothers

collected on different mountains ($F_{1,212}$=3.98, p=0.04) (Tables 25, 26) (Figure 15). The data did not show significant differences due to which elevation group ($F_{1,212}$=1.64, p=0.20) they came from, or due to the temperature ($F_{1,212}$=0.28, p=0.60) they were maintained in. The covariate CL also did not show a significant effect ($F_{1,212}$=2.24, p=0.14). All interaction terms were not significant ($F_{1,212}$<3.28, p>0.07) (Table 25).

Table 25: Three-way ANCOVA results comparing juvenile sprint speed between mountain, temperature, and elevation groups

ANCOVA	**n=221**	**r^2=0.14**	**dependent variable: sqrt(asn(sqrt(m/s)))**		
Source	**SS**	**df**	**MS**	**F**	**p=**
Mountain	0.02	1	0.02	3.98	**0.047**
Temperature	0.002	1	0.002	0.28	0.60
Elevation	0.01	1	0.01	1.64	0.20
Mtn*Temp	0.01	1	0.01	1.06	0.30
Mtn*Elev	0.02	1	0.02	2.91	0.09
Temp*Elev	0.02	1	0.02	3.28	0.07
Mtn*Temp*Elev	0.003	1	0.003	0.56	0.45
CL	0.01	1	0.01	2.24	0.14
Error	1.14	212	0.01		

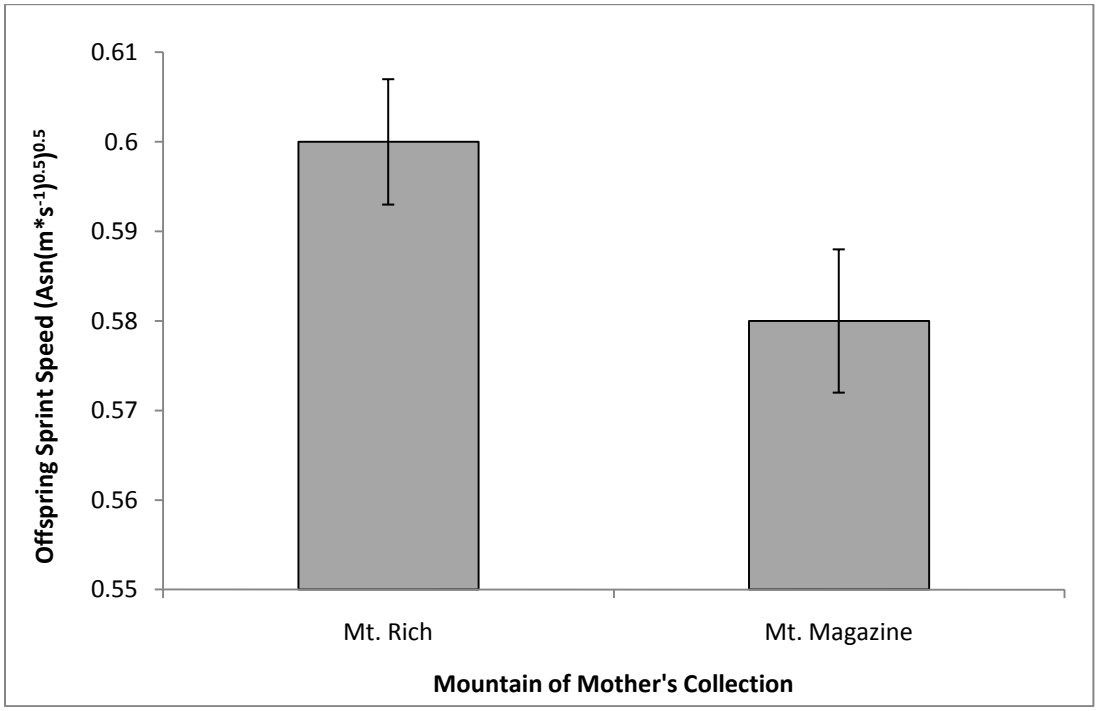


Figure 15: Offspring sprint speed (±SE) between mountain of mother's capture groups

Table 26: Mean juvenile sprint speed values for comparisons between elevation and temperature groups within mountain locations

Juvenile (Asn(sqrt(m/s)))^0.5	Mean ± SE
Mt. Rich	0.60 ± 0.007
High Elevation	0.60 ± 0.013
Cold	0.60 ± 0.009
Room	0.60 ± 0.042
Low Elevation	0.60 ± 0.007
Cold	0.58 ± 0.008
Room	0.61 ± 0.014
Mt. Magazine	0.58 ± 0.008
High Elevation	0.55 ± 0.011
Cold	0.55 ± 0.010
Room	0.56 ± 0.024
Low Elevation	0.60 ± 0.011
Cold	0.55 ± 0.014
Room	0.63 ± 0.013
All Spiders	0.60 ± 0.005

3.8 Thermal Strategy Comparisons

Rabidosa rabida has more characteristics in common with thermal generalists (Figure 16) and with thermal conformers (Figure 17) than with the alternative thermal strategies. Ecological specialists, including thermal specialists are thought to evolve in environments that are relatively homogeneous while generalists are thought to evolve in habitats that are relatively heterogeneous in space and time (Kassen 2002). Due to fitness advantages obtained by performing certain tasks within a relatively narrow temperature range, thermal specialists tend to show a narrow range of temperature preference as opposed to thermal generalists which tend to show wider thermal preferences (Gilchrist 1995). Thermal specialists often maintain a narrow range of body temperatures due either to staying in homogeneous environments or to physiological thermal regulation (Willott & Hassall 1998). Most ectothermic animals are thermal generalists and have adapted physiologies to function at the temperatures they experience (Heinrich 1977). *Rabidosa rabida* experiences a range of thermal environments across its wide

range. This spider is a nocturnally active predator that does not have insulating morphology. It spends its most active time up in the vegetation where it is exposed to ambient temperatures and winds. Because of these behaviors and characteristics *R. rabida* appears to be a thermal generalist.

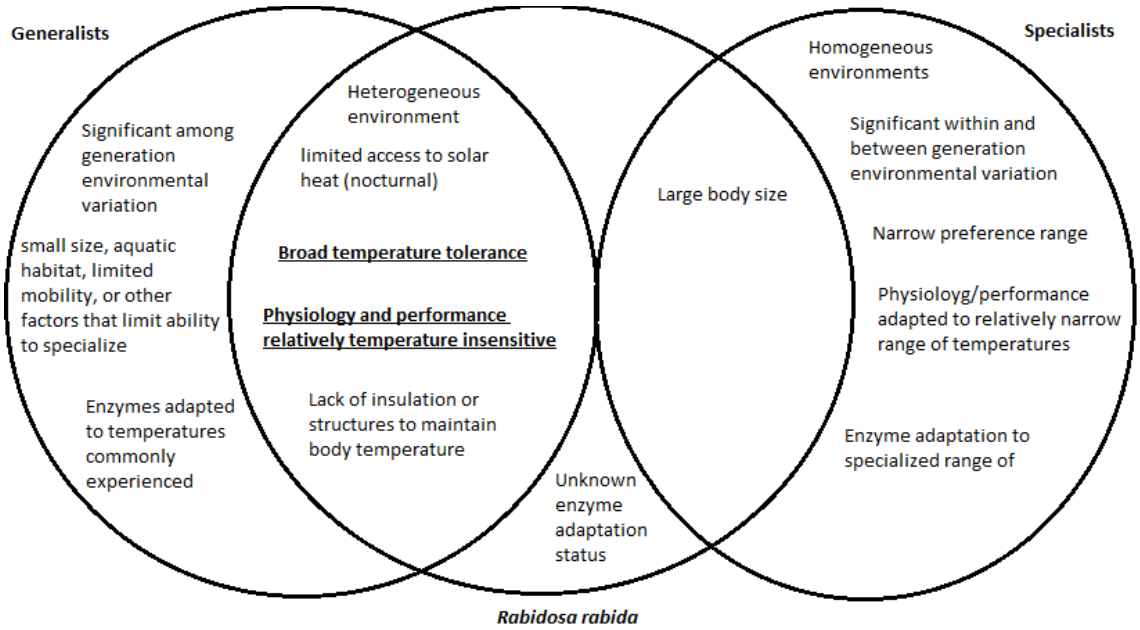


Figure 16: Traits described in thermal generalists and specialists compared with traits of *Rabidosa rabida*. Bold and underlined characters were first described in this study

Thermal regulators use physiological, morphological, and behavioral characteristics to maintain a body temperature at some preferred level. In arthropods, flight muscles are examples of a characteristic used to produce heat. Dark coloration, insulating morphology, and leg length along with behaviors such as basking or stilting have all been described as methods for raising or lowering body temperature (Heinrich 1996). Physiology of thermal regulators is often adapted to the range of temperatures that their body is maintained at or near. Thermal conformers often are found in temperatures that are included within their preferred thermal range. Thermal conformers often have relatively slow metabolism compared to thermal regulators because they do not require large amounts of energy to maintain body temperature. *Rabidosa rabida* has no known behavioral or morphological adaptations for producing or

maintaining body heat. This spider also has a low metabolic rate which is more consistent with thermal conformers than thermal regulators (Anderson 1970).

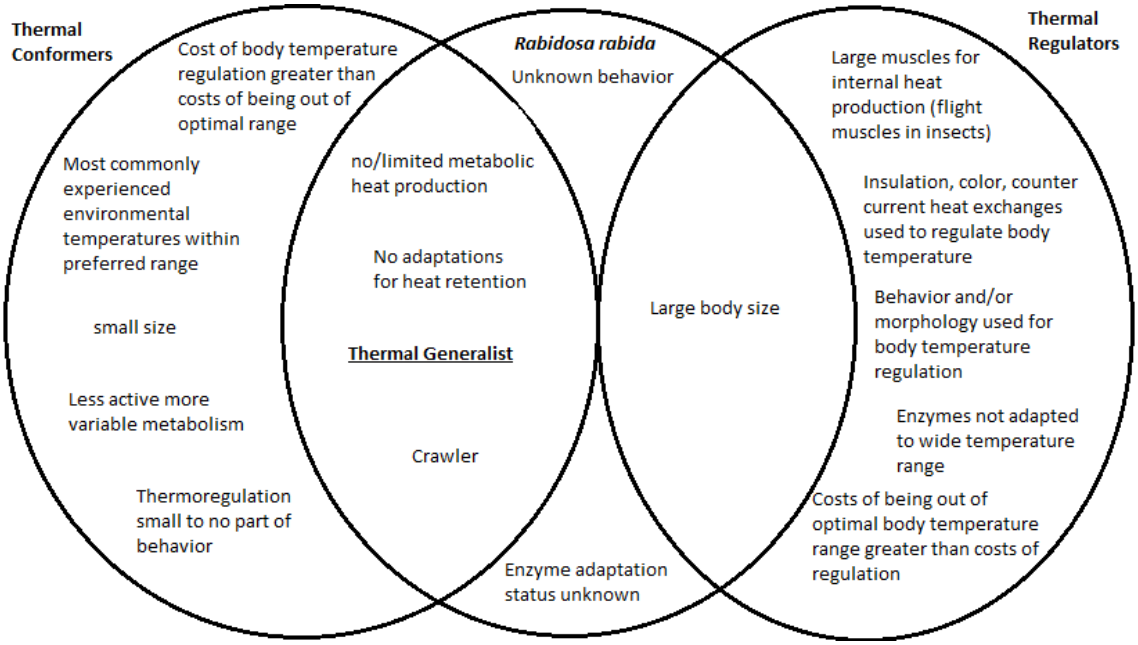


Figure 17: Traits described in thermal conformers and regulators compared to traits of *Rabidosa rabida*. Bold and underlined characters were first described in this study.

A Q_{10} estimate was calculated using the sprint speed data from section 3.7 using the following formula (Schmidt-Nielsen 1997).

$$Q_{10} = (R2/R1)^{(10/(T2-T1)}$$

In this equation R1 is the reaction rate or performance measure taken at temperature 1 (T1), while R2 is the reaction rate or performance rate measured at temperature 2 (T2).

This Q_{10} estimate was calculated over each 5 degree interval. X^2 goodness of fit tests were run comparing Q_{10} estimates between sex, elevation, and mountain groups and between temperature ratios following methods from Sokal and Rohlf (1995). For the sex comparison female Q_{10}s were used as the expected value. For the elevation groups high elevation Q_{10} estimates were used as the expected and for the mountain Mt. Magazine estimates were used

as the expected values. The degrees of freedom were calculated by subtracting 2 from the number of Q_{10} comparisons used. Statistic values were compared to critical values (Rohlf & Sokal 1995). *Rabidosa rabida* exhibited thermal sensitivity as seen through a temperature coefficient of around the mean value observed of 1.96 in all groups across all temperature ranges (Table 28). All groups showed the greatest thermal sensitivity at the lowest temperature range (15-20°C) and an apparent increase in sensitivity at the 25-30°C temperature range with the exception of males which showed an apparent increase at a higher temperature range (30-35°C). The lowest sensitivities were found at the 20-25°C and 30-35°C temperature ranges (Table 28). There were no significant differences in Q_{10} estimates between sexes (X^2=1.72, df=2, p>0.1), mountain groups (X^2=0.91, df=2, p>0.5) or elevation groups (X^2=0.27, df=2, p>0.5). There was also no significant difference between the different 5 degree temperature ranges (X^2=1.19, df=1, p>0.1) (Table 27).

Table 27: X^2 goodness of fit test results comparing Q_{10} estimates between elevation, sex, mountains, and five degree temperature ranges

	Elevations	**Sexes**	**Mountains**	**Temperature Ratios**
X^2	0.27	1.72	0.91	1.19
df	2	2	2	1
p	>0.5	>0.1	>0.5	>0.1

Table 28: Q_{10} estimates for sprint speed across five degree temperature intervals for comparisons between elevation, sex, and mountain groups

Q_{10} of Sprint Speed ± SE

Q_{10} Ratio	**Low Elevation**	**High Elevation**	**Male**	**Female**	**Mt. Rich**	**Mt. Magazine**	**Total**
20/15	2.64±1.036	2.97±1.323	2.26±1.765	2.56±0.851	3.65±1.621	2.49±0.867	2.87±0.797
25/20	1.37±0.667	1.54±0.595	1.03±0.438	1.86±0.794	1.44±0.622	1.40±0.573	1.44±0.418
30/25	2.41±1.156	1.85±0.941	1.94±1.001	2.10±0.964	2.34±0.973	1.88±1.022	2.07±0.679
35/30	1.30±0.689	1.57±0.822	2.25±1.266	1.07±0.510	1.16±0.535	1.85±1.042	1.46±0.499

3.9 Thermal Ecology Discussion

Three of the measures commonly used to describe thermal ecology of a species are thermal preference, critical thermal limits, and locomotor performance across temperature. Thermal preference was used both as a description of thermal ecology and in a search for intra-specific variation. The ecological significance of laboratory measures of thermal preference have been called into question and are thought to overestimate the true mean preferred body temperature of the animal in many cases (Watson 2008). Because this spider was only allowed to acclimate for 30 minutes this estimate is of the acute thermal preference (measured within 2 hr or less of placement in thermal gradient) not the final preference which must be estimated using a longer period of time (Reynolds & Casterlin 1979). Despite this, this measure was able to show differences between groups and so has been a useful measure for the purposes needed here.

Intra-specific variation was seen in the thermal preference between spiders collected at different mountains. There is a small temperature difference between the mountains in the colder times of the year. The lower thermal preference shown by spiders from Mt. Magazine, while small, could suggest that these spiders would become active slightly before the spiders at Mt. Rich or at close to the same time if Mt. Magazine is slightly cooler at the beginning of the active season. Further field observations are needed to confirm this.

Critical thermal limits were also used to describe thermal ecology and look for intra-specific variation. The critical thermal maximum of this spider in the low 40Cs is probably greater than the temperature experienced at night when it is active. Even during the day when this temperature might be encountered simple behaviors could allow the animal to avoid dangerous temperatures. The more important of the critical thermal limits is probably the lower thermal limit which was roughly estimated to be around freezing. Temperatures below freezing are likely encountered by this spider during the winter months. This would suggest that there is some behavior that would allow this spider to avoid either experiencing this temperature or

needing to be active during exposure. This spider was not observed to be active at temperatures near freezing confirming this observation.

Intra-specific variation was found in the measure of critical thermal maxima. There is a difference in critical thermal maximum between the sexes which could suggest physiological differences resulting from reproductive behaviors and a slight physiological sexual dimorphism in this spider. There is also a difference between the elevation groups. Spiders collected at lower elevation, where they would be expected to encounter greater temperatures than at high elevation, showed a greater thermal maximum than spiders from high elevation. The difference is again very small as is the temperature difference between the elevations.

The final thermal ecology descriptive measure looked at in this chapter is locomotor performance across temperature. Sprint speed, used here as a measure of maximum locomotor performance, is often used as a measure of physiological performance relating to temperature (Huey & Stevenson 1979). Sprint speed is also assumed to affect fitness by influencing factors such as predator escape, prey capture, mate location, and many other fitness influencing behaviors. There are examples showing the connection between fitness and sprint speed in reptiles but few examples have been published for spiders. There are a few studies which suggests that sprint speed may accurately represent fitness for many spiders as well (Formanowicz in process).

In this study sprint speed was shown to be significantly affected by temperature. This is an expected result as locomotor performance has been repeatedly shown to be affected by temperature in all ectotherms (Van Berkum 1988; Huey & Kingsolver 1989; Xiang et al. 1996). There was significant variation shown in the sprint speed between spiders collected from different elevations with the spiders from higher elevation having a greater mean sprint speed than those from lower elevation. Comparing the elevation groups across temperatures, the groups differ significantly only at temperatures in the mid 20s near those most likely experienced while hunting in the upper vegetation. At temperatures other than 25°, the sprint

speed varies significantly between elevation groups at all but the lowest temperature run where the mean sprint speed is nearly identical. This difference in sprint speed could be the result of differences in optimal temperature or optimal performance. A more complete measure of performance across temperatures in order to determine optimal performance is necessary to determine what is causing this difference. Despite the type of thermal performance differences, there is variation in the thermal ecology measure that is most often associated with fitness and performance of ecologically important behaviors such as predator escape and prey capture.

Sprint speed using juvenile spiders showed variation between spiders whose mothers were captured on different mountains. This could suggest that there are some developmental differences or that spiders from different mountains react more or less strongly to different temperatures. Juveniles from different mountains differed with respect to high elevation and being raised at cold temperature. This again suggests that not only is the higher elevation a few degrees colder than the low elevation but also that cold temperatures are more ecologically significant than high temperatures to this spider.

Rabidosa rabida was shown to have more characteristics in common with thermal generalists and thermal conformers than with specialists and regulators. This is also not surprising considering their lack of physical adaptations for heat production and control and their nocturnal hunting behaviors. The thermal sensitivity as measured from sprint speed showed that this spider has a temperature coefficient right around 2 for temperatures that are not below 20. Other arthropods have had Q_{10} values reported as being around 2 with variation above and below this value depending on sensitivity (Reichle 1968; Duncan & Dickman 2001). The fact that there was an apparently greater thermal sensitivity across the 25-30 degree range suggests that it is at these temperatures that the effect of temperature begins to show greater influence. This trend was seen for all groups except for male spiders which showed a greater thermal sensitivity between 30° and 35°C than was seen between 25° and 30°C. Spiders, with the exception of males, run faster as temperature increases from 20° to 25°C but the greatest

increase was found in the increase from 25° to 30°C which again are the temperatures that this spider would be expected to encounter in nature during the warm season when it is active. This could also suggest that there would be measureable differences in performance across a single night when temperatures may drop from the upper twenties to lower twenties. Further study of field behavior and field body temperature measurement is needed to confirm these findings.

This first description of thermal ecology has provided the first evidence of intra-specific variation in this ecologically important temperate arthropod predator. Variation is seen in some but not all measures between elevation and mountain groups. Variation is also seen between sexes in some measures though these do not represent comparisons between temperature environments but do suggest sexual dimorphism that might affect future attempts to look at thermal ecology in this spider. Other measures should be explored which directly influence the ecological importance of this animal. These measures might also show variation and provide evidence of what effects a temperature difference of 2° - 3°C will have on this animal and which measures might be most useful when looking for variation between other temperate arthropod predators.

CHAPTER 4

OTHER ECOLOGICALLY IMPORTANT MEASURES

4.1 Introduction

There are multiple types of variation that can be found between populations of a single species. Some variation has a genetic basis and can provide a basis for selection and adaptation to local environments. Other variation can occur without any genetic difference and is caused by environmental effects on gene expression (Stearns & Koella 1986). Variation of both types can be found in the form of behavioral or performance differences which could affect fitness and ecology. If the variation found within a species does have a genetic basis then there is potential for selection on the variance to cause evolution (Simons & Roff 1994). Both types of variation can tell a great deal about the effect of an environmental variable such as temperature on the ecology of an animal. In either case choosing an appropriate variable to test for variation can make a great difference in whether something interesting is found.

One measure that has been suggested as being ecologically important for cursorial spiders is prey capture. The effect of temperature on spider hunting performance is interesting because not only are spiders important predators in many terrestrial ecosystems, but hunting performance is also linked strongly to fecundity showing ecological and evolutionary importance of this variable (Schmalhofer & Casey 1999). The effects of temperature on hunting by spiders has been dominated by web-building spiders and spiders found in desert environments (Riechert & Tracy 1975; Henschel 1990; Turner et al. 1993). These studies have shown the effect of temperature on several aspects of prey capture. Unfortunately the literature concerning the effects of temperature on prey capture in temperature cursorial spiders is much less common. The best example of thermal ecology of hunting performance in a cursorial spider is the work done by Schmalhoefer (1999; 1999) on two species of crab spiders (Araneae: Thomisidae). In this study hunting performance is measured as the ability to capture prey because most spiders, including cursorial spiders such as *Rabidosa rabida*, are sit-and-wait predators rather than

actively pursuing hunters (Uetz 1992; Schmalhofer & Casey 1999).

The final measure used in this section to explore intra-specific variation in this temperate arthropod predator is a measure of immunity. Since innate immunity requires some physiological costs to produce, testing for immunity as a function of ecological and environmental factors is gaining popularity in ecology (Le Moullac & Haffner 2000; Sandland & Minchella 2003; Cotter et al. 2004; Lee 2006). Measures of innate immunity enzymes have been suggested as useful indicators of immunocompetence in arthropods (Mucklow et al. 2004). One innate immunity system important in invertebrates is the phenoloxidase or melanization cascade (Nappi & Christensen 2005).

Phenoloxidase (PO) is an enzyme involved in the oxidation of phenols to quinones which then polymerize into melanin. This reaction produces many intermediate substances and the pigment melanin itself which are toxic to microorganisms making it an important part of invertebrate innate immunity (Soderhall & Cerenius 1998; Nappi & Ottaviani 2000; Nappi & Christensen 2005). Like some insects (Gillespie et al. 1997), in spiders PO resides in the hemolymph in an inactive form called pro-Phenoloxidase (PPO) (Jiravanichpaisal et al. 2006). In order for the PPO cascade to take place the PPO zymogen must be altered from the inactive pro-PO to Phenoloxidase (PO) the active form. The activation of PPO into PO is stimulated by small amounts of microbial components such as lipopolysaccharides and peptidoglycans from bacteria cell walls and beta-1,3 glucans from fungi (Jiravanichpaisal et al. 2006).

This study aimed to determine the effect of a relatively small temperature difference on an ecologically important temperate arthropod predator. Two measures, which show ecological importance, were measured in this section including prey capture and immunity at different temperatures. Through these measures the possibility of intra-specific variation across a small temperature difference will be explored and information about the effect of temperature on ecology was gathered. It was predicted that temperature would be found to have a significant effect on both of these measures as environmental factors have been shown to affect both of these variables in other animals (Schmalhofer & Casey 1999; Cornet et al. 2009).

4.2 Predation Methods

Spiders were collected from Rich Mountain and Mount Magazine following methods described in section 2.2. For testing spiders were allowed to acclimate to one of three temperatures (15, 25, or 35). After 12 hours of acclimation spiders were tested in upside down 25cm diameter convex container with a 2.5cm hole cut in the top and a flat bottom. Playground sand covered the bottom of the container. Twenty four containers were set up in each temperature. A single spider was placed in each container and given five minutes to acclimate to the container. After acclimation two crickets were added to eight of the containers, four crickets to eight other containers, and eight crickets to the final eight containers. Clear tape covered the hole in the container after adding crickets to prevent escape and prevent wind disturbance. Spiders were allowed to capture as many crickets as they could over a 24 hour period. After the allotted time had passed the number of crickets killed was counted. Spiders that died were removed prior to analysis. The carapace length of each spider was measured before testing and used to run an analysis of variance using the carapace length measure as the dependent variable and the elevation, mountain, sex, and temperature groups as main factors. Before running this ANOVA parametric assumptions were tested using a probability plot with linear smoother and Bartlett's and Levine's tests for homogeneity of variances. Mean and standard errors were calculated for each group and graphed for comparison. Seventy spiders were used in this analysis (Table 29).

Table 29: Sample size for predation test

Sample Size	15°C	25°C	35°C	All Temps
All Spiders	24	24	22	70
Mt. Rich	12	11	11	34
High Elevation	7	3	6	16
Male	4	2	2	8
Female	3	1	4	8
Low Elevation	5	8	5	18
Male	3	3	3	9
Female	2	5	2	9
Mt. Magazine	12	13	11	36
High Elevation	6	7	6	19
Male	2	3	3	8
Female	4	4	3	11
Low Elevation	6	6	5	17
Male	2	2	2	6
Female	4	4	3	11

4.3 Predation Results

An analysis of variance showed that the mean carapace length did not differ significantly between temperature ($F_{2,46}$=0.097, p=0.91), sex ($F_{1,46}$=3.181, p=0.08), elevation ($F_{1,46}$=2.419, p=0.13), or mountain ($F_{1,46}$=2.198, df=1/46, p=0.14) groups. All interaction terms were also non significant ($F_{2\text{-}1,46}$<0.67, p>0.45) (Table 30). The means of the number of crickets killed by females was greater than the mean killed by males by more than two standard error units. The mean number of crickets killed by spider from Mount Rich was greater than the mean number killed by spiders from Mount Magazine by a difference of less than two standard error units. The mean number of crickets killed by low elevation spiders was greater than the mean number killed by high elevation spiders but this difference was less than two standard error units (Figure 18) (Table 31).

Table 30: Three-way ANOVA results comparing carapace Lengths between temperature, sex, and mountain groups

Dep Var: CL		**n=70**		**r^2=0.27322**	
Source	**SS**	**df**	**MS**	**F**	**p value**
Temperature	0.001	2	0.000	0.097	0.91
Sex	0.014	1	0.014	3.181	0.08
Elevation	0.011	1	0.011	2.419	0.13
Mountain	0.010	1	0.010	2.198	0.14
Temp*Sex	0.002	2	0.001	0.183	0.83
Temp*Elev	0.001	2	0.000	0.101	0.90
Temp*Mtn	0.006	2	0.003	0.662	0.52
Sex*Elev	0.003	1	0.003	0.560	0.46
Sex*Mtn	0.000	1	0.000	0.009	0.93
Elev*Mtn	0.002	1	0.002	0.412	0.52
Temp*Sex*Elev	0.007	2	0.004	0.792	0.46
Temp*Sex*Mtn	0.002	2	0.001	0.209	0.81
Temp*Elev*Mtn	0.001	2	0.001	0.142	0.87
Sex*Elev*Mtn	0.001	1	0.001	0.317	0.58
Temp*Sex*Elev*Mtn	0.003	2	0.002	0.351	0.71
Error	0.209	46	0.005		

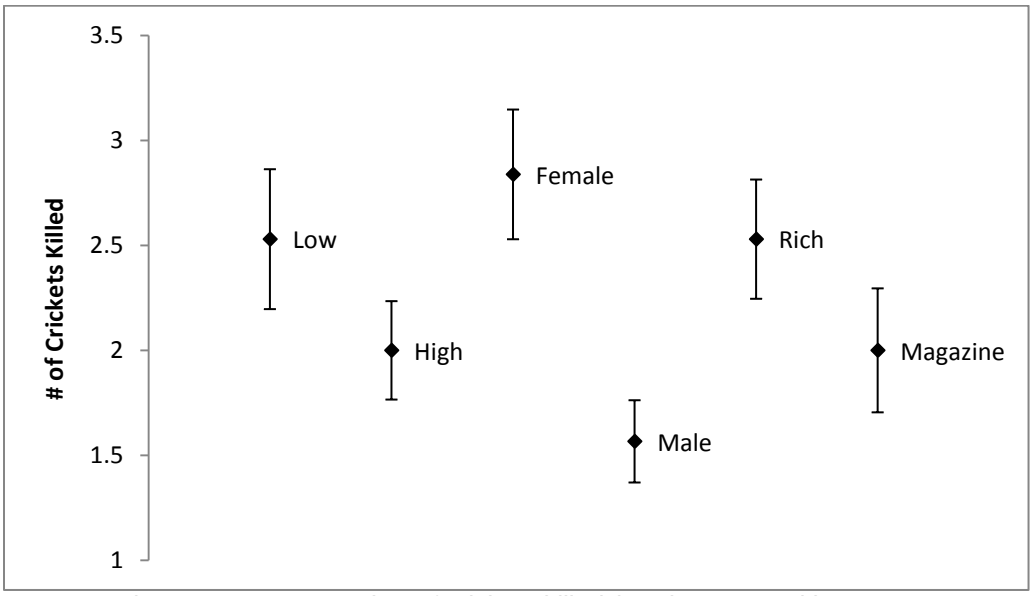


Figure 18: Mean number of crickets killed (±SE) compared between elevation, sex, and mountain groups

Table 31: Mean number of crickets killed for comparisons between sex, elevation, and mountain groups within temperatures

Temperature	15°C	25°C	35°C	All Temps
Total	1.75 ± 0.320	2.13 ± 0.291	3.00 ± 0.378	2.27 ± 0.20
Mt. Rich	1.92 ± 0.379	2.64 ± 0.56	3.09 ± 0.513	2.53 ± 0.284
High Elevation	1.57 ± 0.429	2.00 ± 0.577	2.83 ± 0.543	2.13 ± 0.315
Male	0.75 ± 0.250	1.50 ± 0.500	3.00 ± 1.00	1.50 ± 0.423
Female	2.67 ± 0.333	3	2.75 ± 0.750	2.75 ± 0.366
Low Elevation	2.40 ± 0.678	2.88 ± 0.743	3.40 ± 0.980	2.89 ± 0.449
Male	1.33 ± 0.333	1.67 ± 0.667	2.67 ± 0.667	1.89 ± 0.351
Female	4.00 ± 0.00	3.60 ± 1.030	4.50 ± 2.500	3.89 ± 0.70
Mt. Magazine	1.58 ± 0.529	1.69 ± 0.208	2.91 ± 0.58	2.03 ± 0.272
High Elevation	1.83 ± 0.910	1.71 ± 0.286	2.33 ± 0.422	1.95 ± 0.320
Male	0.50 ± 0.500	1.00 ± 0.000	1.67 ± 0.333	1.13 ± 0.227
Female	2.50 ± 1.258	2.25 ± 0.250	3.0 ± 0.577	2.55 ± 0.455
Low Elevation	1.33 ± 0.615	1.67 ± 0.333	3.60 ± 1.167	2.12 ± 0.461
Male	1.50 ± 0.500	1.00 ± 1.00	3.0 ± 1.00	1.83 ± 0.543
Female	1.25 ± 0.946	2.00 ± 0.00	4.0 ± 2.00	2.27 ± 0.662

The mean number of crickets killed by spiders increased as temperature increased across all temperatures and all initial prey densities except for the 4 cricket density (Tables 1,2,3, and 4). Due to missing data statistical analysis was not reliable. The lowest temperature (15°C) and the highest temperature (35°C) differed by greater than two standard errors. The middle temperature (25°C) differed from the 15°C temperature by less than two standard error units and differed from the 35°C temperature by greater than two standard error units (Figure 19). The mean number of crickets killed between initial prey densities 2, 4, and 8 are shown below but were not able to be analyzed statistically due to missing data (Tables 32, 33, 34)

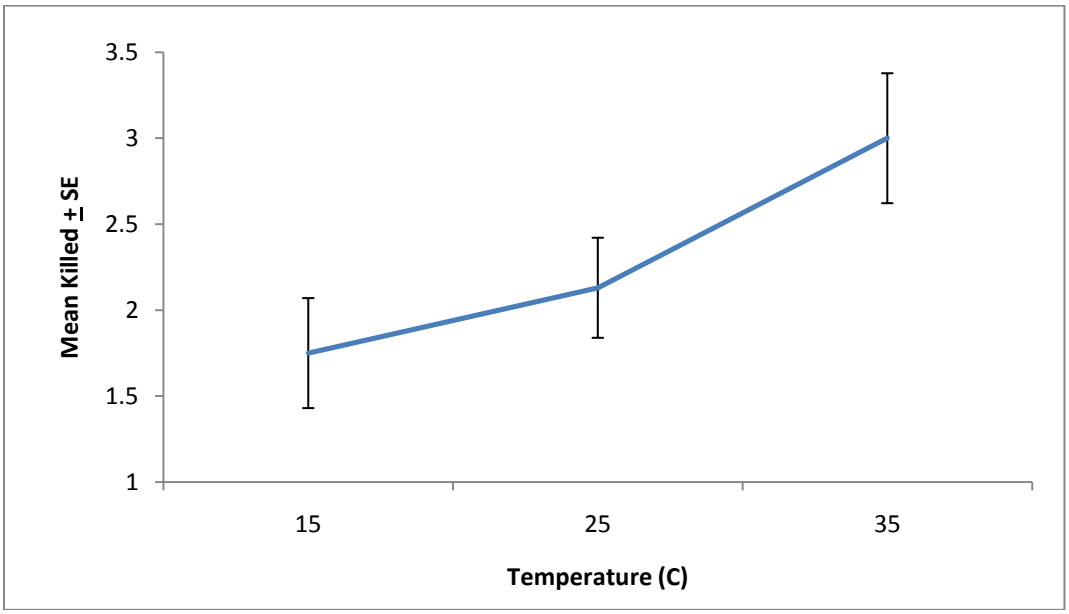


Figure 19: Mean (± SE) number of crickets killed across three temperatures

Table 32: Mean number of crickets killed at initial prey densities of 2 for comparisons between sex, elevation, and mountain groups within temperatures

Initial Density of 2 Crickets

Temperature	15°C	n	25°C	n	35°C	n	All Temps	n
Total	1.0 ± 0.327	8	1.75 ± 0.164	8	1.86 ± 0.143	7	1.52 ± 0.152	23
Mt. Rich	1.25 ± 0.479	4	1.50 ± 0.50	2	1.75 ± 0.250	4	1.50 ± 0.224	10
High Elevation	1.0 ± 0.577	3	N/A	0	1.5 ± 0.50	2	1.20 ± 0.374	5
Male	0.50 ± 0.50	2	N/A	0	N/A	0	0.50 ± 0.50	2
Female	2	1	N/A	0	1.50 ± 0.50	2	1.67 ± 0.333	3
Low Elevation	2	1	1.50 ± 0.50	2	2.0 ± 0.0	2	1.80 ± 0.20	5
Male	2	1	1	1	2	1	1.67 ± 0.333	3
Female	N/A	0	2	1	2	1	2.0 ± 0.0	2
Mt. Magazine	0.75 ± 0.479	4	1.83 ± 0.167	6	2.0 ± 0.0	3	1.54 ± 0.215	13
High Elevation	1.0 ± 1.0	2	1.67 ± 0.333	3	2.0 ± 0.0	2	1.57 ± 0.297	7
Male	N/A	0	1	1	2	1	1.50 ± 0.50	2
Female	1.0 ± 1.0	2	2.0 ± 0.0	2	2	1	1.60 ± 0.40	5
Low Elevation	0.50 ± 0.50	2	2.0 ± 0.0	3	2	1	1.50 ± 0.342	6
Male	N/A	0	N/A	0	N/A	0	N/A	0
Female	0.50 ± 0.50	2	2.0 ± 0.0	3	2	1	1.50 ± 0.342	6

Table 33: Mean number of crickets killed at initial prey densities of 4 for comparisons between sex, elevation, and mountain groups within temperatures

Initial Density of 4 Crickets

Temperature	15°C	n	25°C	n	35°C	n	All Temps	n
Total	2.25 ± 0.453	8	1.75 ± 0.453	8	2.86 ± 0.459	7	2.26 ± 0.268	23
Mt. Rich	2.25 ± 0.750	4	2.0 ± 0.707	4	3.33 ± 0.667	3	2.45 ± 0.413	11
High Elevation	2.0 ± 1.0	2	2	1	4.0 ± 0.0	2	2.80 ± 0.583	5
Male	1	1	2	1	4	1	2.33 ± 0.882	3
Female	3	1	N/A	0	4	1	3.50 ± 0.50	2
Low Elevation	2.50 ± 1.50	2	2.0 ± 1.0	3	2	1	2.17 ± 0.601	6
Male	1	1	1	1	2	1	1.33 ± 0.333	3
Female	4	1	2.50 ± 1.50	2	N/A	0	3.0 ± 1.0	3
Mt. Magazine	2.25 ± 0.629	4	1.50 ± 0.646	4	2.50 ± 0.646	4	2.08 ± 0.358	12
High Elevation	1.50 ± 0.50	2	2.0 ± 1.0	2	2.0 ± 1.0	2	1.83 ± 0.401	6
Male	1	1	1	1	1	1	1.0 ± 0.00	3
Female	2	1	3	1	3	1	2.67 ± 0.333	3
Low Elevation	3.0 ± 1.0	2	1.0 ± 1.0	2	3.0 ± 1.0	2	2.33 ± 0.615	6
Male	2	1	0	1	4	1	2.0 ± 1.155	3
Female	4	1	2	1	2	1	2.67 ± 0.667	3

Table 34: Mean number of crickets killed at initial prey densities of 8 for comparisons between sex, elevation, and mountain groups within temperatures

Initial Density of 8 Crickets

Temperature	15°C	n	25°C	n	35°C	n	All Temps	n
Total	2.0 ± 0.756	8	2.88 ± 0.693	8	4.13 ± 0.811	8	3.00 ± 0.454	24
Mt. Rich	2.25 ± 0.750	4	3.60 ± 0.980	5	4.25 ± 1.031	4	3.38 ± 0.549	13
High Elevation	2.0 ± 1.0	2	2.0 ± 1.0	2	3.0 ± 1.0	2	2.33 ± 0.494	6
Male	1	1	1	1	2	1	1.33 ± 0.333	3
Female	3	1	3	1	4	1	3.33 ± 0.333	3
Low Elevation	2.5 ± 1.50	2	4.67 ± 1.202	3	5.50 ± 1.50	2	4.29 ± 0.808	7
Male	1	1	3	1	4	1	2.67 ± 0.882	3
Female	4	1	5.5 ± 1.50	2	7	1	5.5 ± 0.866	4
Mt. Magazine	1.75 ± 1.436	4	1.67 ± 0.333	3	4.0 ± 1.414	4	2.55 ± 0.755	11
High Elevation	3.0 ± 3.0	2	1.5 ± 0.50	2	3.0 ± 1.0	2	2.5 ± 0.885	6
Male	0	1	1	1	2	1	1.0 ± 0.577	3
Female	6	1	2	1	4	1	4.0 ± 1.155	3
Low Elevation	0.50 ± 0.50	2	2	1	5.0 ± 3.0	2	2.6 ± 1.40	5
Male	1	1	2	1	2	1	1.67 ± 0.333	3
Female	0	1	N/A	0	8	1	4.0 ± 4.0	2

4.4 PPO Methods

Spiders used in this test were collected using the methods described in section 2.2 except that they were not set onto a feeding cycle upon being brought to the lab and were put immediately into the immunity test. Thirty-five spiders were used in the analysis of adult immunity enzyme levels (Table 35). Measurements of carapace length, width, and body length were taken along with mass.

Table 35: Sample size for adult immunity test

	n
Mt. Rich	25
High Elevation	14
Male	7
Female	7
Low Elevation	11
Male	8
Female	3
Mt. Magazine	10
High Elevation	5
Male	1
Female	4
Low Elevation	5
Male	0
Female	5
All Spiders	35

For the immunity enzyme assay spiders were placed into individual 50mL centrifuge tubes and placed in a freezer for 10-15min or until the spider lost the ability to move. Spider tubes were picked blindly from a bag to be sampled. The spider then had its pedicel severed and the hemolymph which came out collected with a micropipette and placed in a clean tube. The cells of the hemolymph were lysed by being vortexed with a small number of glass beads. The slurry was then centrifuged and the supernatant removed from the beads and cellular debris. A 5 µL sample of the hemolymph extract was diluted with 50 µL of 50mM pH 7.5 phosphate buffer to create the PPO sample. Five µL of this dilute PPO sample was diluted by 50 µL of phosphate buffer and used in a Bradford protein assay along with samples of bovine albumin samples of known protein concentration (Kruger 1996; Mydlarz et al. 2008). The Bradford assay was used to calculate the concentration of protein in each hemolymph sample. All kinetic enzyme measurements were calculated using a Synergy 2 multi-Detection microplate reader with Gen5 software (Biotek Instruments). PPO assays were run at 490nm and the Bradford assay was run at 595nm.

PPO activity was measured in a 96 well plate by adding 5 µL of PPO sample to 55µL phosphate buffer, 25µL of 0.1mg/mL trypsin was added and incubated for 10 minutes, followed by the addition of 30µL of 10mM Dopamine and the development of the colored product followed at 490 nm for a period of 10 minutes. During this time all reactions were linear. PO activity was measured using the same methods described above except that 25 µL of trypsin was replaced with the same volume of DI water. Both the PPO and PO wells were done in duplicate and the results from duplicate wells averaged. A control (C) well was also run for each spider using the same methods as used for the PPO except that the 5 µL of PPO sample was replaced by 5 µL added to the buffer volume. A blank (B) well was also run for each spider using the same methods as used for PPO except that instead of trypsin and Dopamine 55 µL of DI water was added. Six wells were required to run each of the above wells for each spider allowing 16 spiders to be fit onto each 96 well plate.

Mean and standard error values were calculated and graphed for each group. The combined activity of PO and PPO were calculated individually. PPO was calculated by subtracting the PO, B, and C wells from the PPO well. PO was calculated by subtracting the B and C well values from the PO well. To get the combined activity of both active and inactive PO enzyme the PPO and PO measurements were added and 1 was added to this value. An analysis of covariance was run using the combined activity of PO and PPO as the dependent variable. Elevation, sex, and mountain of capture were used as main factors and body mass was used as the covariate. Interaction terms were removed due to degrees of freedom issues. Homogeneity of variances was tested using Bartlett's and Levine's tests and normality was tested using a normal probability plot with a linear smoother and a Shapiro-Wilk test of normality. Thirty-five spiders were used in the analysis of adult immunity enzymes (Table 35).

Juvenile spider hemolymph was sampled by putting the spider into a freezer until the spider lost the ability to move and then crushing its body with a plunger. The cells of the spider were lysed and diluted with 50 µL of phosphate buffer. This solution was vortexed and centrifuged. Thirty microliters of the supernatant was removed and diluted again with 30 µL of phosphate buffer creating the PPO sample for the juveniles. This sample was used in both the PPO assay and the Bradford protein assay. All other methods of measuring PPO activity were run using the same methods used on the adults except that

measurements were taken over a 30min time period instead of 10 as in the adults. Statistical analysis was run the same for juveniles as adults. The dependent variable for the ANCOVA was the square root transformed combined PO and PPO activity plus 1. The main factors were elevation, mountain, and rearing temperature groups. Body mass was used as the covariate. Thirty seven spiders were used in this analysis (Table 36). Homogeneity of variances was tested using Bartlett's and Levine's tests and normality was tested using a normal probability plot with a linear smoother and a Shapiro-Wilk test of normality.

Table 36: Sample size for juvenile immunity test

	n
Mt. Rich	17
High Elevation	3
Room	1
Cold	2
Low Elevation	14
Room	5
Cold	9
Mt. Magazine	20
High Elevation	13
Room	6
Cold	7
Low Elevation	7
Room	4
Cold	3
All Spiders	37

4.5 PPO Results

The mean concentrations of PO and PPO enzymes in all groups fall within two standard errors of each other. No significant differences were found due to sex ($F_{1,30}$=0.52, p=0.48), or mountain groups ($F_{1,30}$=0.01, p=0.94). Body mass also did not show a significant effect on immunity enzyme concentration ($F_{1,30}$=0.49, p>0.6). There is an apparent difference in immunity enzyme concentration between elevation groups below α=0.3 ($F_{1,30}$=1.30, p=0.26) level and within one standard error of each other graphically (Table 37) (Figure 20).

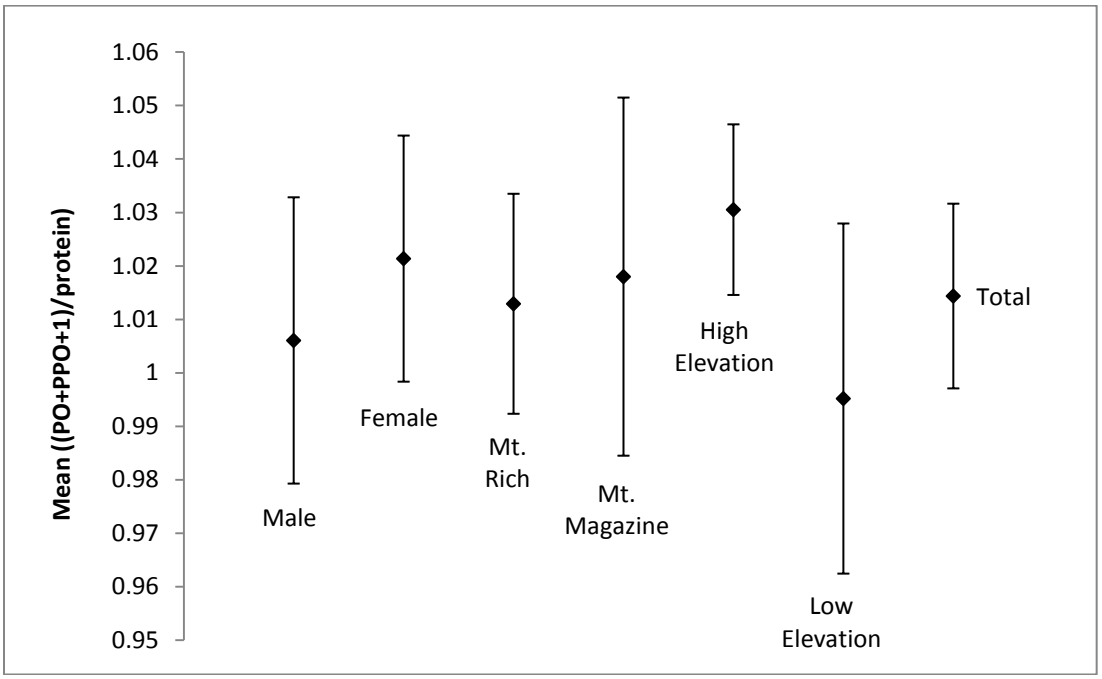


Figure 20: Mean (± SE) adult immunity enzyme concentration compared between sex, mountain, and elevation groups

Table 37: Three-way ANCOVA results comparing adult immunity enzyme concentrations between elevation, sex, and mountain groups

Dep Var: Combined	**n=35**	**r^2=0.04976**			
Source	**SS**	**df**	**MS**	**F**	**p**
Elevation	0.015	1	0.015	1.30	0.26
Sex	0.006	1	0.006	0.52	0.48
Mountain	0.0001	1	0.0001	0.01	0.94
Mass	0.005	1	0.005	0.49	0.49
Error	0.338	30	0.011		

Juvenile immunity enzyme concentrations did not show any significant differences between rearing temperature groups ($F_{1,28}$=1.1, p>0.3), elevation groups ($F_{1,28}$=0.48, p=0.49), and mountain groups ($F_{1,28}$=0.04, 0.84). Interaction terms did not show any significant effects on immunity enzyme concentration ($F_{1,28}$<0.65, p>0.30). The rearing temperature groups do show an apparent difference with the juveniles kept at “Room” temperature showing a greater mean than those kept at a colder temperature. These means did not fall outside of two standard errors of each other (Figure 21) (Table 38). The covariate body mass did show a significant effect on immunity enzyme concentration in the

juveniles ($F_{1,28}$=4.7, p=0.04). All other factors and interaction terms were not significant ($F_{1,28}$<1.1, p>0.31) (Table 39).

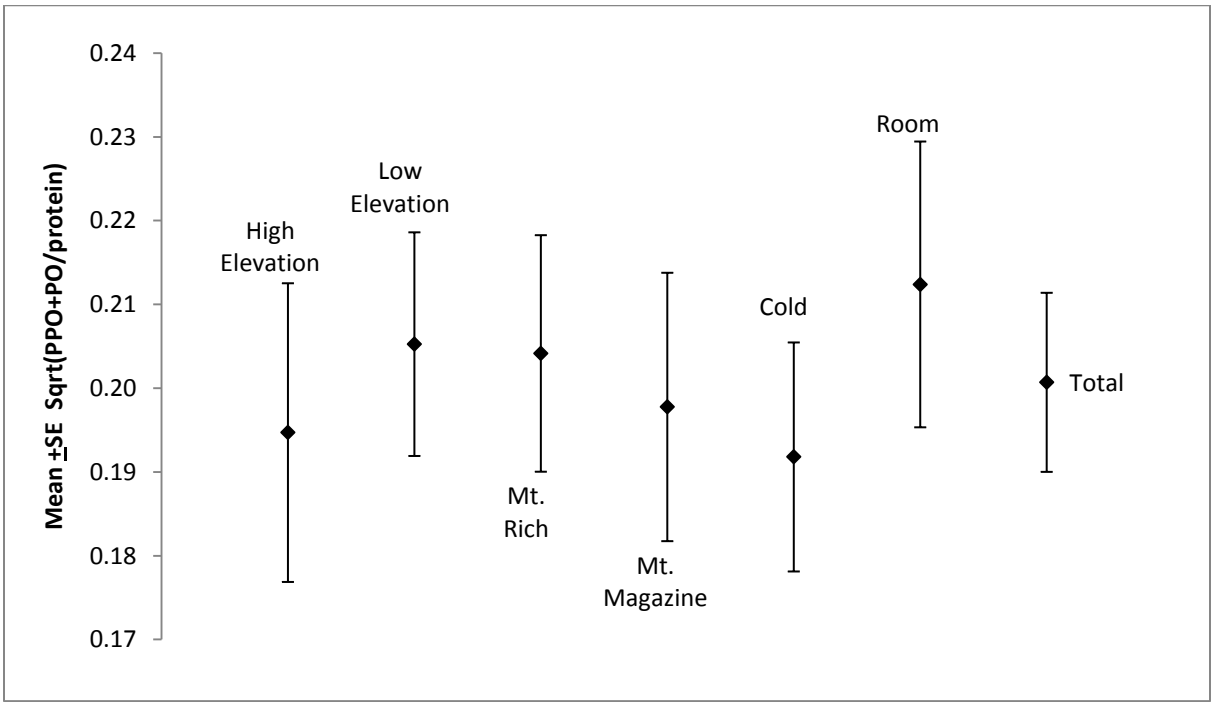


Figure 21: Mean (±SE) offspring immunity enzyme concentration compared between elevation, mountain, and temperature groups

Table 38: Mean immunity enzyme concentration for comparisons between temperature, elevation, and mountain groups

	Mean + SE
Mt. Rich	0.20±0.014
High Elevation	0.21±0.040
Room	0.23
Cold	0.21±0.067
Low Elevation	0.20±0.016
Room	0.24±0.016
Cold	0.18±0.020
Mt. Magazine	0.20±0.016
High Elevation	0.19±0.021
Room	0.21±0.039
Cold	0.18±0.021
Low Elevation	0.21±0.027
Room	0.18±0.031
Cold	0.25±0.04
All Spiders	0.20±0.011

Table 39: Three-way ANCOVA results comparing juvenile immunity enzyme concentration between elevation, mountain, and temperature groups using body mass as a covariate

Dep Var: SQRTCombined		**n=37**	**r^2=0.27354**		
Source	**SS**	**df**	**MS**	**F**	**p=**
Elevation	0.002	1	0.002	0.48	0.49
Moutain	0.0002	1	0.0002	0.04	0.84
Temperature	0.004	1	0.004	1.1	0.31
Elev*Mtn	0.001	1	0.001	0.16	0.69
Elev*Temp	0.0001	1	0.0001	0.03	0.85
Mtn*Temp	0.001	1	0.0001	0.28	0.60
Elev*Mtn*Temp	0.003	1	0.003	0.64	0.43
Mass	0.019	1	0.019	4.7	**0.04**
Error	0.11	28	0.004		

4.6 Further Intra-Specific Variation Discussion

Prey capture and immunity are two measures that have important ecological consequences. Perhaps the most ecologically relevant measure for this spider is predation across temperature. The reason this generalist predator is so ecologically important and interesting is that is consumes large amounts of relatively large arthropod herbivores. The test done here represent a preliminary test following the methods used by Walker and Rypstra (2001) and adding the temperature variable. The data used here were not corrected for body size because the carapace length body size did not show any significant differences between any of the groups used in the analysis. Some of the variation observed could still be explained by body size but not a significant portion.

Prey capture was shown to increase with an increasing temperature as was expected from studies on other ectotherm predators (Thompson 1978). The warmest temperature tested showed the greatest number of crickets killed and was significantly greater than the other two temperatures. There was also variation between the sexes as was originally described by Walker and Rypstra (2001). Statistical analysis of this data was not possible due to limited sample sizes and lack of replication. Further testing is needed before the effect of temperature on predation can be accurately described between elevation and mountain groups as well as in the crossing of these groups. Several improvements can be made to this test to help in such an analysis.

An increase in sample size is needed in order to ensure that there are multiple spiders representing each category needing to be compared. Space was limiting in this test so either better space usage or multiple runs of the same test need to run to improve sample size. The initial prey density variable was used in this test as it was in the original. This measure greatly increases the numbers needed to run meaningful statistical comparisons between groups that differ in temperature. The initial prey density variable should be removed from this test to make the sample sizes necessary more reasonable. Of the three initial prey densities used only the highest density showed a majority of spiders not eating all crickets offered to them. Because of this only the larger initial prey density should be used in future testing. The size of the crickets used was potentially an issue in this preliminary test also. Larger numbers of smaller crickets might provide greater resolution of the trends in prey capture seen across temperature. A single sex might be considered for the next run of this test also in order to make the necessary number of spiders more reasonable. Despite the large number of changes that will be necessary to run this test and analyze it statistically there is an increasing trend in the number of crickets killed as temperature increases. This suggests that, as expected, temperature does influence prey capture. An improved experimental design will provide opportunity to get a more accurate description of this trend.

Innate immunity concentrations are another important ecological variable because an animal must be able to defend itself against infection in order to devote energy towards fitness raising activities rather than defense and recovery. The PPO complex has been used in many invertebrates to measure disease resistance (Mucklow et al. 2004; Newton et al. 2004). There were not any significant differences between groups regarding the concentration of the PPO enzyme active or inactive. There is a very slight difference seen between the elevation groups though it does not fall outside of two standard errors. This difference could be the effect of temperature on prey availability or consumption. Prey capture was shown to be affected by temperature in the previous test and metabolic differences are also expected across temperature. Nutrition has been shown to affect immunity in both vertebrates as well as invertebrates (Ullrey 1993; Siva-Jothy & Thomson 2002; Yang 2007) and could explain this slight though not statistically significant difference. This hypothesis would be supported by the juvenile immunity

measure which showed that spiders in room temperature, which ate more than spiders in cold, showed a slight but not significant increase over the cold temperature group much like the difference seen between elevation groups. The spiders from mothers captured at different elevations did not show the same trend suggesting that it is not a genetically based difference. This study was one of the first looking at Lycosid immunity in response to environmental variables. Other measures of immunity and factors such as behavior and the number of immunity challenges experience in nature should be examined in the future to give a better view of the immune defenses and strategies used by this ecologically important spider and add to the literature on spider immunity (Elliot & Hart 2010).

Thermal preference, tolerance, and locomotor performance have been shown to differ between groups from different temperature environments. Now an innate immunity factor and a preliminary test of predation have been shown not to have significant variation between thermal environments. The next step in looking at potential effects of small temperature differences was to determine what type of variation is being seen in these measures. If they are heritable, then they could be subject to selective pressures exerted by this small temperature difference. In the next chapter heritability estimates were made to determine how much of this variation can be explained by genetic factors and how much is explained by environmental factors alone.

CHAPTER 5

HERITABILITY

5.1 Introduction

Heritability is a measure of the proportion of variance that can be attributed to genetic causes. Two types of heritability estimates are found in the literature. Broad sense heritability, also known as degree of genetic determination, is the proportion of the total phenotypic variance that can be attributed to genetic causes and includes both additive and dominance effects. Broad sense heritability can also be thought of as the proportion of variation found between siblings due to genetic causes. Estimates of narrow sense heritability focus on the variation between parents in a population and include the proportion of variance due to additive genetic effects. Despite which estimate is used heritability estimates fall between 0 representing a situation where variance is attributable almost entirely to environment and 1 where variance between phenotypes is attributable entirely to genetics (Freeman & Herron 2004).

Narrow sense heritability is the more commonly used estimate because it allows for a prediction of how a population will respond to selection. In fact heritability of a trait is a requirement to fulfill the second of Darwin's four basic postulates for evolution. That postulate states that for evolution to take place variation among individuals must be, at least in part, passed from parent to offspring (Darwin 1859; Freeman & Herron 2004). At Darwin's time genetics were not understood but now that postulate has been modernized to state that heritable variation is required for evolution to take place (Simons & Roff 1994). In this study looking at populations of temperate arthropod predators in locations that vary by a small temperature difference heritability estimates are needed to determine if the variation in traits described so far are attributable in part to genetics and thus able to evolve due to the potential selective pressure exerted by temperature.

There are numerous examples of estimates of heritability often referring to narrow sense heritability when not explicitly stating otherwise. The traits most often examined are morphological such

as color or size (Woods 1998; Ellers & Boggs 2002). The ability to perform a given task can also be a heritable trait. Livestock have been popular subjects of heritability estimates of performance looking at speed and endurance in horses (Hintz 1980), milk production and offspring survival in cows (Brinks et al. 1962), and even growth and fat production in pigs (Kennedy et al. 1985) and other farm animals. Arthropods (Prout & Barker 1989; Simons & Roff 1994), plants (Ruttencutter et al. 1979; Ashraf 1986; Soleri & Smith 2002), and even humans (Jensen 1967; Stins et al. 2004) have all been subjects of tests looking at heritability. Arachnid estimates have been far less numerous but there are a few examples (Shaffer & Formanowicz Jr. 2000).

Heritability only explains a portion of the variance seen in a population. Variation not attributable to genetic causes can often be explained by environmental causes. The estimate of the proportion of variance in a population due to environmental factors is called environmentality (Rushton et al. 2007). In this section heritability and environmentality estimates are made for several ecologically important measures made earlier. The environmentality estimates made here represent the proportion of variance due to being maintained in different temperature environments. The measures for which estimates were made include carapace length and width as well as body length and two leg segment length as measures of growth. Sprint speed and PPO concentration will also examined using both parent offspring regression and variance partitioning methods to make broad and narrow sense heritability estimates where possible for these ecologically important performance variables. I expect that the heritability estimates both narrow and broad will be low as has been described for many other organism (Bennett & Huey 1990; Rastogi et al. 2000; Rolff et al. 2005; Kenway et al. 2006) and the environmentality estimate will be relatively large as would be expected with a low heritability.

5.2 Heritability Methods

Females collected in August were set up in the lab in five feeding groups. They had their hunger standardized with four medium to large crickets for 24 h with all remains removed after 24h. After this, each spider was fed two medium crickets each week with the remains removed after 24h. Water was given in excess with the small shell vial and cotton stopper method used in all containers. Spiders that produced egg sacks were maintained on the same feeding regime until the eggs hatched at which time

they were removed from their feeding group and set aside. Cultures of wingless *Drosophila melanogaster* were maintained in the lab for feeding the offspring.

Once the offspring began to disperse from the females, they were collected in 44.4 or 59.1cc plastic containers with moistened white aquarium sand covering the bottom with a clear plastic lid. Each container was marked with the mother's number and the offspring number. The young were maintained in these containers at a lab temperature of 25.6°C for approximately one month while other sibs were collected from their mothers. During this time all spiders set up in their own containers received a fruit fly for 24h and additional moisture as needed every three days. After this time they were grouped into cold, lab and hot temperature groups and placed in a location that maintained the temperature at 20°, 25.6°, or 28°C on the same feeding cycle with increased number of flies and changing species of *Drosophila* provided each feeding as spider size increased. Offspring were maintained until they were used in the sprint speed, size, and immunity measures. The "hot" group raised at 28°C was removed from all but the immunity analysis due to excessive mortality. Measurements from tests using the young spiders were compared with measures from their mothers and a heritability estimates were calculated for sprint speed using parent-offspring regression repeating the mother's measurement for each offspring value. The slope of this regression was multiplied by 2 for full sib estimates and by 4 for half sib estimates to give a range of narrow sense heritability. Regressions of mother on cold group offspring and mother on room group offspring were made and averaged together to obtain a narrow sense heritability estimate for the sprint speed. Standard errors for regression coefficients were multiplied by the same factor as the slope to calculate a standard error for the heritability estimate. Comparisons among siblings were also made for sprint speed, size (CL, CW, BL, Mt, Ti), and immunity measures using variance partitioning and the following ANOVA equation.

$$Measure = \mu + TempGroup + Mother\# + error$$

Variance components were calculated using the following equations and values from the analysis of variance following methods from (Falconer & Mackay 1996). The between family mean squares difference is divided by the total sample size of all offspring used in the analysis. The environmental mean squares difference is divided by the mean number of offspring per family.

$Var(between) = Between\ family\ variance = ((MSmother\# - MSTempgroups))/\#\ total\ offspring$

$Var(env) = Variance\ due\ to\ temperature\ groups = ((MSTempgroup - MSerror))/\ mean\ family\ size$

$Var(within) = Within\ families\ variance\ (error) = MSerror$

$Var(total) = Var(between) + Var(env) + Var(within)$

The MS designation used here represents mean squares values obtained in the analysis of variance. The between families variance was obtained from the MS from the mother# factor. The environmental variance was obtained from the MS from the temperature group variable. The within family variance was obtained using the MS from the error term. Heritability and environmentality estimates were calculated using the following equations.

$h^2_{between} = \mathrm{Var(between)}/\ \mathrm{Var(total)}$

$h^2_{within(error)} = \mathrm{Var(within)}$

$h^2_{env} = \mathrm{Var(env)}/\ \mathrm{Var(total)}$

The equations listed above were used for broad sense heritability estimates for CL, CW, BL, Mt, Ti, sprint speed, and immunity measures. Standard error of heritability estimates were calculated using the following formula adapted from Lynch and Walsh (1998).

$$SE(h2) = (1/R)(1-t)(1+n-1)*t)((2/Nn(n-1))\text{^}0.5)$$

In this equation R=the coefficient of relatedness, n=mean sample size of each family, N=number of families, and t=proportion of variance used to make the heritability estimate.

5.2.1 Size Measures Methods

Spiders had their carapace length, carapace width, body length, metatarsus length, and tibia length measured using a micrometer. Two hundred and twenty one juveniles from 18 mothers were used

to estimate broad sense heritability for CL, CW, BL, Mt, and Ti using methods from Falconer and Mackay (1996). Eighteen families were used and the mean family size was 12.2 (Table 40).

Table 40: Offspring sample size for heritability estimates

	n
Mt. Rich	107
High Elevation	13
Cold	9
Room	4
Low Elevation	94
Cold	58
Room	36
Mt. Magazine	114
High Elevation	53
Cold	33
Room	20
Low Elevation	61
Cold	27
Room	34
Total Offspring	221
Mothers	18
Mean Family Size	12.2

5.2.2 Heritability of Size Measures Results

The between family estimate of broad sense heritability of the carapace length measures was between 0.001 and 0.002 with standard errors of 0.057 and 0.114. The within family estimate was between 0.033 with a standard errors of 0.0385. The proportion of explained variance attributable to rearing temperature conditions was estimated to be 0.967 with a standard error of 0.0121. The between family variation was significant ($F_{17,202}$=4.38, p<0.00001) as was the variation attributable to environmental factors associated with different temperature groups ($F_{1,202}$=364.3, p<0.00001) (Table 41).

Table 41: Two-way ANOVA results comparing carapace lengths and providing means squared terms for calculation of between and within family variance

CL	**n=221**			**r^2=0.70**	
Source	**SS**	**df**	**MS**	**F**	**p**
Between	5.75	17	0.34	4.38	**<0.00001**
TempGroup	28.11	1	28.11	364.27	**<0.00001**
Within	15.59	202	0.08		

The between family estimate of broad sense heritability of the carapace width measure was between 0.001 and 0.003 with standard errors of 0.057 and 0.115. The within family proportion of variance was 0.060 with a standard error of 0.0464. The proportion of explained variance attributable to rearing temperature conditions was estimated to be 0.939 with a standard error of 0.0216. The between family variation was significant ($F_{17,202}$=3.37, p=0.00002) as was the variation attributable to environmental factors associated with different temperature groups ($F_{1,202}$=193.1, p<0.00001) (Table 42).

Table 42: Two-way ANOVA results comparing carapace widths and providing means squared terms for calculation of between and within family variance

CW	**n=221**			**r^2=0.55**	
Source	**SS**	**df**	**MS**	**F**	**P**
Between	2.94	17	0.17	3.37	**0.00002**
TempGroup	9.93	1	9.93	193.08	**<0.00001**
Within	10.39	202	0.05		

The between family estimate of broad sense heritability of the body length measure was between 0.0007 and 0.001 with standard errors of 0.057 and 0.114. The within family estimate of was 0.023 with a standard error of 0.0357. The proportion of explained variance attributable to rearing temperature conditions was estimated to be 0.976 with a standard error of 0.0087. The between family variation was significant ($F_{17,202}$=4.26, p<0.00001) as was the variation attributable to environmental factors associated with different temperature groups ($F_{1,202}$=514.8, p<0.00001) (Table 43).

Table 43: Two-way ANOVA results comparing body lengths and providing means squared terms for calculation of between and within family variance

BL	**n=221**			**r^2=0.77**	
Source	**SS**	**df**	**MS**	**F**	**p**
Between	24.97	17	1.47	4.26	**<0.00001**
TempGroup	177.29	1	177.29	514.79	**<0.00001**
Within	69.57	202	0.34		

The between family estimate of broad sense heritability of the metatarsus length measure was between 0.001 and 0.003 with standard errors of 0.057 and 0.115. The within family estimate was 0.071 with a standard error of 0.0493. The proportion of explained variance attributable to rearing temperature conditions was estimated to be 0.928 with a standard error of 0.0251. The between family variation was

significant ($F_{17,202}$=3.15, p=0.0001) as was the variation attributable to environmental factors associated with different temperature groups ($F_{1,202}$=162.9, p<0.00001) (Table 44).

Table 44: Two-way ANOVA results comparing metatarsus lengths and providing means squared terms for calculation of between and within family variance

Mt	**n=221**			**r^2=0.53**	
Source	**SS**	**df**	**MS**	**F**	**p**
Between	1.94	17	0.11	3.15	**0.0001**
TempGroup	5.89	1	5.89	162.94	**<0.00001**
Within	7.30	202	0.04		

The between family estimate of broad sense heritability of the tibia length measure was between 0.001 and 0.002 with standard errors of 0.002 and 0.003. The within family estimate was 0.043 with a standard error of 0.0012. The proportion of explained variance attributable to rearing temperature conditions was estimated to be 0.956 with a standard error of 0.0004. The between family variation was significant ($F_{17,202}$=3.60, p=0.00001) as was the variation attributable to environmental factors associated with different temperature groups ($F_{1,202}$=274.5, p<0.00001) (Table 45).

Table 45: Two-way ANOVA results comparing tibia lengths and providing means squared terms for calculation of between and within family variance

Ti	**n=221**			**r^2=0.67**	
Source	**SS**	**df**	**MS**	**F**	**p**
Between	6.10	17	0.36	3.60	**0.00001**
TempGroup	27.40	1	27.40	274.50	**<0.00001**
Within	20.17	202	0.10		

5.2.3 Heritability of Sprint Speed Methods

Sprint speed was measured using methods previously described (section 3.?) in the lab at room temperature of 35.6°C. Physical measurements were recorded along with the sprint speed. Mothers were sprinted on a 2m track measuring 0.5m splits. The lanes of the mothers' track were clear tubing approximately 7cm diameter. Juveniles were run on a 1m track measuring 0.2m splits. The lanes on the juveniles' track were 0.75cm diameter and were also composed of clear plastic. A stick prod of appropriate size, with a cover on the end of the stick, was used to chase spiders. A regression of carapace length on sprint speed was run using this data and the residuals from that regression were

subtracted from the sprint speed measure to correct for body size variance. Seventeen parent-offspring comparisons were used in the room temperature parent-offspring regression and twenty families were used in the cold parent-offspring regression. Broad sense heritability estimates were made using 221 juveniles from 18 families with an average of 12.2 juveniles from each mother (Table 40).

5.2.4 Heritability of Sprint Speed Results

The narrow sense heritability estimate taken from the mean of two parent-offspring regressions was (-0.0193) to (-0.0386) with standard errors of 0.085 and 0.169 or 0 heritability (Figure 22). The between family estimate of broad sense heritability of the sprint speed measure was between 0.001 and 0.002 with standard errors of 0.061 and 0.123. The within family estimate was 0.031 with a standard error of 0.0408. The proportion of explained variance attributable to rearing temperature conditions was estimated to be 0.968 with a standard error of 0.0124. The between family variation in this analysis was significant ($F_{17,202}$=4.38, p=<0.00001). The variation attributable to environmental factors associated with different temperature groups was also significant ($F_{1,202}$=364.27, p<0.00001) (Table 46).

Table 46: Two-way ANOVA results comparing spring speeds and providing means squared terms for calculation of between and within family variance

Sprint		**n=221**		**r^2=0.70**	
Source	**SS**	**df**	**MS**	**F**	**p**
Between	0.006	17	0.0003	4.38	**<0.00001**
TempGroup	0.027	1	0.0269	364.27	**<0.00001**
Within	0.015	202	0.0001		

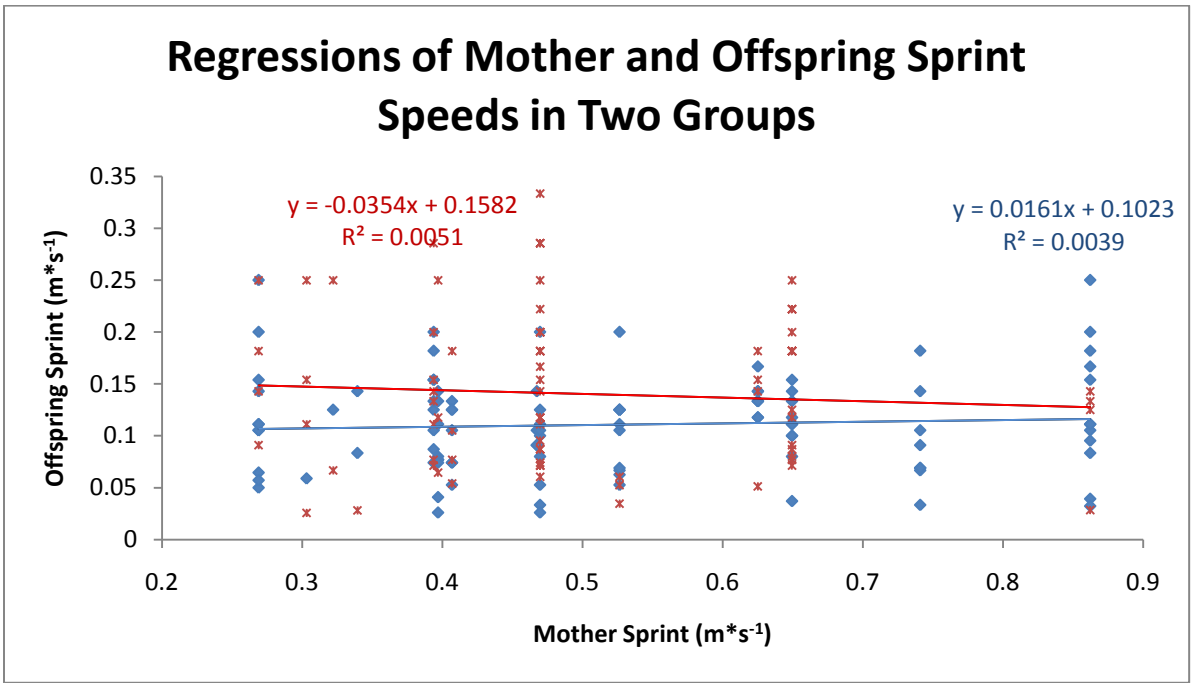


Figure 22: Regression of mean offspring sprint speed on sprint speed of mothers for two groups. Solid diamonds are the cold group and stars are the room group. The equation in the left corner is for the room group and in the right is from the cold group

5.2.5 Heritability of PPO Activity Methods

Juveniles were run through PPO assay methods described in chapter 3. Instead of severing the pedicel as done with adults the juveniles were placed into the freezer until inactive and then crushed with a plunger and vortexed with glass beads. Following pulverization, 50microliters of 50mM pH 7.5 phosphate buffer was added and the solution was mixed with a vortex mixer and centrifuged. After removal from the centrifuge, 30 micro liters of hemolymph solution was transferred to a clean tube and 30 micro liters of buffer was added. This dilution was vibrated and centrifuged. The resulting solution was used in the PPO test as described in chapter 3 and also in the Bradford protein test also described in chapter 3. The only difference between the tests besides the method of hemolymph extraction was that a 30 minute measure was used instead of the 10 minute time used in chapter 3. The same PO+PPO calculation used in chapter three was made and used in variance partitioning to estimate broad sense heritability. Forty four juveniles from eighteen mothers were used to estimate broad sense heritability for immunity enzyme concentrations. The mean number of offspring per family was 2.4 (Table 47).

Table 47: Offspring sample size for immunity heritability estimate

	n
Mt. Rich	18
High Elevation	3
Cold	2
Room	1
Low Elevation	15
Cold	10
Room	5
Mt. Magazine	26
High Elevation	17
Hot	2
Cold	8
Room	7
Low Elevation	9
Hot	1
Cold	3
Room	5
Total Offspring	**44**
Mothers	**18**
Mean family size	**2.4**

5.2.6 Heritability of PPO Activity Results

The between family estimate of broad sense heritability of the immunity enzyme measure was between 0.005 and 0.010 with standard errors of 0.362 and 0.723. The within family estimate was 0.652 with a standard error of 0.1608. The proportion of explained variance attributable to rearing temperature conditions was estimated to be 0.345 with a standard error of 0.2157. The between family variation in this analysis was not significant ($F_{17,24}$=0.782, p=0.70). The variation attributable to environmental factors associated with different temperature groups was also not significant ($F_{2,24}$=2.321, p=0.12) (Table 48).

Table 48: Two-way ANOVA results comparing PPO concentrations and providing means squared terms for calculation of between and within family variance

Immunity	**n=44**		**r^2=0.42**		
Source	**SS**	**df**	**MS**	**F**	**p**
Between	0.017	17	0.001	0.782	0.70
TempGroup	0.006	2	0.003	2.321	0.12
Within	0.030	24	0.001		

5.2.7 Results Summary

Table 49: Summary table of all heritability estimates made in this chapter

h^2 estimates (Full sib - Half sib)

Measure	CL	CW	BL	Mt	TI	Sprint	Immunity
Between Families	0.001 - 0.002	0.001 - 0.003	0.0007 - 0.001	0.001 - 0.003	0.001 - 0.002	0.001 - 0.003	0.005 - 0.010
Within Families	0.033	0.060	0.023	0.071	0.043	0.031	0.652
Temperature Group	0.967	0.939	0.976	0.928	0.956	0.968	0.345
Regression	N/A	N/A	N/A	N/A	N/A	(-0.0193) - (-0.0386)	N/A
Different From Zero	No	No	No	No	No	No/No	No

5.3 Heritability Discussion

Estimates of narrow sense heritability for the sprint speed measure made here used a parent-offspring regression repeating the mother's measurement for each offspring value. This method assumes a parent offspring correlation of zero. This represents a source of error as the actual correlation between parent and offspring is somewhere between zero and one (Kempthorne & Tandon 1953). Another common way to deal with this issue is to take the means of offspring measurements which assumes a parent offspring correlation of one. In this case the repeated parent method was chosen because the expected correlation was expected to be closer to zero than one.

Estimates of broad sense heritability had several potential sources of error. Some estimates were greater than one while others were negative. This shows the need to explain some of the potential sources of error that could result in the biologically meaningless estimate values. The mean number of offspring per sib was used as the denominator to estimate broad sense environmentality which could also be a source of error as the sib sizes were unbalanced. The family sizes were most unbalanced in the sprint speed estimate ranging from 2 to 30. This calls both the broad and narrow sense heritability estimates into question. Large sample sizes and, except for some sibs of extreme size, relatively similar sib sizes should be sufficient to allow for confident interpretation of these results despite the statistical issues.

This study took measurements from mothers as adults and from offspring as juveniles. This requires the assumption that the genetic control of these measures does not vary with life stage and that the methods for measuring used on both adults and juveniles are comparable. These assumptions are believed to be reliable as they been made in previous estimates of heritability using arachnids (Shaffer & Formanowicz Jr. 2000). Narrow sense heritability estimates using parent-offspring regression were not used because the assumption of that genetic factors influencing growth and size do not vary across life stages has been found not to be trustworthy in many arthropods (James et al. 1995; Flatt et al. 2005).

Determining whether the variation described between groups of this spider is heritable is important because only genetically based traits have potential for evolution in response to selection. More specifically the narrow sense heritability estimates will determine if selective pressures can cause

evolution of these measures. Broad sense estimates of heritability will only determine a maximum amount of variation that can be attributed to genetic factors of any kind.

Broad sense heritability estimates showed low and not significantly different from zero estimates for all size measures. This suggests that methods of heritability estimation used here were not strong enough resulting in large standard errors which call the accuracy of the estimates into question. Low heritabilities were predicted from other ectotherm size estimates (Lutz & Wolters 1989; Prout & Barker 1989) and were expected at least in the broad sense estimates. The thermal ecology measure generally understood to have the greatest ecological meaning was sprint speed. This measure is thought to be linked to several physiological processes linked to fitness and performance. It was expected that the phenotypic variation of this measure would be found to be influenced more by environmental factors such as temperature than by additive genetic effects. Despite this it was expected that narrow sense heritability estimates would be found to be small but still significantly different from zero and thus able to evolve in response to selective pressures exerted by temperature. The estimates of heritability of sprint speed given in this test do not support that hypothesis and do not show a narrow or broad sense heritability estimate for family effects that is greater than one standard error away from zero. The standard errors calculated for these estimates are large and better methods for estimating heritability and the standard error could be explored to confirm these results.

The questions raised about the effectiveness of these methods do not mean that these estimates cannot tell something about the influence of genetic factors on phenotypes in the spiders from Arkansas only that these conclusions need to be interpreted carefully due to potential statistical issues. There is variation in both size measurements and sprint speed in these spiders but that this variation is not attributable to genetic influences. This could suggest that temperature has been such a strong selective force that this trait has been driven to a nearly fixed status showing little current genetic variation in the factors influencing size and sprint speed. Another possibility is that the large error found in these estimates may be hiding a small but significant heritability. Other estimates for sprint speed in the literature suggest that this measure has had a low but significant broad sense heritability in other populations of ectotherms (Bennett & Huey 1990; Shaffer & Formanowicz Jr. 2000) and suggest that the

lack of variation explained by genetic factors is not due to sprint speed not being influenced by genetic factors.

The final estimate of heritability made in this section was made for the immunity factors. This measure showed very little variability in chapter 3 and so there was not any expectation of significant heritability attributable to genetic factors to be seen here. As expected the estimates were low and not significantly different from zero. Heritability estimates, both broad and narrow, made for arthropods in the literature showed low heritability estimates (Cotter & Wilson 2002; Lambrechts et al. 2004) so again the lack of variation explained does not suggest the lack of genetic influences only the lack of variation in the genetic factors which influence PPO in this population of spiders. The effects of environmental factors on innate immunity components have rarely been studied and never in wolf spiders making this a potentially interesting future direction for research.

Broad sense heritability estimates did not show estimates significantly different from zero for any body size measure or for the sprint speed or immunity measures despite other measures in other arthropods reported in the literature. These results suggest that these mountain locations do not have enough heritable variation to be a good study sites to look for evolution of these traits despite the variation described in previous chapters. These negative results do suggest that if there are genetic influences on these traits in this population they are small leaving a great deal of variation to be explained by environmental factors such as temperature. This is supported by the environmentality estimates which were consistently large and significantly different from zero.

CHAPTER 6

CONCLUSION

This study has made a first description of the thermal ecology of the ground running wolf spider *Rabidosa rabida* (Chapter 3). In addition to the description of thermal biology for this spider, intra-specific variation among four populations of this species which occupy slightly different thermal environments in the mountains of Arkansas is described (Chapters 2, 3 and 4). This variation was shown not be attributable to genetic factors (Chapter 5) and is therefore likely the result of environmental factors, such as temperature, which may have exerted a strong selective pressure on these populations limiting genetic variation in regards to these measures of thermal performance. Despite their ecological importance (Riechert 1974), high fecundity (Li & Jackson 1996), short generation times (Foelix 1996), and ancient ancestry (Foelix 1996) there is little information available on the thermal biology of any arachnid.

One potential application of this thermal biology data is to habitat selection (Huey 1991). Many factors play a role in habitat selection by organisms including foraging efficiency (Steele 1993), predation risk (Gilliam & Fraser 1987), humidity (Verhoef & Witteveen 1980), and temperature (Bevelhimer 1996). An important first step in examining the effect of any factor on habitat selection is determining whether individuals have the ability to detect variation in that factor in the environment and whether individuals exhibit preferences for one condition or another. If a spider cannot detect temperature differences or if due to being a thermal conformer it does not show a preference for one thermal environment over another then temperature would not be an important factor for determining habitat selection. In this study I found that *R. rabida* is not only able to detect temperature differences but they also exhibit a preference for body temperature in the laboratory (chapter 3).

After determining that an animal can choose between different thermal environments, a next step in the exploration of thermal habitat choice would be to look at whether performance of ecologically important actions is improved at one thermal environment as compared to another thermal environment. Thermal performance is often described using a plot of physiological or behavioral activity as a function of temperature called a thermal performance curve (Gilchrist 1995). Performance curves are bounded by critical temperatures, which are the temperature extremes beyond which the action being measured does not take place. This bounded curve has a single intermediate peak representing the temperature at which optimal performance is observed. Often the optimal value peak is located closer to the warm critical temperature with a steep drop in performance from the optimal value to the ceasing of the performance due to high temperature (Huey & Kingsolver 1993). This curve can also be used to describe thermal sensitivity or the slope of the curve at a given point (Van Berkum 1986; Huey & Kingsolver 1989). In this study, I found that thermal performance increased as temperature increased. Spider sprint speed showed the greatest change across temperature between 15° and 20°C and between 25° and 30°C for all spider groups except for male spiders which showed greater sensitivity at a higher temperature range (Chapter 3). This increased thermal sensitivity suggests that it is in these temperature ranges that choosing a slightly warmer temperature would provide the greatest performance benefit. The 25°-30° range is likely to be a temperature range commonly experienced by this spider in the mountains of Arkansas (Chapter 2).

It might be expected that if temperature were the only factor influencing habitat selection that this spider would find the thermal habitat closest to its optimum temperature which appears to be in the mid to upper 30°C's. This temperature would most likely be found during the day when solar radiation is available for a basking ectotherm (Heinrich 1996). This spider, however, is not found hunting in the vegetation during the day but only at night when the temperatures are cooler. As was noted earlier many factors influence habitat choice and these

factors may be able to explain this discrepancy between the nocturnal activity and the thermal performance of this spider.

Predator avoidance is one potential explanation for nocturnal activity (Metcalfe et al. 1999). *Rabidosa rabida* is well camouflaged and has the ability to move quickly to avoid most predators like birds and small mammals. The predator that might influence this spider to be nocturnal is the parasitoid wasps which are commonly seen killing these spiders in Arkansas. Parasitoid wasps are common and important predators of spiders (Cobb & Cobb 2004). The interaction between this species and parasitoid wasps has not been well described. Other potential causes for a nocturnal activity could be the potential drawbacks of increased temperature. While performance increases with temperature so does water loss (Ahearn & Hadley 1969; Duncan & Dickman 2001) and metabolic needs (Huey & Kingsolver 1989; Porter & Tschinkel 1993; Knies & Kingsolver 2010). The effect of temperature on these factors needs to be explored over a longer period of time than was used in this study and may help to explain this spider's habitat choice.

This last suggestion brings up another question. Why would a spider hunt up in the vegetation as opposed to on the ground where it would be less exposed to wind and temperature changes? The choice of activity up in the vegetation could be the result of prey availability or as has been suggested before that it is an example of niche partitioning and avoidance of inter specific interactions with other large wolf spiders that live on the forest floor (Brady & Mckinley 1994). The influence of temperature on foraging location choice has only rarely been explored and likely represents a tradeoff with the factors described above (Kronk & Riechert 1979).

Temperature is an important factor in determining habitat choice for ectotherms. Unfortunately there is a lack of information about many organisms' thermal biology including the non web building spiders (Schmalhofer & Casey 1999). A greater understanding of the thermal biology of these spiders and other arachnids will not only contribute to studies of habitat choice

but also allow us to be able to find changes in thermal biology occurring in the future. Thermal biology of all animals needs to be looked at across small temperature differences closer to the temperature ranges experienced in nature and not the ranges across which we are certain to find differences. And as always we need to include multiple factors when looking at habitat choice or thermal selection. Temperature interacts with every ecological and biological factor making it an important and interesting factor which influences life in ways we are still describing.

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BIOGRAPHICAL INFORMATION

Ryan Stork was born in October 1982 in Atlanta Georgia. Ryan attended Harding University in Searcy, Arkansas where he earned a BS in Biology and gained an interest in invertebrate biology thanks to the Harding faculty and a University subscription to the Journal of Arachnology. Following Graduation from Harding, Ryan enrolled at UTA in a BS to Ph.D. track and developed his research and academic interests continuing his interests in arthropod ecology. While at UTA he married Katy, his wife whom he met at Harding, and had a daughter Annalee Karen Stork. He plans to teach and to continue his research in thermal ecology of spiders.