

OVERVIEW OF TWO INTRODUCED SPIDERS, *TEGENARIA AGRESTIS* WALCKENAER
AND *TEGENARIA DUELLICA* SIMON (AGELENIDAE), IN WASHINGTON STATE: LIFE
HISTORY DEVELOPMENT, INTERACTIONS AND MEDICAL IMPORTANCE

By

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Abstract

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The European spider *Tegenaria agrestis* was introduced into Washington State in the 1930s and is a concern today due to the suspicion that its bite causes necrotic lesions. The introduction of the closely related congener species, *Tegenaria duellica*, in the 1960s may influence populations of *T. agrestis* through ecological similarities and competitive interactions. I examined three aspects of the potential medical importance of *T. agrestis*. First, the bacterial diversity was surveyed to determine if the spider carries any pathogenic bacteria, second, the ability of the spiders to transfer methicillin-resistant *Staphylococcus aureus* (MRSA), and third, the hemolytic activity of the venom. We found ten genera of bacteria on the exterior surface of the spiders, and none of the spiders exposed to MRSA transferred this pathogen. The hobo spider venom was not deleterious to vertebrate red blood cells. Because the occurrence of intraguild predation between closely related spiders can effect population numbers, an investigations of the interactions between these species were conducted under laboratory and field conditions. Studies were, the time taken to utilize a congener web for prey capture; antagonistic interactions between adult female conspecifics and congeners; antagonistic interactions between similarly sized juvenile

congeners; and field experiments to assess the survivorship of conspecific and congener: adult males and adult females and similarly sized juveniles in a simplified habitat. Both species readily accepted prey on the web of its congener. Laboratory and field trials indicated that *T. duellica* was the dominant surviving species throughout the season. Differences in microhabitat selection were found with overall similarities in macrohabitat. Because of overlaps in biological parameters and their propensity to share similar niches, comparative trials were conducted to investigate overlaps in habitat selection around homes in urban environments, assess the seasonal life history and record the growth and development of the congeners in the United States. A two year life cycle is described for both species. *Tegenaria duellica* maintained a size advantage over *T. agrestis* from March through August however; the majority of *T. duellica* were smaller than *T. agrestis* during the months of September and October.

Table of Contents

	Page
Acknowledgements	iii
Abstract	iv
List of tables	viii
List of figures	ix
CHAPTER ONE	
Introduction	1
Literature Cited	4
CHAPTER TWO: Comparative development and life history of two introduced spiders <i>Tegenaria agrestis</i> Walckenaer and <i>T. duellica</i> Simon (Agelenidae) in urban environments in Washington State	
Abstract	8
Introduction	9
Material and Methods	11
Results	14
Discussion	16
Literature Cited	18

CHAPTER THREE: Interactions between spider congeners *Tegenaria agrestis* Walckenaer and *T. duellica* Simon (Araneae: Agelenidae)

Abstract	29
Introduction	30
Material and Methods	32
Results	36
Discussion	38
Literature Cited	41

CHAPTER FOUR: A PCR-based survey of bacterial diversity of *Tegenaria agrestis* Walckenaer (Araneae: Agelenidae) and investigation of potential pathogen acquisition and transfer, with hemolytic venom analysis

Abstract	52
Introduction	53
Material and Methods	56
Results	60
Discussion	62
Literature Cited	68

LIST OF TABLES

CHAPTER TWO

1. Categories of spider habitats	21
2. Collections from western and eastern Washington	22
3. Seasonal distribution of adult female, male and juvenile	23
4. Number of molts	24
5. Growth rate of <i>Tegenaria agrestis</i>	25
6. Growth rate of <i>Tegenaria duellica</i>	25

CHAPTER THREE

1. Web acceptance	44
2. Laboratory antagonistic trial, juveniles	45

CHAPTER FOUR

1. Bacteria found on the hobo spider	74
2. Natural and cultured bacteria	75

LIST OF FIGURES

CHAPTER TWO

1. Collections from western and eastern Washington	26
2. Seasonal distribution of adult female	27
3. Seasonal distribution of adult male	27
4. Seasonal distribution of juvenile	27
5. The maximum carapace width	28
6. The minimum carapace width	28

CHAPTER THREE

1. Home in Spokane.....	46
2. Field enclosures design	47
3. Field enclosures at the WSU Tukey research farm	48
4. Habitat supports in field enclosures	49
5. Laboratory antagonistic interactions	50
6. Field antagonistic interactions	51

Dedication

This dissertation is dedicated to Nana, a true friend when one was needed.

CHAPTER ONE

GENERAL INTRODUCTION

The first record of *Tegenaria agrestis* Walckenaer in the United States was in Seattle, Washington in 1930 (Exline 1936, Exline 1951). European distribution is widespread from Europe to central Asia (Platnick 2009). The congener species, *Tegenaria duellica* Simon (= *T. gigantea* Chamberlin and Ivie, (Brignoli 1978, Roberts 1995, Platnick 2009), was introduced into western WA in 1960 (Crawford and Locket 1976). The European distribution of *T. duellica* is described as Holarctic (Platnick 2009), with populations being recorded in Portugal, Spain, France and Britain (Croucher et al. 2007). In northwestern United States and southwestern Canada, *T. duellica* has spread south along the coastal regions of British Columbia, to Washington and Oregon west of the Cascade Mountains with isolated population found east of the mountain range (Vetter et al. 2003). The current range of *T. agrestis*, originally named the aggressive house spider, includes Washington, Oregon and Idaho (Baird and Akre 1993) Montana, Utah, Nevada, and Wyoming (Baird and Stoltz 2002) as well as Colorado and southern British Columbia (Vetter et al. 2003).

Although no medical concerns are associated with *T. agrestis* in Europe (Binford 2001), conjectures have been made in Washington, Oregon and Idaho, since the late 1980s, due the suspicion that its bite causes necrotic tissue lesions (Vest 1987a). The concerns surrounding this introduced spider grew as it became one of the more common spiders found around homes (Akre and Myhre 1991) and a small mammalian study indicated that possible reactions would result in necrosis of the soft tissue (Vest 1987b). Yet, venom analysis did not find any chemical that

would induce tissue necrosis in mammals (Binford 2001, Gomez and Binford, personal communication).

Both *Tegenaria* species are considered synanthropic in Europe and in introduced areas of the United States (Akre and Myhre 1991, Roberts 1995, Bennett 2002). Of the spiders found in North America, only *Loxosceles* spp. are known to possess venom that causes necrotic arachnidism (Swanson and Vetter 2006). However, spiders have been proposed to vector bacteria as an explanation for necrotic arachnidism (Atkinson et al. 1995, Binford 2001 Fagan et al. 2003, Baxtrom et al. 2006). There are many examples of bacterial infections in necrotic lesions initially thought to be induced by spider bites (Soe et al. 1987, Peel et al. 1999, Dominguez 2004, Lui et al. 2005, Weissfeld et al. 2005, Benoit and Suchard 2006, Vetter et al. 2006, El Fakih et al. 2008). Community-acquired MRSA is a prevalent and widespread pathogen which causes necrotic tissue lesions that is often misdiagnosed as resulting from spider bites (Dominguez 2004, Baxtrom et al. 2006, Vetter et al. 2006, Cohen 2007). Still, there is little evidence linking spiders to such bacterial species. The medical connotation surrounding *T. agrestis* and its prevalence around homes continues to cause concerns for homeowners. It has been postulated that the larger *T. duellica* may compete with the smaller *T. agrestis* (Bennett 2002) and a reduction in the number of *T. agrestis* has been noted in areas where the spiders overlap (Vetter et al. 2003, Crawford, personal communications) but the mechanism is unknown.

Overlaps in ecological and behavioral parameters may cause competition with conspecifics, especially during increases in density, as with the territorial *Agelenopsis aperta* (Gertsch), where larger species were found to displace a smaller species from their web through agonistic

encounters (Riechert 1978). The size of the individuals in an encounter may be a factor that indicates the outcome, often with a high rate of web takeover occurring due to the larger body size of the invader compared to the web owner (Eichenberger et al. 2009). Antagonistic interactions between arthropod predators, such as spiders, include intraguild predation and cannibalism which occurs in natural and human altered environments (Reitz and Trumble 2002, Denno et al. 2004, Rypstra and Samu 2005, Snyder and Evans 2006). Intraguild predation is described as a combination of competition and predation (Polis and Myers 1989) but also as a mechanism leading to competitive displacement, a severe form of interspecific competition where an invading species eliminates another species that is utilizing the same environmental resources (Reitz and Trumble 2002). Larger intraguild predators often cannibalize smaller conspecifics (Polis et al. 1989, Langellotto and Denno 2006) or other small competing predators and those with a lesser foraging strategy (Denno et al. 2004, Rypstra and Samu 2005) and competitive displacement often occurs between closely related species (Reitz and Trumble 2002). The body size differs between the adults of these introduced congeners: male and female *T. duellica* have a range of 12-18 mm body length while male and female *T. agrestis* are smaller at 7-14 mm. (Vetter et al. 2003). Biological parameters, such as requirements for habitat location, possess the possibility of competition leading to displacement for closely related species (Nyffeler et al. 1986). Reproductive potential, seasonal development (Hann 1990) and body size (Eichenberger et al. 2009) could determine if an established invading species would withstand the introduction of another species by coexisting without interference.

The objective of this study was to examine biological and ecological parameters: microhabitat preference, seasonal life history and comparative development along with behavioral

interactions, of the hobo spider *T. agrestis* and the giant house spider *T. duellica* in order to better understand the outcomes of possible encounters in sympatric locations. In addition, the microbial fauna on the exterior of *T. agrestis* was described due to the surrounding medical concerns and suggestions of possible bites causing tissue necrosis. Two supporting tests were also conducted to assess if *T. agrestis* can mechanically acquire and transfer a highly pathogenic bacterium such as MRSA; and to determine toxicity of the venom to mammals, a standard hemolytic assays was conducted in order to corroborate the results of Binford (2001) and Binford and Gomez (unpublished) venom analysis.

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CHAPTER TWO

COMPARATIVE DEVELOPMENT AND LIFE HISTORY OF TWO INTRODUCED SPIDERS, *TEGENARIA AGRESTIS* WALCKENAER AND *T. DUELICA* SIMON (AGELENIDAE) IN URBAN ENVIRONMENTS IN WASHINGTON

Abstract

The hobo spider *Tegenaria agrestis* has spread across the Pacific Northwest since its introduction into Washington State. The more recent introduction of the closely related giant house spider *Tegenaria duellica* into Washington may influence populations of *T. agrestis* due to their propensity for shared niches. Comparative trials were conducted to investigate overlaps in habitat around homes in urban environments, seasonal life history and development. Differences in microhabitat selection were found between the two species with overall similarities in habitat. A two year life cycle is described for both species in Washington State. We found that the overwintering stages included juveniles and adult females and that the largest populations of all spider stages were in the summer, specifically in July. *Tegenaria duellica* maintained a size advantage over *T. agrestis* from March through August however; the majority of *T. duellica* were smaller than *T. agrestis* during the months of September and October.

Introduction

The potential displacement of native spiders by introduced spiders has been studied due to the significant ecological impacts the introduction has upon native spider populations (Nyffeler et al. 1986, Hann 1990, Eichenberger et al. 2009). In the Pacific Northwest, we have had the opportunity to investigate the potential competition of an established introduced species, the hobo spider, by its congener, the more recently introduced giant house spider. Overlapping biological parameters, such as habitat requirements suggest that competition could lead to displacement, especially for closely related species (Nyffeler et al. 1986). Reproductive potential, seasonal development (Hann 1990) and body size (Eichenberger et al. 2009) are determining factors in evaluating the potential interactions between an established species and a more recent introduction.

The first record of *Tegenaria agrestis* Walckenaer in the United States was in Seattle, Washington, in 1930, (Exline 1936, Exline 1951). It is widespread in its distribution from Europe to central Asia (Platnick 2009) and its current range in North America includes Washington, Oregon and Idaho (Baird and Akre 1993) Montana, Utah, Nevada, and Wyoming (Baird and Stoltz 2002) as well as Colorado and southern British Columbia (Vetter et al. 2003). Seasonal maturation of male *T. agrestis* occurs in the fall in Europe and in late summer and early fall in the United States (Akre and Myhre 1991). Adult females have been found throughout the year in Europe (Jones 1983, Roberts 1995) but are thought to mature in late summer in the United States and may survive until early spring (Akre and Myhre 1991). Juvenile *T. agrestis* have been found throughout the year and will overwinter to mature the following season (Bruce 1988, Akre and

Myhre 1991). Egg cases of *T. agrestis* were produced only in the fall under laboratory conditions (Akre and Myhre 1991) and this was confirmed by field observations (Bruce 1988). The eggs that overwintered hatched in early spring from April through June (Bruce 1988; Akre and Myhre 1991). In Europe, sexual maturity of *T. agrestis* is reached after nine molts (Foelix 1996), however, in the United States the number of molts was recorded as between 12 and 15, but a final molt to maturity was not determined (Akre and Myhre 1991).

A congener species *Tegenaria duellica* Simon (= *T. gigantea* Chamberlin and Ivie) (Brignoli 1978, Roberts 1995), was introduced into western Washington in 1960 (Crawford and Locket 1976). This spider has mostly spread south along the coastal regions of British Columbia, to Washington and Oregon west of the Cascade Mountains since its introduction, but isolated populations have been found east of the mountain range (Vetter et al. 2003). In Europe it is described as Holarctic (Platnick 2009), being recorded in Portugal, Spain, France and Britain (Croucher et al. 2007). Female adult *T. duellica* have been found throughout the year in Europe (Jones 1983, Roberts 1995) and in the United States (Akre and Myhre 1991). Male *T. duellica* mature in late summer and fall in Europe (Jones 1983, Roberts 1995) and similarly in the United States with collections being made from June to September (Akre and Myhre 1991). Very little biological information exists on the juvenile stages of *T. duellica* in Europe (Jones 1983) and North America (Bennett 2002).

Both *Tegenaria* species are considered synanthropic in Europe and in introduced areas of the United States (Akre and Myhre 1991, Roberts 1995, Bennett 2002), but are also found in more natural habitats (Bennett 2002, Salomon 2007). Body size differs between the adults of these

spiders: male and female *T. duellica* have a range of 12-18 mm body length while male and female *T. agrestis* are smaller at 7-14 mm. (Vetter et al. 2003). It has been postulated that the larger *T. duellica* may compete with the smaller *T. agrestis* (Bennett 2002) and a reduction in the number of *T. agrestis* has been noted in areas where the spiders overlap (Vetter et al. 2003, Crawford, personal communications) but the mechanism is unknown. In this paper we examine biological and ecological parameters: microhabitat preference, seasonal life history, and spider development in order to better understand the possible interactions and comparative development of these two closely related, introduced spiders.

Materials and Methods

Spider Collection

Spiders were collected from March to September in 2007 and again in 2008 around the outside of homes in urban environments in Spokane (Spokane County), Pullman (Whitman County), Bellevue (King County) and Puyallup (Pierce County) Washington State. Spiders were located by searching for potential habitat around landscape materials using webbing as an indicator. Positive identification of adult spiders was accomplished by examination of sclerotized genitalia according to Vetter and Antonelli (2002). Immature spiders were identified to genus using Ubick et al., (2005), followed by an examination of sternal patterns as delineated in Vetter and Antonelli (2002). Spiders were individually held in 13 x 11 x 6 cm containers and provided with food and water twice weekly as maintenance, unless experimental procedures specified otherwise. The diet consisted of fruit flies and crickets. Spiders and egg cases were maintained

in an environmental chamber with an average temperature of 26 °C on a 12:12 L:D cycle and average relative humidity of 61%.

Microhabitat preference

Tegenaria agrestis is found throughout Washington, while *T. duellica* is established in western, Washington with small, isolated populations and individual spiders collected in parts of eastern, Washington. All stages of both species are often found in western Washington in urban environments around the same home, side by side in the same habitat. To assess for an overlap in selected harborage around homes, which may play a role in competition or displacement between the species, habitat site of the spiders collected was recorded throughout the collecting season. A categorical list of habitat locations was determined after the first year of collecting. A chi-square two way contingency test was used to compare the percentage of the selected microhabitat preference for homogeneity between the two species.

Seasonal life history

The expanding range of *T. agrestis* across the northwestern United State has been monitored since 1993; with a well established population recorded in Washington (Baird and Akre 1993, Baird and Stoltz 2002, Vetter et al. 2003). Collections of *T. duellica* have recently become more common than *T. agrestis* along most of the coastal areas of British Columbia, Washington and Oregon where populations of the two species overlap (Vetter et al. 2003). In order to examine the range of *T. duellica* beyond the coastal regions of Washington, *T. agrestis* and *T. duellica* were collected from March to September in 2007 and again in 2008 with the number of spiders recorded for each species and stage in previously defined sites in western and eastern

Washington. To better understand what stages of development each species is at during a given point throughout the season and at what stages or instars of conspecific and congener a spider may encounter as a possible intraguild predator, the stage (i.e., adult female, male or juvenile) of each species was recorded for each month of collection. For each spider, measurements were taken of the width and length of the carapace and species and gender were recorded.

Spider Development

Egg cases were collected in February 2008 to better understand development of *T. agrestis* and *T. duellica* in the United States, where there is an inconsistency in the number of molts for *T. agrestis* and a paucity of information for *T. duellica*. The egg cases were maintained in an environmental chamber until they eclosed, at which time the emerging spiders were counted and recorded. Due to the high number of hatchlings, the spiderlings were allowed to cannibalize to reduce overall numbers before they were separated; three to four individuals were placed in a container so molting was more easily monitored. Cannibalism continued in the container until only one spider remained. Molts were recorded, initially from the mass molting in the enclosures, and then individually as spider numbers were reduced to a single spider. After each molt, the width and length of the carapace was recorded. Due to the small size of the spiders, the starting carapace measurements for all spiders was recorded as a width of <1mm and length of 1mm. Species were determined using sternal patterns as described in Vetter and Antonelli (2002) until genitalia could be used for certain identification.

Results

Microhabitat preference

Spiders were collected from March to September in 2007 and again in 2008 with data from both years combined for analysis. Habitat sites for a combined 1192 spiders collected, *T. agrestis* (n=759) and *T. duellica* (n=433), were recorded from the outside of 146 homes in urban environments in Washington. The preferred microhabitat with the highest percentage for both *T. agrestis* and *T. duellica* were landscape block or rock walls (36% and 44% respectively). The other common sites of harborage for *T. agrestis* were, objects on rock beds (25%) and long grass (14%). Common selected harborage sites for *T. duellica* were objects on rock beds (12%) and stacked wood or boards (13%) (Table 1). A Chi-square two way contingency test for homogeneity of microhabitat preference between congener species indicated a difference in selected microhabitats between *T. agrestis* and *T. duellica* ($\chi^2 = 115.94$, $P < 0.001$).

Seasonal life history

There was a total of 2373, *T. agrestis* (n= 1382) and *T. duellica* (n= 991), spiders collected in Washington from March to September in 2007 and 2008. The number of spiders collected in western Washington was: *T. agrestis* (n= 526) and *T. duellica* (n= 948), and in eastern Washington: *T. agrestis* (n= 856) and *T. duellica* (n= 43) (Table 2) (Figure 1).

Throughout the season cannibalism and intraguild predation are important factors controlling population size for both *T. agrestis* and *T. duellica*. The stage of the individual, e.g., young spiderlings or reproductive adults, is a factor dependant on the outcome of their interaction.

There was a total of 2388 *T. agrestis* and *T. duellica* spiders collected from March to September during 2007 and 2008. The stages collected for were as follows: *T. agrestis* adult female (n=398), male (n=262) and juvenile (n=727), and *T. duellica* adult female (n=348), male (n=87) and juvenile (n=566; Table 3). Adult female populations for both species increased in June with the highest number collected in July and a subsequent decrease in August (Figure 2). No adult males for either species were collected in March or May, but three *T. duellica* were collected in April; however, numbers of adult males increased rapidly from June to July and dropped steadily through August and September (Figure 3). Regular collections of juveniles for both species were made in every month; with the largest numbers collected in June and July (Figure 4).

The maximum width of the carapace recorded for the collected *T. agrestis* and *T. duellica* shows the size advantage *T. duellica* has over *T. agrestis* from March through August with an equal size maximum for September and October (Figure 5). The minimum recorded carapace widths for the collected *T. agrestis* and *T. duellica* show an equal size for both species from March through July with an equal increase in August for both but a size discrepancy of 2 mm favoring *T. agrestis* over the normally larger *T. duellica* in September and October (Figure 6).

Spider Development

The hatching of the egg cases occurred in March for *T. agrestis* and in June for *T. duellica*. Of the egg cases collected, the number of spiders that were maintained until adulthood was, 3 males and 1 female for *T. agrestis*, and 8 males and 5 females for *T. duellica*. An average of ten molts was recorded for *T. agrestis* (9.75) and nine for *T. duellica* (9.38) to reach reproductive maturity (Table 4). In addition to the field collected egg cases, seven *T. duellica* females produced an egg

case in their housing containers the second week of July which hatched approximately 22 and 28 days later in the first week of August, with an average emergence of 24 spiders and a range of 14 to 50. Two additional egg cases were laid the last week in August (25 August) and eclosed the third week in September (20 September), a total of 26 days, no counts of the emerging spiders were recorded. The number of hatchlings recorded from the sixteen *T. agrestis* egg cases was averaged at 107 (44-164), eleven egg cases of *T. duellica* were recorded with an average 41 (14-75). Under our laboratory environmental conditions and feeding regimen, 17 spiders reached maturity over a period of sixteen month from March 2008 to August 2009. This indicates a life cycle that spans two seasons for the two species in the United States, with all of the *T. agrestis* spiders maturing after sixteen months and the first *T. duellica* spider maturing after twelve months. Those *T. duellica* from egg cases produced later in the year, in July and August, matured after fourteen months. Growth rates, measured by carapace width, indicates that *T. duellica* females may reach maturity as early as the 6th instar and males as early as the 7th instar (Table 5); *T. agrestis* reached maturity at the 8th instar and 10th instar for females and males, respectively (Table 6). *Tegenaria duellica* molted through a maximum of 12 instars.

Discussion

While a difference in microhabitat preference was detected between *T. agrestis* and *T. duellica*, the overall macrohabitats are found side by side and therefore does overlap. Both species are described as synanthropic in the United States with small populations occurring in more natural areas (Bennett 2002), and similarly in Europe, where *T. duellica* is found around homes, sheds, gardens, under logs and rocks and *T. agrestis* is found in low vegetation, under stones and bark

and in disturbed areas such as alongside railroad tracks, but is not as commonly found inside homes (Jones 1983, Roberts 1995). One observation on microhabitat preference between congeners made throughout the field collections in this study was that *T. duellica* was found under more wood or boards than *T. agrestis* which was more commonly found under rocks or bricks (MGW, personal observation). Studies have examined the potential for an invading spider to displace a native species due to similarities in preferred habitat locations (Hann 1990, Nyffeler et al. 1986). Although differences in microhabitat selection exist, these differences are likely to be negligible when resources are limiting.

Higher numbers of *T. duellica* were collected in western Washington compared to *T. agrestis*, similar to initial reports of the distribution of *T. agrestis* and *T. duellica* in the Pacific Northwest (Vetter et al. 2003). This study does confirm that *T. duellica* does occur in eastern Washington and that isolated populations are established in Pullman and Albion. As the *T. duellica* moves across Washington, encounters may occur with *T. agrestis* and over time affect the numbers of *T. agrestis* established there as it has on the coast. The seasonal distribution of stages, juvenile and adult males and females overlap for the two species, with increases of females and juveniles collected in June and males collected in July. One determinant of an encounter is the size of the interacting individuals, with larger spiders out-competing the smaller species (Eichenberger et al. 2009). The measurement of the carapace width taken after each monthly collection provides a range of sizes for each species throughout the collecting season. A definite size difference exists between the species with *T. duellica* having the size advantage over *T. agrestis* for six of the months that collections occurred. However, during the months of September and October, mostly small juvenile *T. duellica* were collected compared to the larger juvenile yearlings of *T. agrestis*.

The spiders raised from eggs took approximately 12-16 months to mature, depending upon species, but both *T. agrestis* and *T. duellica* will take two seasons to become reproductively mature. We experienced some difficulties in the rearing process which resulted in a loss of spiders. However, of the seventeen spiders that were raised to maturity, we determined approximately nine molts for *T. duellica* to become an adult with a range of 7-12 for males and 6-11 for females and ten molts for *T. agrestis* with a range of 10-11 for males and 8 for females. Yet, with the small number of *T. agrestis* (n=4) that remained, it is possible that the nine molts to reach maturity determined in Europe is the same here in the United States. There are overlaps in macrohabitat, seasonal distribution and life cycle between *T. agrestis* and *T. duellica*. With these similarities in biological and ecological parameters we are beginning to better understanding the possible interactions that may occur in the field. A closer examination of their encounters is still needed to determine the mechanisms to the proposed competitive displacement of *T. agrestis* by *T. duellica*.

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Table 1: Categories of spider habitats around homes in urban environments. A total of 1192 spider were collected in 2007 and 2008: *Tegenaria agrestis* (n=759) and *T. duellica* (n=433). Numbers of individual spiders collected in a habitat are listed with the percent of the total of each species in a given habitat (e.g., of the 759 *T. agrestis* collected, 36% were found in landscape blocks or rock walls). A Chi-square two way contingency test for homogeneity indicated a difference in habitat preference between *T. agrestis* and *T. duellica* ($\chi^2 = 115.94$, $P < 0.001$).

Habitat	<i>T. agrestis</i>	Percent	<i>T. duellica</i>	Percent
Landscape block or rock wall	274	36	191	44
Objects on rock bed	187	25	52	12
Long grass	106	14	28	6
Stacked wood or boards	24	3	55	13
Under drain board	24	3	29	7
Stacked blocks or bricks	30	4	28	6
In or under vegetation	14	2	24	5
Debris	19	3	8	2
Deep bark	20	3	5	1
Other	61	8	13	3
Total	759	101	433	99

Table 2: Number of *Tegenaria agrestis* (Ta) and *T. duellica* (Td) collected from western and eastern Washington State during the months of March through September 2007 and 2008.

Washington	2007		2008		Total collected	
	T. a.	T. d.	T. a.	T. d.	T. a.	T. d.
Eastern	566	19	290	24	856	43
Western	220	297	306	651	526	948

Table 3: The sampled distribution of adult female, male and juvenile, *Tegenaria agrestis* (Ta) and *T. duellica* (Td) collected from March to September during 2007 and 2008 in Washington State.

Month	Female		Male		Juvenile	
	Ta	Td	Ta	Td	Ta	Td
March	14	16	0	0	30	59
April	9	21	0	3	24	50
May	17	0	0	0	66	59
June	71	77	21	1	188	138
July	127	137	147	47	240	146
August	65	51	86	31	155	83
September	95	46	8	5	24	31
Total	398	348	262	87	727	566

Table 4: The number of molts for spider species, *T. agrestis*, 3 males and 1 female and for *T. duellica*, 8 males and 5 females, to reach sexual maturity under laboratory conditions.

<i>T. agrestis</i>			<i>T. duellica</i>		
	Average	Range		Average	Range
Male (n=3)	10.3	10-11	Male (n=8)	10.25	7-12
Female (n=1)	8	8	Female (n=5)	8	6-11
Total	39/4=9.75		Total	122/13=9.38	

Table 5: Growth rate measured as width of carapace (mm) for *T. agrestis*, (n=13). Spiders that matured to a reproductive stage at an instar are indicated as female (F) and male (M).

Instar	Number	Average	Range	Mature
1	13	<1		
2	13	>1		
3	13	1.7	1-2	
4	13	2	1-2.5	
5	13	2.15	1.5-2.5	
6	13	2.5	2-3	
7	12	2.75	2-3	
8	9	3	2-3.5	1F
9	7	3.14	3-3.5	
10	4	3	3-3.5	2M
11	4	3	3-3.5	1M
12	4	3	3-3.5	

Table 6: Growth rate measured as width of carapace (mm) *T. duellica*, (n=16). Spiders that matured to a reproductive stage at an instar are indicated as female (F) and male (M).

Instar	Number	Average	Range	Mature
1	16	<1		
2	16	1.28	1-2.5	
3	16	1.88	1-3	
4	16	2.16	1.5-3.5	
5	16	2.44	1.5-3.5	
6	16	2.88	1.5-4.5	1F
7	15	3.4	2-5	1M, 2F
8	14	3.71	2-5	
9	13	3.88	2-5	1M, 1F
10	13	4.13	2.5-5	2M
11	13	4.5	3-5	2M
12	13	4.8	3-6	2M

Figure 1: Number of *Tegenaria agrestis* and *T. duellica* collected from western and eastern Washington State during the months of March through September 2007 and 2008. Percent values are given from the total 2373 spiders collected.

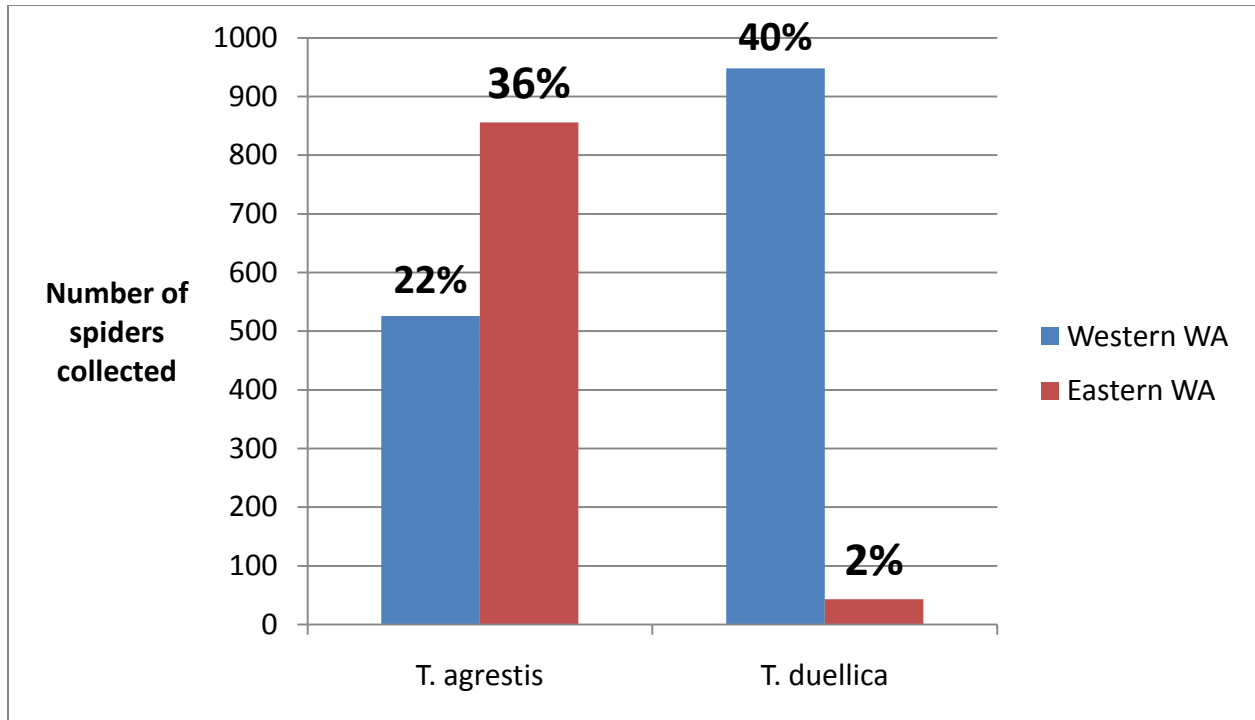


Figure 2: The seasonal collection of adult female *T. agrestis* and *T. duellica* from March through September during 2007 and 2008.

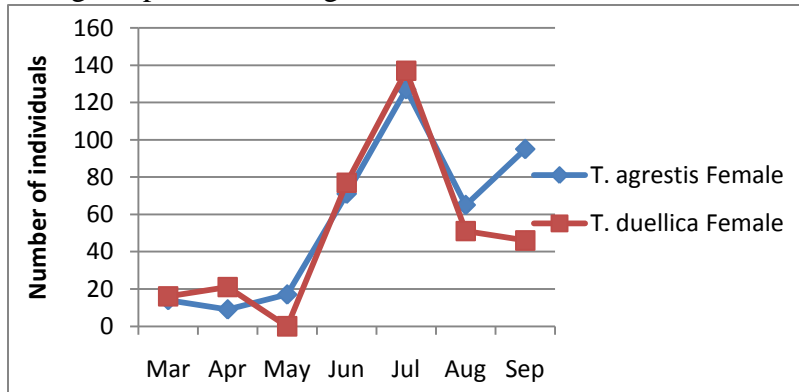


Figure 3: The seasonal collection of adult male *T. agrestis* and *T. duellica* from March through September during 2007 and 2008.

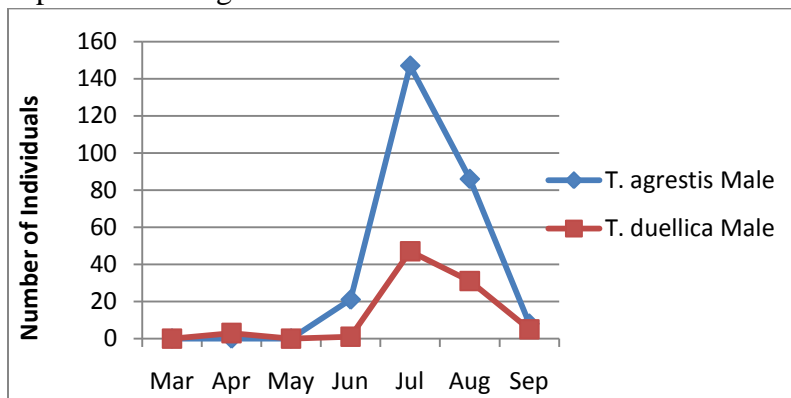


Figure 4: The seasonal collection of juvenile *T. agrestis* and *T. duellica* from March through September during 2007 and 2008.

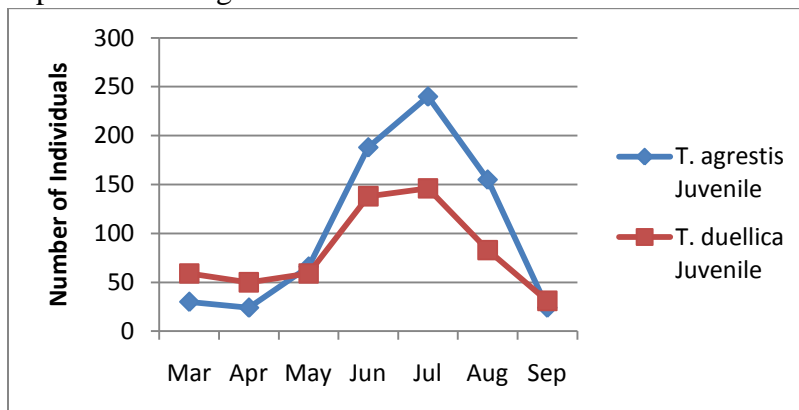


Figure 5: The maximum carapace width recorded after collection for *T. agrestis* and *T. duellica* during the months of March through October for 2007 and in 2008.

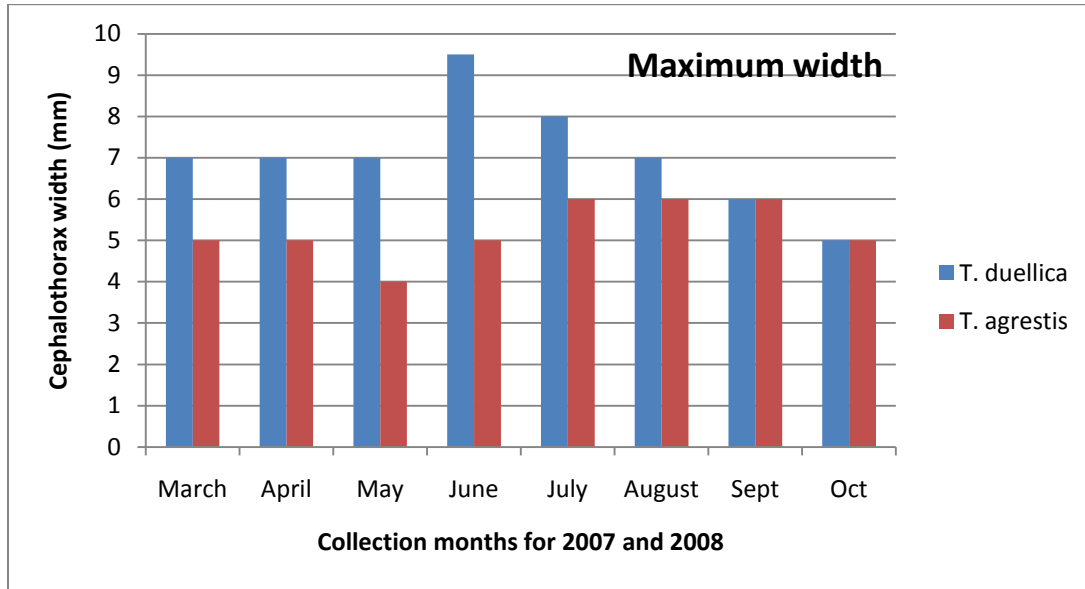
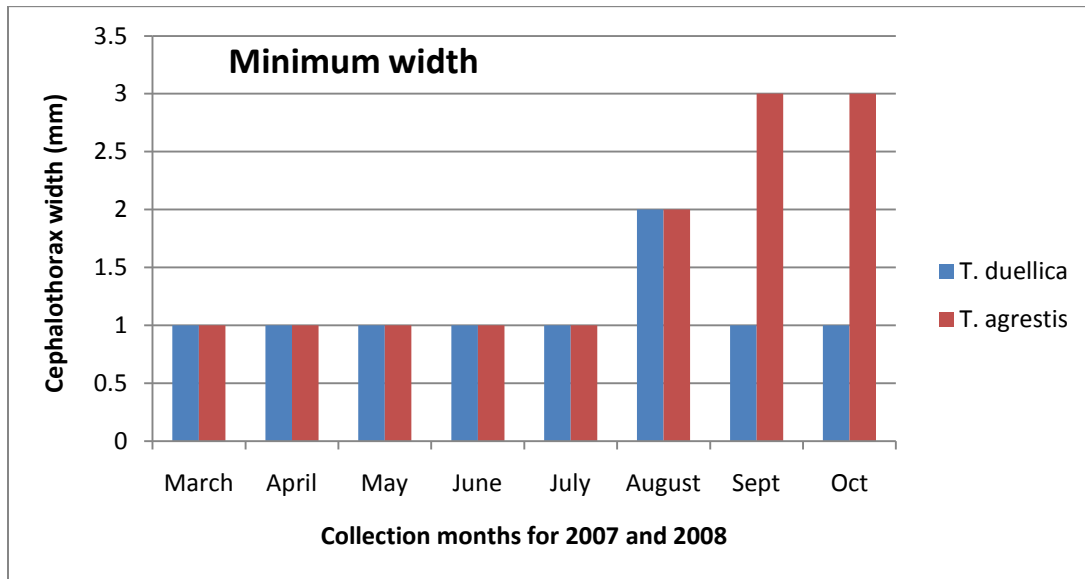


Figure 6: The minimum carapace width recorded after collection for *T. agrestis* and *T. duellica* during the months of March through October in 2007 and in 2008.



CHAPTER THREE

INTERACTIONS BETWEEN SPIDER CONGENERS *TEGENARIA AGRESTIS*

WALCKENAER AND *T. DUELLICA* SIMON (ARANEAE: AGELENIDAE)

Abstract

The occurrence of intraguild predation and cannibalism between closely related or conspecific spiders can effect population numbers or lead to displacement. An investigation of the interactions between two introduced European congeners, *Tegenaria agrestis* and *T. duellica*, were conducted under laboratory and field experimental conditions. Assessments were made in the laboratory on the time taken to utilize a congener web for prey capture, antagonistic interactions between adult female conspecifics and congeners, and between similarly sized juvenile congeners. Field experiments assessed the survivorship of three groups of congeners: adult male, adult female and similarly sized juveniles in a simplified habitat which simulated the simple repeated habitats often found around homes in urban environments. Both species readily accepted prey on the web of its congener. Laboratory trials indicated that intraguild predation and cannibalism occurs for both species, but between pairs of similarly sized juvenile spiders, *T. duellica* has a more significant survivorship than *T. agrestis*. No differences in survivorship between stages were observed in the field experiment but *T. duellica* was the dominant surviving species throughout the season.

Introduction

Antagonistic interactions between arthropod predators, such as spiders, include intraguild predation and cannibalism which occurs in natural and human altered environments (Reitz and Trumble 2002, Denno et al. 2004, Rypstra and Samu 2005, Snyder and Evans 2006). Intraguild predation is described as a combination of competition and predation (Polis et al. 1989) but also as a mechanism leading to competitive displacement, a severe form of interspecific competition where an invading species eliminates another species that is utilizing the same environmental resources (Reitz and Trumble 2002). Larger intraguild predators often cannibalize smaller conspecifics (Polis et al. 1989, Langellotto and Denno 2006) or other small competing predators and those with a lesser foraging strategy (Denno et al. 2004, Rypstra and Samu 2005), and competitive displacement often occurs between closely related species (Reitz and Trumble 2002). Introduced and invasive spiders are likely to affect endemic spider species (Snyder and Evans 2006) through antagonistic interactions of closely related species (Burger et al. 2001, Reitz and Trumble 2002).

Many studies have shown that antagonistic interactions between spiders that share similar ecological niches are important for coexistence (Polis et al. 1989, Balfour et al. 2003). For example, Rypstra and Samu (2005) showed that the wolf spider *Pardosa milvina* Hentz preferred either a cricket or a smaller spider species, *Hogna hulluo* Walckenaer, over a conspecific in a choice experiment. Females of the European spider, *Tegenaria atrica* Koch, are solitary and only tolerate conspecifics during mating season or with the emergence of offspring and a short 20 day period before dispersal; other than these periods, cannibalism has been shown to occur (Pourié

and Trabalon 1999). The decline of an endemic New Zealand widow spider, *Latrodectus katipow* Powell, was explained by differences in seasonal and reproductive potential that allowed the introduced Theridiidae *Steatoda capensis* (Cambridge) to displace the widow spider in overlapping areas (Hann 1990). In North America, the native *Steatoda borealis* (Hentz) was described as being displaced by the introduced *S. bipunctata* (Linnaeus) due to similarities in microhabitat, prey selection, diel timing, seasonality and sexual behaviors (Nyffeler et al. 1986). Overlaps in ecological and behavioral parameters may cause competition with conspecifics, especially during increases in density, as with the territorial *Agelenopsis aperta* (Gertsch), where larger species were found to displace a smaller species from their web through agonistic encounters (Riechert 1978). The size of the individuals in an encounter may be a factor that indicates the outcome, the larger the body size of the invader compared to the web owner the higher the rate of web takeover (Eichenberger et al. 2009).

The objective of this study was to examine ecological and behavioral interactions of the hobo spider *Tegenaria agrestis* Walckenaer and the giant house spider *T. duellica* Simon. (= *T. gigantea* Chamberlin and Ivie, (Brignoli 1978, Roberts 1995, Platnick 2009). The first record of *T. agrestis* in the United States was in Seattle, Washington, in 1930, (Exline 1936, Exline 1951). During the late 1980s, reports of necrotic lesions in humans in Washington, Oregon and Idaho were suspected to be the result of bites by *T. agrestis* (Vest 1987). The concerns surrounding this introduced spider grew as it became one of the more common spiders found in and around homes (Akre and Myhre 1991) with a rapidly expanding range from Washington, Oregon and Idaho (Baird and Akre 1993) to Montana, Utah, Nevada, Wyoming (Baird and Stoltz 2002), Colorado, and southern British Columbia (Vetter et al. 2003). The closely related giant house spider

Tegenaria duellica was introduced into western Washington in 1960 (Crawford and Locket 1976) and has since been recorded in Oregon, Idaho, Utah and British Columbia (Vetter et al. 2003). It has been suggested that the larger *T. duellica*, with a 12-18 mm body length, may compete with the smaller *T. agrestis*, whose body length is 7-14 mm (Bennett 2002) and a reduction in the number of *T. agrestis* has been noted in overlapping areas (Crawford and Vest 1988, Crawford, personal communications). We conducted laboratory and field experiments with adult and similar sized juveniles to investigate the interactions between these two introduced species to better understand the impact of *T. duellica* on *T. agrestis* due to possible antagonistic interactions in sympatric locations.

Materials and Methods

Spider Collection

Spiders were collected from around the outside of homes in urban environments in Spokane (Spokane County), Pullman (Whitman County), Bellevue (King County) and Puyallup (Pierce County) Washington. Spiders were located by searching potential habitat around landscape materials using webbing as an indicator. Positive identification of adult spiders was accomplished by examination of sclerotized genitalia according to Vetter and Antonelli (2002). Immature spiders were identified to genus using Ubick et al., (2005), followed by an examination of sternal patterns according to Vetter and Antonelli (2002). Spiders were individually held in 13 x 11 x 6 cm containers and provided with food and water twice weekly, unless experimental procedures specified otherwise. The diet consisted of fruit flies and crickets.

Spiders were maintained in an environmental chamber with an average temperature of 26 °C (25-28 °C) on a 12:12 L:D cycle and average relative humidity of 61%.

Web acceptance

The primary function of a web is to capture food but it is also used in chemical communication between conspecifics (Roland 1984). *Tegenaria* species produce a flat sheet web funneled into a tubular retreat that may follow an existing crack or vegetation. In this experiment, newly established webs were used to determine if *Tegenaria* spiders would use the web of a congener to capture prey. Forty-three juvenile spiders, for each *T. agrestis* and *T. duellica*, were collected and housed for one week in individual containers, allowed to establish a new web, and were not fed. The experiment began when the web owner was removed from the container and the congener species was introduced and allowed to explore the web and container for five minutes before being offered a cricket as prey. Time was recorded from the moment the cricket was introduced until the spider grasped the prey or after five minutes had passed. The introduced spider was then returned to its own web with the captured prey upon completion of the trial. A two sample, t-test was used to determine the difference in prey capture time between species.

Laboratory survival trials

To test whether aggression exists between individual adult female spiders, conspecifics (n=10 for each species) were randomly paired to assess for cannibalism and congeners (n=10) were randomly paired to assess for intraguild predation. Spiders were fed 24 hours prior to introduction. A conspecific or congener was introduced into the enclosure and allowed to interact with the web owner for a ten day period without food. The congener enclosure used was always

T. agrestis, the initial web owner, because this spider is established with an expanding range that is larger than that of *T. duellica*. Control enclosures (n=5 for each species) consisted of randomly selected spiders that remained in their original container and were also only provided with water during the ten day period. Mortality was recorded at the end of the first day and every day following. A one-way ANOVA was computed to compare survival time for conspecifics, congeners and single spiders.

Because the hobo spider is much smaller than the giant house spider in the adult stage and because important interactions are likely to occur in the juvenile stages, immature *T. agrestis* (n=41) and *T. duellica* (n=41) of similar cephalothorax widths were matched in laboratory interactions. Juvenile spiders were selected from the laboratory maintained population and paired with equally sized congeners. These spiders were not always the same instar but were always the same size. Spiders were fed 24 hours prior to introduction. For each interaction the web owner, *T. agrestis*, established a web in the container and then a similar-sized *T. duellica* was introduced into the enclosure. Mortality was monitored daily for two weeks, during which only water was provided. A one proportion summary test was used to determine which species would survive an encounter with a similar-sized congener.

Field survival experiment

Field enclosures were designed to test whether intraguild predation and cannibalism exists in an artificial habitat. The structural simplicity of the field enclosures represent the lack of habitat complexity in repeated structures such as landscape blocks, which may occur around homes in urban environments. The basic design of the field enclosures were 1m² lattice wood frames, 21

cm high, covered in fabric screening with an inset canopy lid. The frames were buried 0.5 cm into the substrate with soil mounded around the exterior perimeter (Figure 2). Eight enclosures were placed at the Washington State University Tukey Horticulture Orchard on a cleared dirt surface in full sun to encourage microhabitat use (Figure 3). Six enclosures were used for adult trials (3 male and 3 female) and two enclosures for juvenile trials. Inside the adult enclosures there were nine, 20 cm² boards that were raised approximately 3.5 cm on small rocks to supported all four corners (Figure 4). The remaining two enclosures, used for the juvenile trials, contained sixteen, 15 cm² boards raised approximately 3.5 cm from the substrate. The raised boards were spaced evenly within the enclosure and allowed the spiders to take refuge under the boards and establish webs. This design mimics the natural habitat where webs are constructed on the underside of objects as was observed around homes.

Preliminary test of field enclosures

Eight enclosures were placed at Tukey farm to test for the density of spiders each enclosure could maintain for the individual species. Each enclosure had three spiders for each habitat, or raised board, as a starting point: female n= 27, male n= 28, juvenile n= 48. Spiders were fed the day before being placed into the enclosure and then every other day for fourteen days. Spider numbers were counted during feeding. The largest decrease in density occurred in the first three days, mostly due to observed cannibalism and intraguild predation, and reached a constant density the last four days. After fourteen days the average of the remaining spiders was: female n= 5, male n= 7, immature n= 7. This was used as the start number for each species in the field experiment plus one to account for miscellaneous mortality: female n=6, male n=8, immature n=8.

Adult male and female spiders were randomly selected from the laboratory maintained population and juvenile spiders were selected based on a size range of the cephalothorax width (3 – 4.5 mm). To standardize hunger levels, spiders were fed one cricket 24 hours before being introduced into the field enclosure. *Tegenaria agrestis* spiders (8 males, 8 females and 16 juveniles) were introduced into separate enclosures, as described above, and allowed to establish webs. The following day, spiders were counted in each enclosure and brought back up to the starting number, if needed; locations of spiders within the enclosures were recorded. *Tegenaria duellica* spiders (8 males, 8 females and 16 juveniles) were then introduced into the enclosures with *T. agrestis*. The number and location of the spiders in each enclosure was monitored the following day and every other day for 14 days. The trial was replicated three times for adults and twice for juveniles with each trial being repeated five times from June to October 2008. A Binary Logistic Regression was used to determine the effects of intraguild predation and cannibalism on the population and survival of the congener species in a simplistic artificial habitat.

Results

Web acceptance

Of the total 86 spiders, 43 per species used in the experiment, 63% *T. agrestis* and 88% *T. duellica* captured prey in a congener's web before the imposed time limit of five minutes had passed (Table 1). All spiders introduced into a foreign web moved freely across the web surface and 75% (n=65) utilized the congener web for prey capture. The average time to prey capture was 22.9 seconds for *T. agrestis* and 18.0 seconds for *T. duellica*, which was not significantly different ($P = 0.663$). Both species will use the web of a congener to capture prey.

Laboratory survival trials

Antagonistic interactions between adult female conspecifics and congeners resulted in significantly higher mortality than the solitary control spiders (Figure 5). Cannibalism occurred after an average of 2.9 days for *T. agrestis*, and 0.8 days for *T. duellica* in laboratory interactions with a conspecific. When the congener species interacted, *T. agrestis* survived intraguild predation by *T. duellica* after an average of 2.7 days. The average survival time for the solitary control spider was 9.6 days for *T. agrestis* and 9.8 days for *T. duellica*.

Of the 41 introductions of juvenile *T. duellica* into an equally sized juvenile *T. agrestis* web, 61% *T. agrestis* and 10% of *T. duellica* did not survive at the end of the fourteen day period (Table 2). Four of the surviving *T. duellica* and three of the surviving *T. agrestis* did not feed on their congener and were removed from the analysis due to unknown causes of mortality. In four pairings, both spiders died and in one pairing both survived the full fourteen days; all five pairings were removed from the trial. The results of the one proportion test indicates that the 86% of *T. duellica* that survived out of the total 29 survivors was a significant survivorship rate over the 13% of surviving *T. agrestis* ($P < 0.001$).

Field survival experiment

The antagonistic interactions of small individual populations of *T. agrestis* and *T. duellica* male, female and juveniles in a simulated field environment, showed no significant differences between stages. However, *T. duellica* was the dominant surviving species ($P < 0.001$) throughout the experimental season (Figure 6). Observations of both predation and cannibalism were

recorded. On average, almost twice as many *T. duellica* (59%) survived at the end of the 14 day period than *T. agrestis* (33%).

Discussion

The interactions between these closely related species indicates a trend toward increased survival for *T. duellica* over *T. agrestis*, independent of size differences, in areas where the two species are sympatric. They may coexist in the field, as seen in laboratory and field trials, but in reduced numbers and lower populations for *T. agrestis* than if it existed independently of *T. duellica*. Cannibalism and intraguild predation occurred between conspecifics and congeners, respectively, even in the presence of offered prey; however *T. duellica* displayed a higher prevalence of antagonistic behaviors.

The web acceptance trial displayed the rapidness to which either species will utilize the web of the other species for prey capture. Observations in the field experiment along with field collections (MGW) concur with these findings. This suggests an ease of web transfer between conspecifics and congeners in antagonistic interactions that may occur in nature or in situations of web abandonment such as adult males who leave to search for a mate or by spiders that have moved due to lack of resources or space limitation. The web of a spider is produced with chemical signals that communicate to conspecifics, however, there may be chemicals that are common with closely related species (Roland 1984). Male *T. duellica* have been observed approaching and courting at the web of female *T. agrestis* (Akre and Myhre 1991) and a “happy and healthy” *T. agrestis* male was collected in the web of a female *T. duellica* (Bennett 2002).

Tegenaria duellica, in the *T. atrica* group, has been determined to be a related sister taxon with *T. agrestis*, with a pairwise distance of 0.2040 (Croucher et al. 2004), which is closer in range than many other *Tegenaria* species.

The introduction of a juvenile *T. duellica* into an equally sized juvenile *T. agrestis* web in the laboratory experiment simulates a web invasion that may occur in nature as an invader begins to establish in a new range. If competition is high and resources are limited, an antagonistic maneuver and takeover of the web of a congener would be advantageous for survival if the invader could utilize the web. In similarly sized immature spider interactions, *T. duellica* survived more often than *T. agrestis* when paired in antagonistic trials indicating an advantage in attack or territoriality. Yet with varying sizes of adults and juvenile stages collected for both species during the season, both spiders may have a period of size advantage over the other during individual incidents of contact.

The fact that *T. duellica* had a higher rate of survival than *T. agrestis* in the field trials for adult males, females may be explained by its larger size, yet the same results were found between similarly sized immature spiders in both laboratory and field trials. It has been suggested that the movement of *T. duellica* into areas where *T. agrestis* is currently established, is reducing the numbers of *T. agrestis* (Bennett 2002), which often becomes the most common spider around homes (Akre and Myhre 1991). This observation has been made by those in the field who receive specimens from concerned homeowners. If this is true, the expansion of *T. duellica* could limit the expansion of *T. agrestis*. The two species exist in overlapping ranges of England, Belgium and Scotland (Oxford and Merrett 2000) but it is unclear how they interact. While it

may be expected that the larger *T. duellica* would prey on the smaller *T. agrestis*, there may be territorial or aggressive behavior or differences in seasonal and reproductive potential in *T. duellica* that prevent high populations of *T. agrestis*.

While cannibalism and intraguild predation may be a common occurrence between some generalist predator spiders that are often food limited, this behavior is unlikely if escape or avoidance is possible in a habitat (Langellotto and Denno 2006, Wise 2006). With the often high number of *T. agrestis* and simple repeated habitats that occur around homes, *T. duellica* would have little difficulty in competing with *T. agrestis* for web location and food resources. The elimination of a species by an invader through predation may reduce competition and achieve increased resources but the cost incurred may be injury, disease or death (Rypstra and Samu 2005, Wise 2006). As congeners, encounters in the field are potentially detrimental to both *T. agrestis* and *T. duellica*. However, what was not tested here was the possible advantage a larger *T. agrestis* could have over a smaller *T. duellica*; such encounters may occur at specific time of the season. Interestingly, the lack of interspecific competition was described between *Argiope aurantia* Lucas and *A. trifasciata* (Forsk.) two closely related species that share many resources as well as life histories; yet, the distribution and abundance was noted to possibly be effected more by physical factors and vegetation (Horton and Wise 1983). Questions still exist regarding the physical displacement, natural occurrence of web take over, or intraguild predation of *T. agrestis* by *T. duellica* but, we have shown the ease with which both species use the others web for prey capture and the tendency of *T. duellica* to engage and survive an antagonistic encounter with *T. agrestis*. The passing of time and the movement of *T. duellica* populations will determine if they will establish equilibrium in the United States as they have in Europe.

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Table 1: The average time in seconds for *Tegenaria agrestis* to capture a cricket in the web of *T. duellica* and *T. duellica* to capture prey in the web of *T. agrestis*. Results of the two sample t-test were not significant ($P = 0.663$).

	No.	Total Time	Mean	St. Dev.	SE Mean
<i>T. agrestis</i>	27/ (n=43)	619	22.9	50.6	9.7
<i>T. duellica</i>	38/ (n=43)	685	18.0	33.4	5.4

Table 2: Survivorship of an antagonistic trial, pairing equally sized juvenile *T. agrestis* and *T. duellica* over a fourteen day period. The results of the one proportion test indicates that the 86% of *T. duellica* that survived out of the total 29 survivors was a significant survivorship rate over the 13% of surviving *T. agrestis* ($P < 0.001$). *Indicates trials that were removed from the experiment

	<i>T. agrestis</i>	<i>T. duellica</i>
Start no.	41	41
Died/eaten	25 (61%)	4 (10%)
*Both died	4	4
*Died/not eaten	4	3
*Did not feed	3	4
*Both survived	1	1
Total survived	4 (13%)	25 (86%)

*Indicates trials that were removed from the experiment

Figure 1: Home in Spokane, Washington provided as an example of the habitat simplicity in repeated structures, such as landscape blocks, utilized by spiders around homes in urban environments. Note the lack of vegetation behind the blocks in the foreground. The blocks do not extend past the left corner of house. Total number of spiders collected under landscape blocks at this location in 2007 was 77 male, female and juvenile *Tegenaria agrestis* along with 21 viable egg cases.



Figure 2: The enclosures used in the field experiment were 1m² lattice wood frames, 21cm high, covered in fabric screening with an inset canopy lid. The frames were placed at the WSU Tukey research farm on a cleared dirt surface and buried 0.5 cm into the substrate with soil mounded around the exterior perimeter.



Figure 3: Eight frames were placed at the WSU Tukey research farm on a cleared dirt surface in full sun to encourage microhabitat use. Six frames were utilized for adult trials (3 male and 3 female) with nine, 20 cm squares raised approximately 3.5 cm on small rocks that supported all four corners and two for juvenile trials which contained sixteen, 15 cm squares raised approximately 3.5 cm from the substrate



Figure 4: The small rocks that supported all four corners of the nine, 20 cm squares in the adult enclosures and the sixteen, 15 cm squares in the juvenile enclosures raised approximately 3.5 cm from the substrate.



Figure 5: Laboratory antagonistic interactions of *T. agrestis* (Ta) and *T. duellica* (Td), conspecific and congener pairings of *T. agrestis* with *T. duellica*, compared to solitary control individuals over a ten day period. Results of a one-way ANOVA, with a pooled standard deviation of 2.6, were significant ($P < 0.001$).

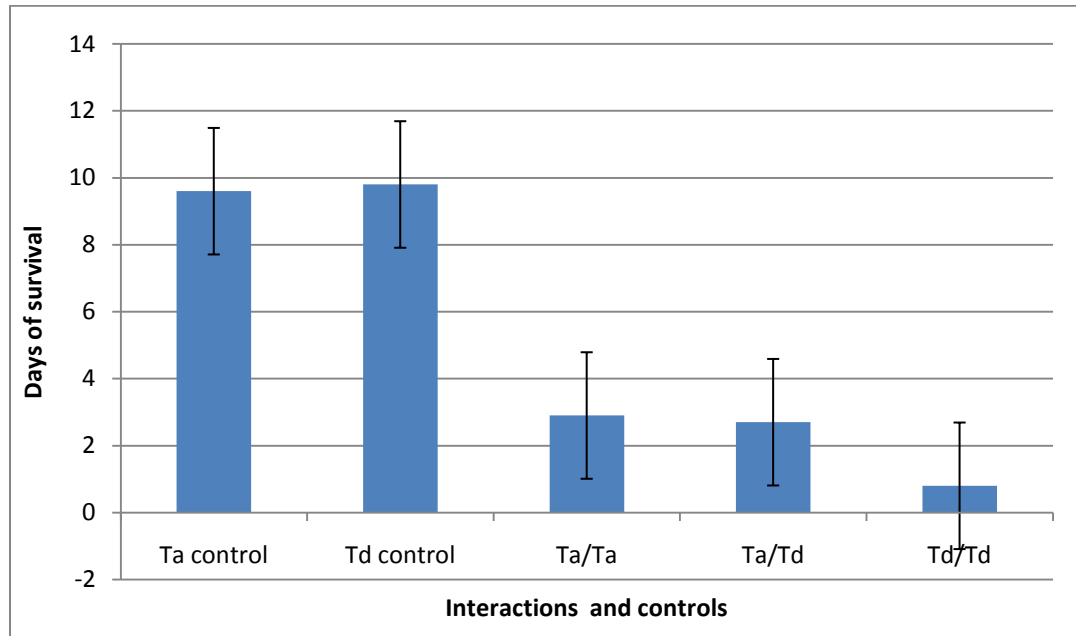
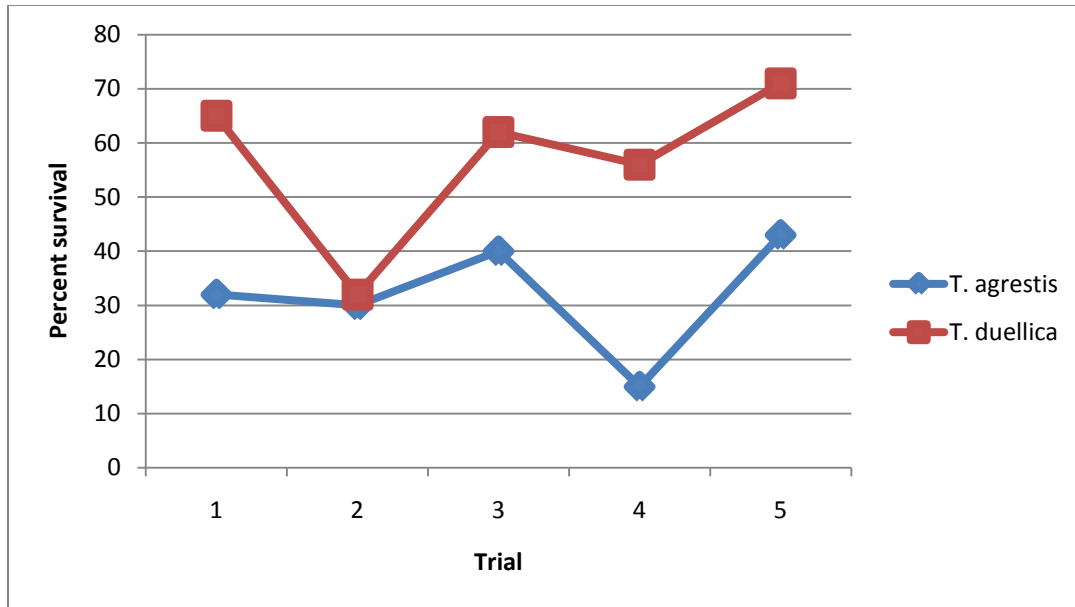


Figure 6: Survival of antagonistic interactions in field experiment of *T. agrestis* and *T. duellica*. Five trials each conducted over a fourteen day period. *Tegenaria duellica* was the overall dominant surviving species with a Binary Logistic Regression result of ($P < 0.001$).



CHAPTER FOUR

A PCR-BASED SURVEY OF BACTERIAL DIVERSITY OF *TEGENARIA AGRESTIS* WALCKENAER (ARANEAE: AGELENIDAE) AND INVESTIGATION OF POTENTIAL PATHOGEN ACQUISITION AND TRANSFER, WITH HEMOLYTIC VENOM ANALYSIS

Abstract

The European spider *Tegenaria agrestis* (hobo spider) has been implicated as a spider of medical importance in the Pacific Northwest since its introduction in the late 1980s. Studies have indicated that the hobo spider causes necrotic tissue lesions through hemolytic venom or through the transfer of pathogenic bacteria introduced by its bite. Bacterial infections are often diagnosed as spider bites, in particular the pathogenic bacteria methicillin-resistant *Staphylococcus aureus* (MRSA). This study examines three aspects of the potential medical importance of hobo spiders in part of its introduced range, Washington State, USA. First the bacterial diversity of the spider was surveyed using a PCR-based assay to determine the spider carries any pathogenic bacteria. Second, an experiment was conducted to determine the ability of the spiders to transfer MRSA. Third, the venom was evaluated to assess the hemolytic activity. We found ten genera of ubiquitous bacteria on the exterior surface of the spiders. In addition, none of the spiders exposed to MRSA transferred this pathogen. Finally, the hemolytic venom assay corroborates with previous studies that found hobo spider venom was not deleterious to vertebrate red blood cells.

Introduction

The misdiagnosis of spider bites is a widespread and common problem that can have far reaching consequences. The conventional description of a spider bite and its symptoms are often based purely on circumstantial evidence without the suspected spider being presented or envenomation being witnessed (Isbister 2004, Benoit and Suchard 2006, Vetter and Isbister 2008). Several medical conditions commonly misdiagnosed as spider bites include bacterial, viral and fungal infections, vasculitis, dermatological conditions, bites and stings from other arthropods, and miscellaneous causes such as allergies or drug reactions, chemical burns, reactions to poisonous plants or diabetic ulcers (Benoit and Suchard 2006). Professionals working in toxicology and poison control clinics report spider bites or necrotic arachnidism as over-diagnosed and poorly defined and almost always without any corroborating evidence (Russell and Gertsch 1983, Vest 1993, White 2003, Isbister 2004). Evidence from studies that refute the diagnosis of necrotic arachnidism is growing as researchers determine that many spiders commonly associated with humans do not cause suspected symptoms; for example, the yellow sac spider, *Cheiracanthium mildei* (Foradori et al. 2005, Vetter et al. 2006a), the white tailed spiders, *Lampona* spp. (White 1999, Isbister and Gray 2003), wolf spiders, (Lycosidae) (Isbister and Framenau 2004) the black house spiders, *Badumna* spp. (Isbister and Gray 2004) and the hobo spider, *Tegenaria agrestis* (Vetter and Isbister 2004, 2008). Numerous studies describe cases in which initially reported spider bite wounds and resulting necrotic tissue lesions are found to be the result of bacterial infections (Soe et al. 1987, Dominguez 2004, Weissfeld et al. 2005, Moran et al. 2006, Vetter et al. 2006b, Cohen 2007, El Fakih et al. 2008).

Of the spiders found in North America, only *Loxosceles* spp. are known to possess venom that causes necrotic arachnidism (Swanson and Vetter 2006). However, spiders have been proposed to vector bacteria as an explanation for necrotic arachnidism (Atkinson et al. 1995, Binford 2001 Fagan et al. 2003, Baxtrom et al. 2006). There are many examples of bacterial infections in necrotic lesions initially thought to be induced by spider bites (Soe et al. 1987, Peel et al. 1999, Dominguez 2004, Lui et al. 2005, Weissfeld et al. 2005, Benoit and Suchard 2006, Vetter et al. 2006b, El Fakih et al. 2008). In all of the described cases, the alleged spider was not presented as evidence. The bacterial species identified include the lesion inducing *Mycobacterium ulcerans* (Atkinson et al. 1995), human wound inhabiting *Photorhabdus luminescens* (Peel et al. 1999), *Photorhabdus asymbiotica* (Weissfeld et al. 2005), *Nocardia brasiliensis* (Lui et al. 2005) *Staphylococcus aureus* (Soe et al. 1987, Lui et al. 2005) and the methicillin-resistant *Staphylococcus aureus* (MRSA) (Dominguez 2004, Weissfeld et al. 2005, Vetter et al. 2006b, El Fakih et al. 2008). Community-acquired MRSA is a prevalent and widespread pathogen which causes necrotic tissue lesions that is often misdiagnosed as resulting from spider bites (Dominguez 2004, Baxtrom et al. 2006, Vetter et al. 2006b, Cohen 2007). Still, there is little evidence linking spiders to these bacterial species. There have been studies that have identified the microbial fauna of several spider species, specifically searching for pathogenic bacteria (Atkinson et al. 1995, Baxtrom et al. 2006). Results of these studies indicate that most spiders harbor common species of non-pathogenic bacteria that are ubiquitous in nature. These examples illustrate that bacterial infections are being attributed to spider bites. Bacterial infections from animal bites, including human bites, can be a minor medical concern or develop into serious medical problems by the introduction of pathogenic bacteria from the mouth of the biter, the skin of the victim and/or environmental exposures to the resulting wound (Brooks, 2005). However, it

remains unclear whether or not spiders are capable of spreading pathogenic bacteria through biting.

The hobo spider, *Tegenaria agrestis*, originally named the aggressive house spider, has been implicated as a spider of medical concern because necrotic lesions have been attributed to the bite of this spider in the Pacific Northwest (PNW) since the 1980s (Vest 1987a, 1987b, Akre and Myhre 1991). This introduced spider is large and highly visible and is therefore often anecdotally reported as being responsible for many types of skin eruptions and lesions when no spider or other arthropod was actually witnessed biting or stinging the victim. The hobo spider is not a medical concern in its native Europe (Binford 2001). In fact, a venom analysis did not find any components that would induce tissue necrosis in mammals (Binford 2001, Gomez and Binford, personal communication). Therefore, an important step in verifying the potential toxicity of the hobo spider bite is to identify the microbial fauna, associated with this spider, which may be vectored during a bite or accidental injury.

We identified the microbial fauna associated with *T. agrestis* as a first step toward establishing if hobo spiders can carry and transfer pathogenic bacteria. In order to estimate bacterial biodiversity, universal primers were used in a PCR-based assay to amplify the prokaryotic 16S rRNA gene. This technique has been used in a variety of natural environments including marine sediments (Marchesi et al. 1998), cerebrospinal fluid (Lu et al. 2000), the termite gut (Hongoh et al. 2003) and the human gut (Ley et al. 2005). We amplified a conserved region of the bacterial 16S rRNA gene that contains enough sequence divergence to accurately distinguish between hundreds of bacterial species within a sample (Marchesi et al. 1998, Osborn et al. 2000). In

addition to the bacterial diversity survey we also used a more direct approach to assess the possibility that hobo spiders can mechanically acquire and transfer a highly pathogenic bacterium such as MRSA. Finally, we conducted standard hemolytic assays to determine toxicity of *T. agrestis* venom to mammals in order to corroborate the results of Binford (2001) and Binford and Gomez (unpublished) venom analysis.

Material and Methods

Spider Collection

To examine the microbial fauna associated with *T. agrestis*, spiders were collected into sterile 50 mL Falcon tubes from around the outside of homes in urban environments in the cities of Spokane, Pullman, Bellevue and Puyallup, Washington State. Spiders were located by searching under rocks, concrete blocks and landscape material using webbing as an indicator for potential habitat. Positive identification of adult spiders was accomplished by examination of sclerotized genitalia according to Vetter and Antonelli (2002). Immature spiders were identified to genus using Ubick et al., (2005), followed by an examination of sternal patterns as delineated in Vetter and Antonelli (2002). Spiders were individually held in sterile containers and tested upon arrival to the laboratory or within 48 hours depending upon collecting location. No food or water was administered prior to the experiments described in the following paragraphs.

Bacterial Diversity Assay

In order to identify external and internal bacterial species associated with *T. agrestis*, we isolated microbes from spiders using the following sampling methods. First, whole spiders were

individually ground with a mortar and pestle in Tris-EDTA (TE) in a salt buffered solution. Second, whole spiders were individually ground with mortar and pestle in Luria-Bertoni (LB) broth in case the bacteria were sensitive to the media in which the spiders were ground. We chose a generalized, bacterial media as the grinding solution. We dissected abdomens and ground them in fresh LB broth to isolate the internal gut-associated microbial fauna. Finally, multiple spiders were individually washed in 5 mL of LB media for 1 minute each to pool bacterial species associated with the exterior of the spiders. Multiple culture methods were used including plating different concentrations of spider wash or ground spider materials onto LB agar or Baird-Parker agar as well as growing different concentrations of spider wash or ground spider materials in LB broth at different temperatures for different amounts of time. Of these methods, only a dilution of 20 μ L of LB spider wash in 5 mL LB broth incubated at 37°C with agitation for three hours produced positive results. A positive control with *Escherichia coli* and negative control with sterile double distilled water (ddH₂O) were run with all treatments.

Amplification and Sequencing of Bacteria

In order to identify microbes associated externally and internally from *T. agrestis* we amplified a conserved region of the 16S rRNA gene with universal bacterial primers. The primers used were: forward primer 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3'), reverse primer 1387R (5'-GGG CGG WGT GTA CAA GGC-3') (Marchesi et al. 1998) and reverse primer 1389R (5'-ACG GGC GGT GTG TAC AAG-3') (Osborn et al. 2000). The predicted sequence length for the PCR product amplified with 63F with 1387R and 63F with 1389R was approximately 1300 base pairs (bp).

Samples from all of the spider treatments (whole ground TE, whole ground LB, ground abdomen LB, whole wash LB, and multiple wash LB) were used as templates for PCR reactions with the bacterial universal primer pairs. In addition to using ground and washed spider media exactly as described as the template for PCR, we also used individual colonies from the cultured samples. The following materials were included in the PCR reactions: 1 μ L of template (treatment solution, cultured medium, or colony), 1 μ L each of 10 μ M universal bacterial primers 63F with 1387R and 63F with 1389R, 12.4 μ L ddH₂O, 2 μ L 10X PCR buffer, 2 μ L 25mM magnesium chloride (MgCl₂), 0.4 μ L 10 mM dNTPs, 0.1 μ L Dimethyl sulfoxide (DMSO) and 0.1 μ L Taq polymerase (Invitrogen, Carlsbad, CA) run on a PTC-100 thermal cycler, MJ Research Inc. (Massachusetts, USA) with the following program: 95°C for 10 minutes, 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute, repeat (95°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute) 35 times, 72°C for 10 minutes and 4°C hold. PCR products were visualized on a 1.5% agarose and TAE gel and cut out for sequencing. PCR products were isolated from the agarose gel fragment with a Bio-Rad Freeze and Squeeze kit (Bio-Rad, Hercules, CA). The resulting isolated PCR products were ligated into a pGEM Teasy kit (Promega, Madison, WI) for amplification. To isolate the amplified plasmids we used a Qiagen mini-prep kit (Qiagen, Valencia, CA). Big Dye cycle sequencing was conducted at the Washington State University Molecular Biology Core Laboratory. The sequences were identified to genus and species using tblastx on the National Center for Biotechnology Information (NCBI) website.

Potential of the Hobo Spider to Transfer MRSA

In order to conduct a direct test of the ability of the hobo spider to acquire and transfer pathogenic bacteria from surfaces and to vector the pathogen during an accidental bite, we

exposed hobo spiders to methicillin-resistant *Staphylococcus aureus* (MRSA). Specifically, we exposed living adult hobo spiders to MRSA saturated substrates for a total of five minutes. Lyophilized MRSA cultures from American Type Culture Collection (ATCC) were reconstituted with tryptic soy broth and incubated overnight in a 37°C shaker bath, following ATCC recommendations. Adult spiders were collected and identified as described previously above. A polyethylene splash apron (Fisher Scientific, Pittsburg, PA) was chosen as the substrate for MRSA because this pathogen has been shown to persist on this specific material for up to 51 days (Neely and Maley, 2000). The apron was cut into disks to fit 15 x 95 mm Fisher brand petri dishes, then glued to the inside of the bottom dish with an Avery permanent acid-free glue stick. The polyethylene disks were then cleaned with 70% ethanol and allowed to air dry before treatment. The treatment included two separate methicillin-resistant *Staphylococcus aureus* cultures and a negative control of LB medium which were swabbed onto the cleaned polyethylene disks and allowed to air dry. Spiders collected in sterile containers (n=30) were manipulated to walk or stand only on the treated and control polyethylene disks in the petri dishes for five minutes. The treated spiders were removed and housed in individual sterile petri-dishes. The spiders were then tested at 1 hr, 2 hrs and 3 hrs post-MRSA exposure for the presence of the pathogenic bacteria. Spiders were forced to walk on a clean polyethylene disk glued to a petri dish then washed with 5 mL of LB broth. After the spider was forced to walk on the clean polyethylene disk, the disk was then swabbed with a LB moistened sterile cotton-tipped stick, which was in turn swabbed onto a blood agar plate. Blood agar plates and LB broth were incubated overnight at 37°C (with shaking for the liquid media) then tested for the presence of MRSA by use of a MRSA agglutination screening kit (Denka Seiken Co., LTD, Tokyo, Japan). Persistence of MRSA on the MRSA saturated treatment disks was also tested as a control.

Hobo Spider Venom Hemolysis Assay

Hemolytic activity of spider venom was determined using the spectrophotometric hemolysis assay of Forrester et al. (1978) modified by Foradori et al. (2005). One venom gland from each of an adult male and female spider was individually homogenized in 50 μ L of a 20 mM Tris buffered saline with 8 mM CaCl_2 , pH 7.4 (TBSC; after Babcock et al. 1981). The entire venom gland homogenate (VGH) was added to a 1.0 mL suspension of 2% sheep red blood cells in TBSC and incubated at 37 °C for 1.5 h with gentle agitation. The suspension was then diluted with 3 mL TBSC and centrifuged at approximately 760 X g for 5 min. The optical densities of the supernatants were measured at 540 nm with a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA). Within each assay, a 1 mL aliquot of the sheep RBC suspension was incubated as stated above, then lysed with 3 mL distilled water, and used as the standard for 100% hemolysis. The values for % hemolysis were determined against total hemolysis (Foradori et al. 2005).

Results

Spider Collection Notes

Hobo spiders were observed in abundance around the urban homes where they were collected. Landscape blocks, bricks, rock-beds, long grass and stacked wood or boards were found to be preferred habitat for both adult and juvenile spiders. To allow for seasonal and temperature variations in microbial fauna, collections took place from June to October 2007 and February to October 2008. During the collection period, 102 *T. agrestis* adults were examined for bacteria.

Bacterial Diversity Assay

We performed a PCR-based assay to identify the bacterial species associated with *T. agrestis*. Bacterial species were identified by comparing the sequences to GenBank (<http://www.ncbi.nlm.nih.gov/BLAST/>) with the tblastx algorithm and confirmed with the Ribosomal Database Project (<http://rdp.cme.msu.edu/index.jsp>). Seventy-three bacterial samples were isolated from the spiders, providing forty-five bacterial sequences for a total of six gram-positive and four gram-negative bacteria genera identified (Table 1). The genera identified, followed by the percent sequence identity, include: *Bacillus* spp. (100%), *Paenibacillus* sp. (100%), *Aeromonas* sp. (99%), *Arthrobacter* sp. (98%), *Pseudomonas* spp. (98%), *Pantoea* sp. (97%), *Staphylococcus* sp. (97%), *Rahnella* sp. (97%), a bacterium in the order Actinomycete (95%), and *Exiguobacterium* sp. (93%). Identification to species level was confirmed, followed by the percent sequence identity, for *B. megaterium* (100%), *P. fluorescens* (100%), *B. circulans* (99%), *P. veronii* (99%), *B. thuringiensis* (97%), *P. poae* (97%), *S. saprophytica* (97%), and *B. simplex* (95%). Descriptions of natural bacterial occurrences and pathogenic capabilities of isolates were found using *The Manual of Clinical Microbiology* 7th, 8th and 9th edition (Murry et al., 1999, 2003, 2007) (Table 2). All of the ten genera identified with *T. agrestis* are ubiquitous bacterial fauna found in natural environments with several occurring on human and animal skin.

Potential of the Hobo Spider to Transfer MRSA

Spiders were exposed to MRSA in order to determine their ability to acquire the pathogen from a MRSA treated surface. This pathogen was chosen because the tissue lesions that it causes are often misdiagnosed as resulting from spider bites (Dominguez 2004, Baxtrom et al. 2006, Vetter et al. 2006b, Cohen 2007). We used a commercially available MRSA screening kit to detect the

acquisition and transfer of MRSA by the hobo spider from polyethylene disks. No MRSA was found either on the spiders or on the clean surfaces to which the MRSA-exposed spiders were subjected. There was no MRSA pathogen carried or transferred to another surface by the MRSA exposed spiders although, MRSA was found to persist on the polyethylene disks.

Hobo Spider Venom Hemolysis Assay

A simple test that can help determine whether or not a spider may cause medically significant bites is a hemolysis assay. Compared to the known hemolytic activity (> 37%) associated with *Loxosceles reclusa* venom gland homogenates (VGH), the potential of *T. agrestis* VGH to cause hemolysis was negligible at 0.62% and 0.93% for male (n= 5) and female (n=7) spiders respectively.

Discussion

We identified the sequences of six gram-positive and four gram-negative bacteria genera associated with the hobo spider, *T. agrestis* in Washington State (Table 1). The microbial fauna identified from our 102 *T. agrestis* specimens collected around the outside of homes in the PNW are all species previously cultured from natural environments. These include outdoor environments as well as the external surfaces of animal and human skin. Because the hobo spider builds its web near the ground it is not surprising that they harbor the non-pathogenic bacteria we identified including species of *Bacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Arthrobacter*, *Actinomycetes* and species in the Family Micrococcaceae. Several of the bacteria we isolated from the spiders are also considered normal human fauna such as species of *Staphylococcus*,

Arthrobacter, and the two species of *Exiguobacterium* which have little or no pathogenic potential. Yet some of these naturally occurring bacteria include species that can be opportunistic or have pathogenic potential, such as species of *Bacillus*, *Rahnella*, *Aeromonas*, *Pseudomonas*, *Staphylococcus*, *Arthrobacter* and *Actinomycetes*. However, none of the eight bacterial species that we cultured from the hobo spider were recognized as a disease producing agent although some of the species have been isolated from human wounds or infections including *Pseudomonas fluorescens*, *P. poae*, *P. veronii*, *Bacillus circulans*, *B. thuringiensis* and *Staphylococcus saprophyticus*. Our results are comparable to the Baxtrom et al. (2006) who found that the majority of the 99 common North American house spiders they analyzed carried *Bacillus* spp. and *Staphylococcus epidermidis* among other diptheroid bacteria. However, by using the 16S rRNA gene and universal primers we were able to culture higher numbers and subsequently a larger diversity for identification with more confidence than could be obtained with phenotypic identifications.

We examined spiders for pathogenic bacteria because infections from opportunistic or lesion producing bacteria are often described as spider bites. Medical professionals are examining for primary or secondary bacterial infections when patients report lesions as possible spider bites. For example, reported cases from Australia of patients with necrotic lesions thought to be the result of spider bites, were actually found to be infected by the lesion inducing microorganism *Mycobacterium ulcerans* (Atkinson et al. 1995). The suggestion that the spider infected the victim with *M. ulcerans* is an interesting possibility but it is just as likely that *M. ulcerans* infected the victim as a secondary infecting agent via a non-spider route (Harvey and Raven 1991). It was concluded that *M. ulcerans* is not associated with spiders or spider bites in

Australia and reported that antibiotics should not be prescribed for suspected spider bites (Atkinson et al. 1995) In another example, four patients who believed that they were bitten by spiders were shown to have *Photorhabdus luminescens* infections; *P. luminescens* is a bacterial species associated with nematodes but is not typically known to induce necrotic lesions in humans although it has been found in human wounds (Peel et al. 1999). Finally, in an alleged spider bite case, two different bacterial species were cultured from the necrotic lesion - *Photorhabdus asymbiotica* and importantly, *methicillin-resistant Staphylococcus aureus* (MRSA) (Weissfeld et al. 2005). These authors describe the possibility of an arthropod vector of *Photorhabdus* because it is found in the guts of entomopathogenic nematodes and often cultured from infections of individuals who have engaged in outdoor activity (Weissfeld et al. 2005). While *P. asymbiotica* is not usually known to induce necrotic lesions, MRSA is well known for causing soft tissue lesions. Of 38 patients requiring surgical debridement of soft tissue infection due to reported spider bites, all exhibited *S. aureus*, and 87 % of those were MRSA (Fagan et al. 2003).

A primary goal of this study was to determine if the hobo spider carried the necrotic lesion inducing bacteria MRSA. While we did not find MRSA on hobo spiders, that might be due to sampling as the MRSA strain is a community or nosocomal spread infection which has not been isolated from natural environments. Based on our ability to detect bacterial diversity and due to the number of MRSA infections initially reported as spider bites, we conducted a more direct experiment in which we exposed hobo spiders to a MRSA-saturated substrate to determine if hobo spiders were capable of acquiring and transferring the bacteria from a contaminated surface. In our experiments, the hobo spider was unable to acquire MRSA after forced contact

with a MRSA-saturated substrate for a full five minutes. This experiment was designed to mimic a natural setting in which the spider was subjected to an environment with a pathogenic bacteria such as MRSA (e.g., hospitals, nursing homes, prisons, athletic departments, and child care centers) (Moran et al. 2006; Klevens et al. 2007). In this experiment, since the spider was not able to acquire MRSA it also did not transfer the pathogen.

We also conducted a hemolytic assay of the venom of Washington State collected spiders. The venom from US hobo spider populations has been compared to populations from the native and introduced regions in Europe with no significant variables found to explain the perceived envenomation concern in the US (Binford 2001). The European populations are not known to cause necrotic arachnidism (Bettini and Brignoli 1978, Binford 2001). Nevertheless, we believed that it was important to conduct a standard venom hemolytic assay on hobo spiders collected for this study. Foradori et al. (2005) examined the venom of 45 spider species for the ability to cause hemolysis or breakdown of red blood cell membranes. Of the spiders included in the assay, only two possessed venom enzymes which caused hemolysis. The venom of the brown recluse spider, *Loxosceles reclusa*, which contains the enzyme sphingomyelinase D has been shown to produce necrotic lesions (Kurpiewski et al. 1981). The venom of the yellow sac spider, *C. mildei* contains phospholipase A₂ an enzyme which may be capable of hemolytic activity (Foradori et al. 2005). The venom of *T. agrestis* has yet to be shown to contain any necrotizing components. Consistent with previous results (Gomez and Binford unpublished), Washington State *T. agrestis* venom did not cause significant hemolytic activity in our assay.

The hobo spider has rapidly expanded its range since its introduction into the Seattle, Washington area in the 1930s (Exline 1936, 1951) and has moved into neighboring states (Baird and Akre 1993, Baird and Stoltz 2002, Vetter et al. 2003). In one studied case that reported a necrotic lesion resulting from a putative *T. agrestis* envenomation; many of the details are unclear (Binford 2001, Vetter and Isbister 2004, 2008). The case described a 42-year old woman with a history of phlebitis who found a crushed brown spider on her ankle beneath her pant leg. The spider was reported as a hobo spider but there is no explanation of how it was identified or by whom. In addition, the woman did not seek medical attention for 79 days after the incident and therefore questions surround the actual cause of the necrosis (Case 2, Vest 1996). Our study focused on identifying putative factors that could correlate *T. agrestis* bites with necrotic arachnidism based on widespread concern and some previous studies that have attributed necrotic lesions to the hobo spider. In the late 1980s, Vest (1987a) described the probable involvement of the hobo spider, *T. agrestis*, as the cause of necrotic lesions. Vest (1987b) further implicated *T. agrestis* in a preliminary study where New Zealand white (NZW) rabbits and one California giant rabbit were subjected to envenomation by forced bite. Vest (1987b) observed that the four NZW rabbits bitten by male *T. agrestis* developed significant dermal lesions. This experiment was repeated by other researchers using approximately 40 NZW rabbits over a two year period (Gomez and Binford, unpublished). They injected 20 μ L of venom intradermally at concentrations of 1:25, 1:75 and 1:150 which resulted in mild reactions but no developing necrosis. They also injected pure undiluted hobo spider venom which produced a modest dermal inflammation but still not the significant necrosis that Vest described (Gomez, personal communication).

One possibility for the discrepancy in results could be that Vest forced the spider on to the skin of the rabbit allowing for possible bacterial introduction and/or digestive fluids that may have been expelled in response to the forced pressure. We have ruled out bacteria as a cause of necrosis in the *T. agrestis* spiders we surveyed which is also supported by Binford (2001). While researchers have focused on spider venom as the tissue necrotizing agent, other factors besides bacteria could also have medical significance. Spider digestive fluids have been examined for potential necrotizing agents. The garden spider, *Argiope aurantia*, was found to possess collagenase in the gut which cleaved connective tissue proteins but did not produce necrotic lesions on rabbit test subjects (Foradori et al. 2001). Peptidases in the digestive fluid of *A. aurantia* were suggested to help inactivate the peptidases produced by its prey, while inhibitors in the gut of the spider may protect the spider from the serine peptidases of the prey during digestion (Foradori et al. 2006). Medical literature is reporting an increasing number of bacterial infections due to overuse of antibiotics and antibacterial cleansers depleting beneficial bacterial flora (Levy 2001). The microbial fauna on human skin can be beneficial to our health but can also be the cause of infection or further injury to an existing wound (Cogen et al. 2008, Dethlefsen et al. 2007).

There is little evidence to support the claims that the hobo spider possesses a necrotizing toxin. Although this introduced spider is common around homes in urban environments in the PNW and is expanding its range, we cannot substantiate its involvement in human necrotic tissue lesions as once suspected (Bennett 2002 Bennett 2004, Vetter and Isbister 2004, 2008). This study confirms previous results and provides further evidence that the hobo spider, *T. agrestis*, is not a spider of medical concern. We have identified the bacteria associated with *T. agrestis* as

ubiquitous environmental fauna and displayed the spider's inability to transfer a pathogenic bacteria. The hemolytic venom assay demonstrates that the spider is incapable of causing severe cellular damage. Finally, the results from the mammalian assay strengthen the hypothesis that the hobo spider does not cause necrotic lesions.

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Table 1: Gram positive and gram negative bacteria genera cultured from the hobo spider, *Tegenaria agrestis* collected in Washington State.

Gram-Positive	Gram-Negative
<i>Bacillus</i>	<i>Pantoea</i>
<i>Staphylococcus</i>	<i>Aeromonas</i>
<i>Exiguobacterium</i>	<i>Pseudomonas</i>
<i>Arthrobacter</i>	<i>Rahnella</i>
<i>Paenibacillus</i>	
<i>Actinomycete</i>	

Table 2: Natural or cultured environmental locations of identified microbes which were also isolated from the hobo spider, *Tegenaria agrestis*, using the *Manual of Clinical Microbiology* 7th, 8th and 9th edition.

Bacteria	Natural or Cultured Environment	Pathogenic Potential
<i>Bacillus</i>	Isolated from soil	Some species opportunistic or obligate pathogens
<i>Pantoea</i>	Isolated from plants	Occasional opportunist in wounds
Actinomycete	Isolated from soil and organic matter	40 genera relevant to human and animal health
<i>Aeromonas</i>	Isolated from wet environments	Potential intestinal pathogen
<i>Staphylococcus</i>	Widespread in nature including human skin	Some infection or disease causing species
<i>Arthrobacter</i>	Present on human skin and in soil	Occasional opportunistic in wounds
<i>Paenibacillus</i>	Isolated from soil	Not opportunistic pathogen
<i>Exiguobacterium</i>	Isolated from and on human skin	Not opportunistic pathogen
<i>Pseudomonas</i>	Worldwide, in moist environments	Occasional opportunist in wounds
<i>Rahnella aquatilis</i>	Isolated from water samples	Opportunistic pathogen