INVASION AND HIGH-ELEVATION ACCLIMATION OF THE RED IMPORTED FIRE ANT (FORMICIDAE: *SOLENOPSIS INVICTA*) IN THE SOUTHERN BLUE RIDGE ESCARPMENT REGION

A Thesis Presented to the Faculty of the Graduate School of Western Carolina University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology.

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ABSTRACT

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The red imported fire ant, *Solenopsis invicta*, is an invasive species in the United States that has rapidly spread throughout the country since its first introduction to Mobile, Alabama, in the 1930s from South America. Within the past decade, researchers have predicted elevational and latitudinal limits on *S. invicta*'s distribution owing to the presumed difficulty of their coping with the severity of high-elevation winters. Building on recent reports of *S. invicta* colonies established at elevations in excess of 1219 meters in Macon and Jackson counties, NC, I subsequently (2016-2017) documented approximately 75 live colonies in the Highlands and Cashiers areas of Macon and Jackson Counties. The presence of these invasive ants at relatively high elevations raises the question of the likelihood of their continued persistence. In this study I explored possible physiological and behavioral adaptation of *S. invicta* to high elevation environments through a comparison of cold and heat tolerance, fat content, and nest-site selection collected along an elevational gradient from piedmont to montane regions in the Carolinas and north Georgia (203-1228 m). For comparison, I also collected physiological temperature tolerance data for the dominant native woodland ant *Aphaenogaster picea*.

Solenopsis invicta occurring at higher elevations exhibited a significantly greater tolerance for cold temperature extremes as compared to lower-elevation conspecifics. This tolerance differential resembles that of the native ant *A. picea*, which naturally occurs along a similar elevational gradient. Whereas *S. invicta* had a significantly higher tolerance for heat and a wider overall tolerance range compared to the native ant *A. picea*, both species exhibited a similar downward shift in thermal tolerances when moving up the elevational gradient. There was no significant difference in colony lipid content along the gradient, suggesting that greater metabolic rates are not needed to sustain these ants through winter dormancy at high elevations. Finally, nest-site selection in proximity to a thermal mass (rocks, logs, concrete footers, etc.) does not seem to improve thermoregulation, and ants at high elevations do not appear to select nest sites based on proximity to a potential thermal buffer. The results are discussed in the context of future projections for the continued spread of *S. invicta* in montane and higher-latitude regions.

CHAPTER ONE: INTRODUCTION

Solenopsis invicta, the red imported fire ant, is one of several fire ant species native to South America. The Latin specific epithet “invicta” translates to "unconquered." This name is appropriate for *S. invicta* given that this is a highly invasive and aggressive species in its non-native environments. Portuguese settlers in Brazil even referred to them as “the actual owners of the Amazon Valley” (Wilson 1971, pg. 1). This species is indigenous to southern Brazil, Paraguay, and northeast Argentina, and has been introduced to many countries over the past two centuries, including the U.S. where it has rapidly spread since its first introduction to Mobile, Alabama, in the 1930s. Owing to the highly aggressive nature of these fire ants, their large colony size, and their potent sting, they are widely regarded as a medical, agricultural, and ecological menace. Wherever they have colonized, they pose a threat to native ants and other insect species, agricultural crops, livestock, human health, and even wildflower populations through disruption of ant-mediated seed dispersal (Zettler et al. 2001; Ness and Bronstein 2004; Tschinkel 2006; Drees 2014).

One of the most common areas in which to find *S. invicta* is rural, agricultural settings, where they can cause extensive damage. Fire ants are omnivorous, and will feed on germinating seeds of agricultural crops, as well as small animals including young livestock (Tschinkel 2006). The ants are also known to girdle trees and are often a pest to nuts, grapes, and tree fruits (Jetter et al. 2002). Not only do their eating habits cause damage but they are also notorious for destroying irrigation lines and infesting farm equipment. Furthermore, many people choose to avoid equipment or areas with high incidence of fire ants due to their noxious sting. *Solenopsis* *invicta* stings may cause localized irritation, swelling, and cause blisters, and can even cause anaphylactic shock to those who are allergic (Drees 2014).

Solenopsis invicta inflicts damage to natural ecological systems as well. The myrmecochorous mutualism between spring ephemeral wildflowers and native ants, such as *Aphaenogaster picea*, is a stunning example of coevolution, but *S. invicta* interfere with this interaction by supplanting native ants (Zettler et al. 2001; Ness and Bronstein 2004). Moreover, when *S. invicta* consume the seed's elaiosome (the lipid rich appendage used to attract native ant dispersers), the seeds are often destroyed or scarified in the process (Zettler et al. 2001; Ness and Bronstein 2004) and intact seeds are often discarded above ground in less than ideal conditions for germination.

Solenopsis invicta have also displaced the native fire ant, *Solenopsis xyloni*, in much of its natural habitat since introduction of the imported species (Jacobson et al. 2006). *Solenopsis xyloni* is native to the southeastern United States and once occupied much of the Carolinas; however, they are rarely found in the southeast presently. Many aerial insectivores prey upon the less aggressive *S. xyloni* and keep their populations in check, but there are few animals that are known to prey on *S. invicta* in the United States. The purple martin, *Progne subis,* is one of the few exceptions, and while they can eat millions of alates (winged sexuals) each year, this is not enough to keep populations low enough to allow *S. xyloni* to bounce back to historical numbers (Jackson et al. 2016). *Solenopsis invicta* is able to supplant native ants and push them out of their native habitats by aggressively spreading across landscapes with few predator encounters and altering phylogenetic structures of native ant communities, but *S. invicta*'s spread is ultimately facilitated by humans (Tschinkel 2006; Lessard 2009).

Habitat disturbance is crucial for the establishment of many invasive species;

globalization and the increase of trade allows *S. invicta* to spread successfully to these disturbed environments via shipping and other modes of transport (Early et al. 2016). Surprisingly, the recent introduction of *S. invicta* in many countries can be traced to populations in the United States rather than South America (Ascunce et al. 2011). Recently invaded countries include China, Taiwan, Hong Kong, Australia, and some countries of the Caribbean, where researchers have found fire ants to have been introduced from the U.S. within the past few decades. The exact year of introduction to these newly invaded areas is unknown, but genetic evidence has allowed the populations to be traced back to the United States. The spread of fire ants from the U.S. highlights the ubiquity of these pests in this country, perhaps especially in and around many major shipping ports. It seems likely that *S. invicta* will continue to spread to new ecosystems across the globe from both native and non-native habitats, as long as the local climate accords with the physiological limits of the species.

Around the world, there is a common environmental habitat shared among all *S. invicta*: warm and highly disturbed habitats (Tschinkel 2006). *Solenopsis invicta* prefer sunny, open areas to site their nests, because the species is extremely thermophilic and the brood incubate in the top of the nest at the warmest hours of the day. Many ectotherms depend on the climate of their immediate surroundings for thermoregulation, which can limit their capacity to persist in relatively cooler habitats. *Solenopsis invicta* has evolved behaviors such as nest building and nest site selection to improve colony thermoregulation, but however innovative these adaptations are, the ectothermic fire ants may be still substantially limited by winter temperatures at high latitudes and elevations, although perhaps less so than once thought. Researchers predicted that *S. invicta* populations in the southeastern U.S. would be restricted from higher latitudes and altitudes by the greater cold temperature extremes because they originated in a subtropical climate (Korzukhin et al. 2001; Tschinkel 2006; Bertelsmeier et al. 2014). For example, Korzukhin et al. (2001) predicted that cold weather would limit *S. invicta* alate production through freeze-kills and stunted reproductive output. Excessive cold also limits colony growth by reducing the number of foragers and colony defenders. Korzukhin (2001) predicted that *S. invicta* would be unlikely to colonize areas with minimum winter air temperatures between -3 and -7 degrees Celsius, including the mountains of western North Carolina. In their predictive range map, these researchers predicted that western North Carolina was unsuitable habitat for *S. invicta* due to the cold temperature extremes at high elevations, making it “improbable” that the ants would ever be able to persist in the region (Korzukhin 2001). This assessment was considered the most “realistic to date” by Tschinkel (2006), however, *S. invicta* colonies have been observed at over 1,220 m in the southern Appalachian mountains (Yeary-Johnson 2014 and pers. obs.)

It is not yet evident if high-elevation colonies are now perennial or if they are at the periphery or beyond their range of environmental tolerance and may soon perish. One recent (2014-2015) study, conducted by a student at the Highlands Biological Station Institute for the Environment program, documented 68 *S. invicta* colonies in Jackson and Macon Counties, several of which were found to be active following the severe winter of 2015 (Karl Yeary, personal communication; Yeary-Johnson 2014). Several counties in western North Carolina are now under quarantine for *S. invicta,* including Macon County as of 2016 and parts of Jackson County as of 2017 (North Carolina Department of Agriculture & Consumer Services 2018; Fig. 1).

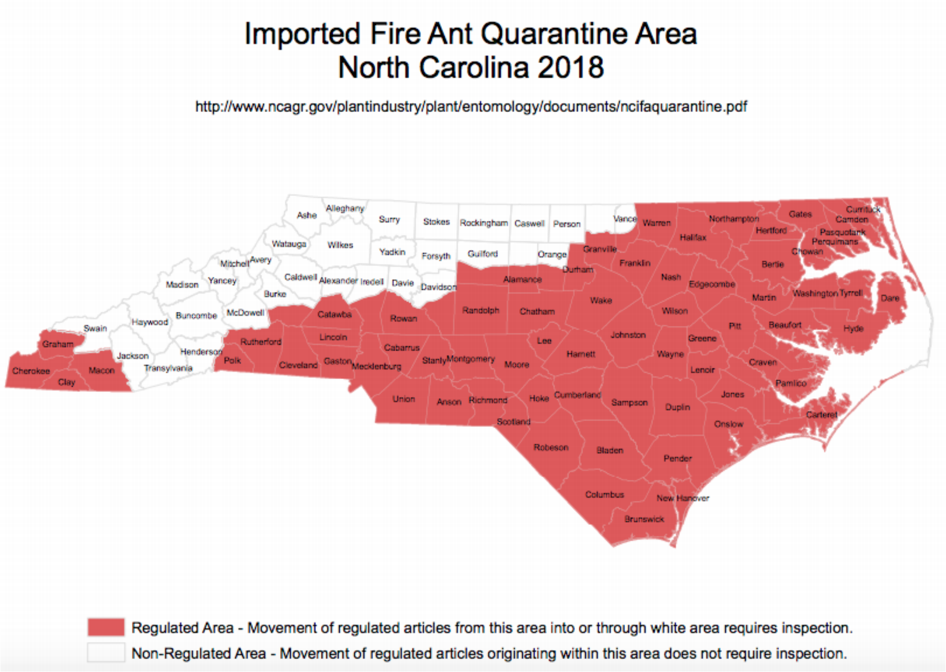

Figure 1. North Carolina quarantine map of the red imported fire ant in 2018. From the North Carolina Department of Agriculture & Consumer Services (Johnson 2018).

One study found that *Solenopsis invicta* collected in South Carolina from different microenvironments varied in their ability to tolerate heat (Boyles et al. 2009). Ants from shaded and unshaded (i.e., cooler and warmer) microenvironments were collected and tested for both heat and cold tolerance and interestingly, ants from both microclimates had similar cold tolerances but differed in their ability to tolerate heat. Even though heat tolerance varied in *S. invicta*, they displayed an inflexible threshold for cold tolerance, raiding the question of whether warmer environments put selective pressure on *S. invicta* for higher heat tolerance. Although more research is needed, there is evidence of thermal tolerance differences of *S. invicta* from varying microenvironments, and it is necessary to research whether thermal tolerances differ on a larger scale, such as an elevational gradient.

Research Questions

The ongoing persistence of fire ants at high elevations in the southern Appalachians raises the question of what the driving mechanism is for this species' survival through cold winters. Is a physiological change or perhaps a behavioral change allowing *S. invicta* to persist at high elevations? Is it a combination of these changes that may allow their continuation? As an initial exploration of the means of high elevation adaptation, I ran a series of studies to examine the extent of physiological and/or behavioral change and then compared my results with ants at low elevations and with a native ant species.

1. How do *S. invicta* critical thermal tolerances along an elevation gradient compare to the native *A. picea* ants?

As a first step toward addressing the question of physiological adaptation to high-elevations, I assessed the thermal tolerance of the non-native *S. invicta* and the native *A. picea*, on an elevational gradient of 203-1228 m across the Blue Ridge Escarpment of upstate South Carolina and western North Carolina, split into three levels: low (0-305 m), mid (457-762 m) and high (915+ m). I hypothesized that *S. invicta* may physiologically adapt or acclimate to colder temperatures, which was tested with a thermal tolerance assay (Smith and Tschinkel 2009; Warren et al. 2016).

The native *A. picea* is one of at least 26 distinct species in the genus in North America (Lubertazzi 2012). Ants in this genus live under rotting logs or other objects on hardwood forest floors and are one of the most abundant invertebrates in these eastern North American forests (Lubertazzi 2012). *Aphaenogaster* are generalist foragers and frequently consume elaiosomes, which facilitates the dispersal of spring ephemeral wildflowers (Lubertazzi 2012; Warren 2015), and are one of the first ants to begin foraging in the spring in eastern North America, later entering diapause at the onset of winter (Lubertazzi 2012). The ease of identification and the abundance of *A. picea* in the Blue Ridge Escarpment region made them a good candidate as a comparison species to *S. invicta*, although they greatly differ in their habitat requirements. *Solenopsis invicta* inhabit disturbed full-sun habitats whereas *A. picea* inhabit forested areas that are often shaded. *Aphaenogaster picea* occasionally occupy forest edges where *S. invicta* are also sometimes found, but it should be noted that differences in the habitats of these two species could amplify differences in thermal tolerance.

Habitat composition in the southern Blue Ridge Escarpment region differs on an elevation gradient, whereas lower elevation collection sites are primarily urban and high elevation collection sites are primarily rural. The ‘urban heat island effect’ is a phenomenon whereby that ambient air temperatures in cities are consistently warmer compared to rural environments (Imhoff et al. 2010). This urban heat island effect possibly intensified the temperature differences that are present on an elevational gradient, as Imhoff et al. (2010) found that urban development increases ambient temperatures by an average of 1.3 degrees Celsius in the winter and 4.3 degrees Celsius in the summer in several major U.S. cities.

Determining minimum and maximum thermal tolerance limits for worker ants can provide insights to their potential range limits and distribution in regard to climate. *Solenopsis invicta* was once thought to be unable to persist at high elevation areas in the southern Appalachians, but a greater cold tolerance at high elevations could indicate that ants are able exhibit similar spring foraging phenology as their low elevation counterparts (Bishop et al. 2017).

Peak performance occurs for an ectothermic organism at intermediate body temperatures;

body temperatures that are too high or too low will result in reduced performance and eventually a loss of motor function (Tschinkel 2006). It is informative to observe both lower and upper thermal tolerance limits for *S. invicta* and examine if there are differences in thermal limits of this species on an elevational gradient. Lower (critical thermal minimum; CT_{min}) and upper (critical thermal maximum; CT_{max}) physiological temperature limits are calculated by recording temperatures at which an organism loses its righting response or motor function (Huey and Stevenson 1979) and are the temperatures at which the ants would be unable to move (e.g. forage for food or perform other worker functions).

Based the fact that *S. invicta* was surviving and persisting at high elevations in the Blue Ridge Escarpment (Yeary-Johnson 2014; pers. obs.), I hypothesized that they would have a lower minimum thermal tolerance at higher elevations (H1a) and that the shift in thermal tolerance would be similar to that of the native ant, *A. picea*, but that tolerance would not be as low as *A. picea* (H1b) because *A. picea* has been present in North America longer and has had longer to adapt to local climates. I also predicted that there would be a decrease in the maximum thermal tolerance of *S. invicta* along an elevational gradient (H1c) and that heat tolerance would be much higher for *S. invicta* compared to *A. picea* (H1d) because *S. invicta* is adapted to a subtropical (warmer) climate. I further predicted that the thermal tolerance range of *S. invicta* would increase as elevations increase (H1e), and that thermal tolerance range increase of *S. invicta* would resemble that of *A. picea* (H1f).

2. Are higher elevation colonies less healthy?

Lipid content of worker ants gives an indication of colony health and can be useful in predicting the reproductive success of a colony (Smith and Tschinkel 1999; Tschinkel 2006;

Warren et al. 2015). Ants usually begin storing fat in the summer and into the fall in preparation for winter dormancy, or diapause (Wilson 1971; Tschinkel 2006) so evaluating lipid content of *S. invicta* colonies on an elevational gradient can help elucidate whether or not colonies at high elevations have less fat due to a shorter foraging time.

Warren et al. (2015) examined several impacts of exurban development on ants in the *Aphaenogaster rudis* complex and found that colony health was not affected by exurban edges but foraging and nest location were influenced. Colony lipid content did not differ significantly between colonies near exurban edges and colonies in undisturbed wooded environments, but fat content was positively correlated with colony size. Since colony health can be measured by colony lipid content (Warren et al. 2015), and ants at higher elevations may have a shorter foraging time due to colder and longer winters resulting in less food for the colony, then I hypothesized that fire ants at high elevations (915 m and above) in the southern Appalachian mountains would exhibit lower lipid content than that of their low elevation (305 m and below) congeners (H2) because they may be unhealthier due to a shorter foraging time and may expend more metabolic energy.

3. Does *S. invicta* compensate for colder environments by selecting warmer microhabitats for colonies?

Alternatively, *S. invicta* may persist high in the southern Appalachian mountains by means of some form of niche construction, in which a species alters the surrounding environment in order to improve fitness (Laland et al. 2016). By altering mound height, for example, fire ants may be able to persist in less than ideal environments and regulate temperature inside the nest (Laland et al. 1999; Tschinkel 2006; Laland et al. 2016). Fire ants use more energy to build their mounds higher in the spring when temperature tracking is most important for brood growth (Penick and Tschinkel 2008). The mounds typically rise above the ground year-round to let cool air in the tunnels while the workers incubate brood at the top (Tschinkel 2006). Choosing the best habitat to suit the population's needs is another facet of niche construction (Laland et al. 1999; Laland et al. 2016). Many social insects rely on beating their wings to ventilate the hive or nest, but ants do not have that option as all of the workers are wingless. Instead, many ants manipulate the placement of their nest to regulate the microclimate (Wilson 1971; Tschinkel 2006).

All worker ants are sterile, so queens are the sole reproductive female in a colony. After the mating flight, where male and female alates mate, the female disperses to a new site where she consumes her own wings and then lays eggs that become her first workers (Tschinkel 2006). *Solenopsis invicta* queens may exhibit a behavioral adaptation to high elevations by selecting nest sites buffered by the cold and the wind (near a pole or large rock for example), which might be most advantageous in the winter. *Solenopsis invicta* colonies at high elevation sites often appear to occur on or adjacent to rocks or similar thermal masses, raising the possibility of microhabitat site selection (Wilson 1971; pers. obs.). Selecting sites on or adjacent to potential thermal masses may buffer the colony from the coldest winter temperatures and winds. As the sun rises, a thermal mass may absorb heat and transfer it to the soil around the fire ant nest.

I hypothesized that *S. invicta* nests situated on a potential thermal source would have higher minimum soil temperatures than nests out in the open (H3a) and that the majority of colonies at high elevations (915+ m) would be sited on or near a thermal source (H3b) because altering mound height may not be enough to control the thermoregulation of the colony.

No matter the mechanism, whether it is physiological adaptation via cold tolerance or higher lipid content, behavioral adaptation via site selection, or a response to climate change, it is highly worthwhile to document and track the incidence of high-elevation *S. invicta* colonies. Eradication of invasive fire ants in North America is now impossible, and as they continue to spread it is important to gain a comprehensive understanding of their ubiquity in the United States and their ability to adapt to novel environments (Bertelsmeier et al. 2014; Simberloff 2014), including high elevations.

CHAPTER TWO: MATERIALS AND METHODS

Collection Sites and Mapping

Solenopsis invicta are recognized by the two nodes on their segmented petiole, elbowed antennae, and fiery sting. *S. invicta* were collected at three elevational ranges, low (0 to 305 m), mid (457 to 762 m), and high (915+ m). The highest elevation population of *S. invicta* recorded in the Carolinas was 1,228 m elevation on a logging trail (N35.281783 W-83.225667) in Franklin, North Carolina. The lowest elevation colony collected for this study was at 203 m in Clemson, South Carolina (N34.6921 W-82.84345), generating an elevational range of 1,025 m.

Fire ant colonies were abundant in disturbed areas along road rights-of-way, highway rest stops, and along agricultural fields (personal observation), and there was little difficulty in meeting the sample size goals set out in this study. For all elevational localities, I visited disturbed habitats and collected the first active colonies that I observed that were more than ten meters away from any other *S. invicta* colony to reduce the possibility of collecting ants from a polygynous colony. If I found no colony after fifteen minutes of searching, I moved on to another site.

Approximately 300 worker ants from 14 colonies at each of three localities (n = 42 colonies) occurring along a piedmont-to-mountain elevational gradient were collected in the Carolinas and Georgia (Fig. 3) from 7 June 2017 to 20 July 2017. The GPS location of each *S. invicta* colony sampled was recorded with a GPS unit and recorded for mapping purposes through Google Maps™ (Google Maps 2018). Ants were collected in a shop-vacuum after all observations were recorded. Each colony was individually placed in reusable plastic bags in a cooler and promptly brought back to the lab (Highlands Biological Statioin) and placed in a refrigerator until thermal tolerance tests were performed, no more than 24 hours after field collection.

Low Elevation

The “low elevation” locality ranged from 0-305 m (0-1000 feet) in elevation and included colonies in Anderson County and Oconee County, South Carolina (Fig. 2). Colonies at low elevations all were collected in urban and suburban areas and were conspicuous due to the red clay soil that they perturbed. Colonies were chosen haphazardly, the main criteria being that they were at least 10 m apart. The majority of the colonies were collected along Tiger Boulevard in Clemson, South Carolina (N34.692611 W-82.847204) in one connected stretch of land. Ants from low elevation *A. picea* colonies were collected as close to the *S. invicta* colonies as possible (Fig. 3), and most were collected near Issaqueena Lake (N34.745902 W-82.86018).

Mid Elevation

The “mid-elevation” locality ranged from 457-762 m (1500-2500 feet) in elevation, targeting colonies in Rabun County, Georgia, and Macon and Jackson Counties, North Carolina (Fig. 2). Nests were less conspicuous at this elevational range, so we collected ants after observing them on roadsides (e.g. in Rabun county, GA) and by targeting known sites of occurrence (pers. obs.), such as the Western Carolina University campus (N35.309325 W-83.18485) and Coweeta Hydrologic Laboratory (N35.059707 W-83.430739). Ants were collected from seven mid-elevation *A. picea* colonies as close to the *S. invicta* colonies as possible (Fig. 3).

High Elevation

The “high elevation” locality included colonies found at 915 m (3000 ft) elevation and above, targeting known colonies in southern Macon and Jackson counties, North Carolina in the Blue Ridge Escarpment area (Fig. 2). High elevation colonies (915+ m) were more elusive. Based on several observations of fire ants in Macon and Jackson counties, I targeted sites with known disturbance and infestation of *S. invicta*, such as the Lonesome Valley residential community in Cashiers, NC (N35.131682 W-83.062290), the Cashiers Recreation Center (N35.110823 W-83.104809), the Chattooga Narrows hiking trail (N 35.040692 W -83.136446), and a private logging road in Franklin, NC (N35.279735 W-83.231491) that all have active *S. invicta* populations (Fig. 2). Ants were also collected from seven high elevation *A. picea* colonies as close to the *S. invicta* colonies as possible (Fig. 3).

Winter Mapping

In the winter of 2017-2018, high elevation colonies were mapped and used to examine site selection preferences in relation to potential thermal buffers. I observed nine pairs of colonies that met the following criteria: each was within ten meters of the other and had approximately the same mound size, and one was in proximity to a potential thermal buffer while the other was not. I marked these colonies with a GPS and mapped them in Google Maps™ (Fig. 4) (Google Maps 2018). I also took observational data on 34 other high elevation colonies and mapped them in Google Maps™ (Fig. 5) (Google Maps 2018).

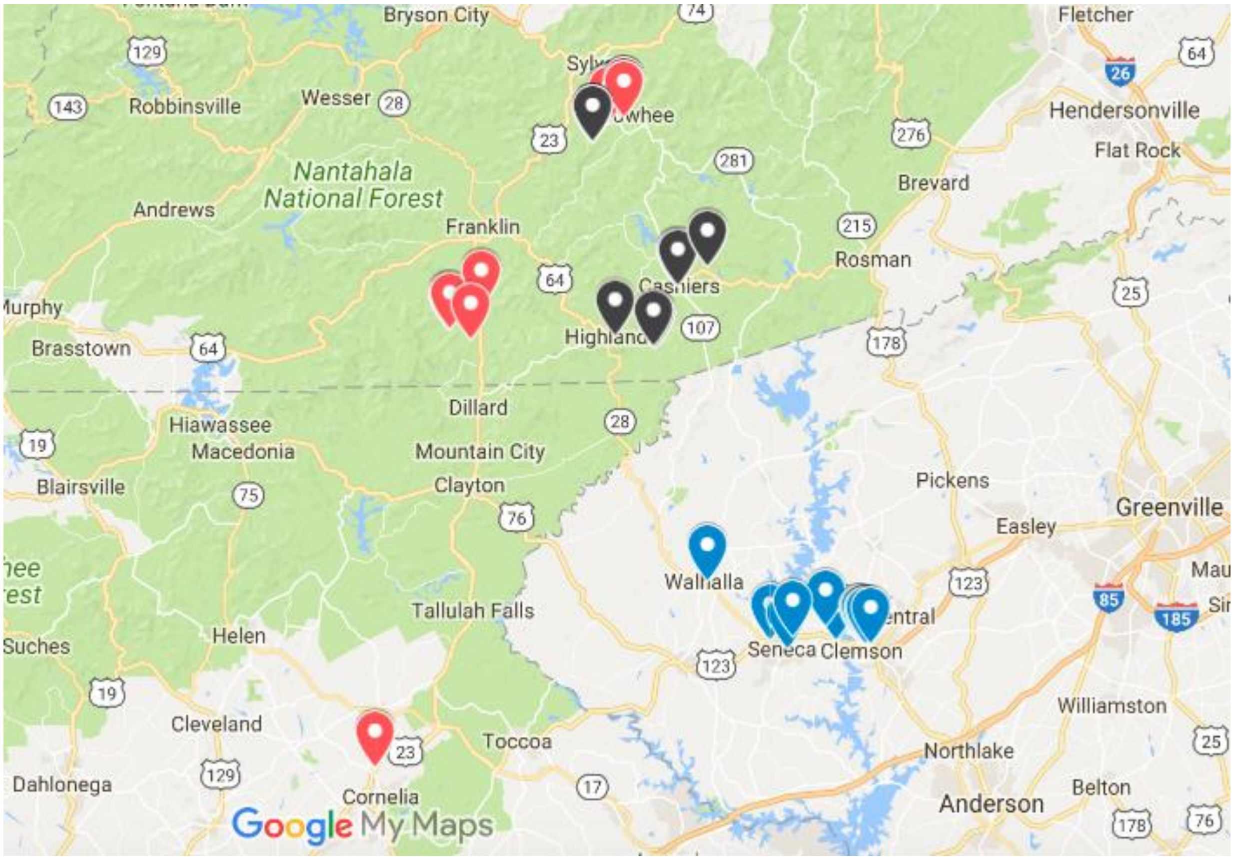

Figure 2. Map of *Solenopsis invicta* colonies collected for thermal tolerance and lipid analysis assays in the summer of 2017. High elevation (915+ m) colonies are black, mid elevation (457-762 m) colonies are red, and low elevation (0-305 m) colonies are blue. ©2017 Google LLC, used with permission. Google and the Google logo are registered trademarks of Google LLC.

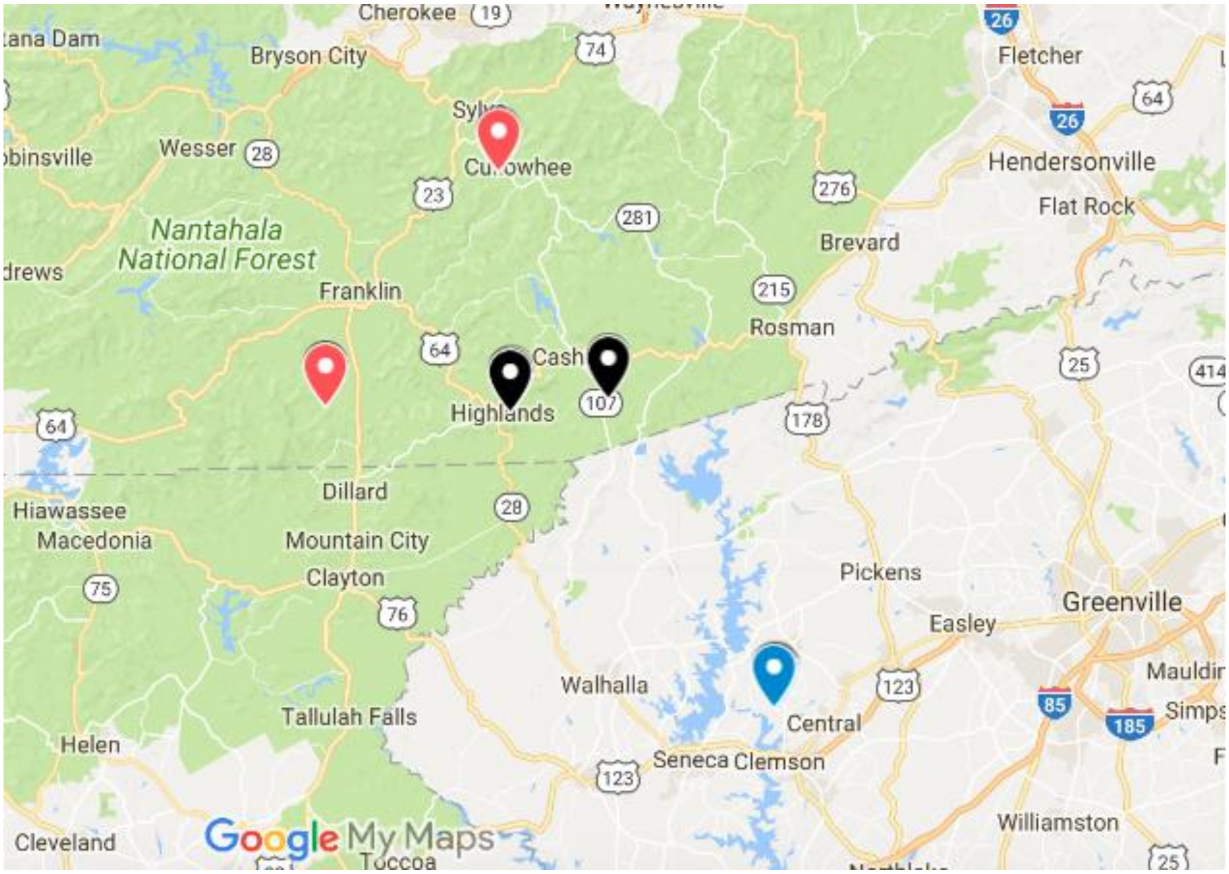

Figure 3. Map of *Aphaenogaster picea* colonies collected for the thermal tolerance and lipid analysis assays in the summer of 2017. High elevation (915+ m) colonies are black, mid elevation (457-762 m) colonies are red, and low elevation (0-305 m) colonies are blue. ©2017 Google LLC, used with permission. Google and the Google logo are registered trademarks of Google LLC.

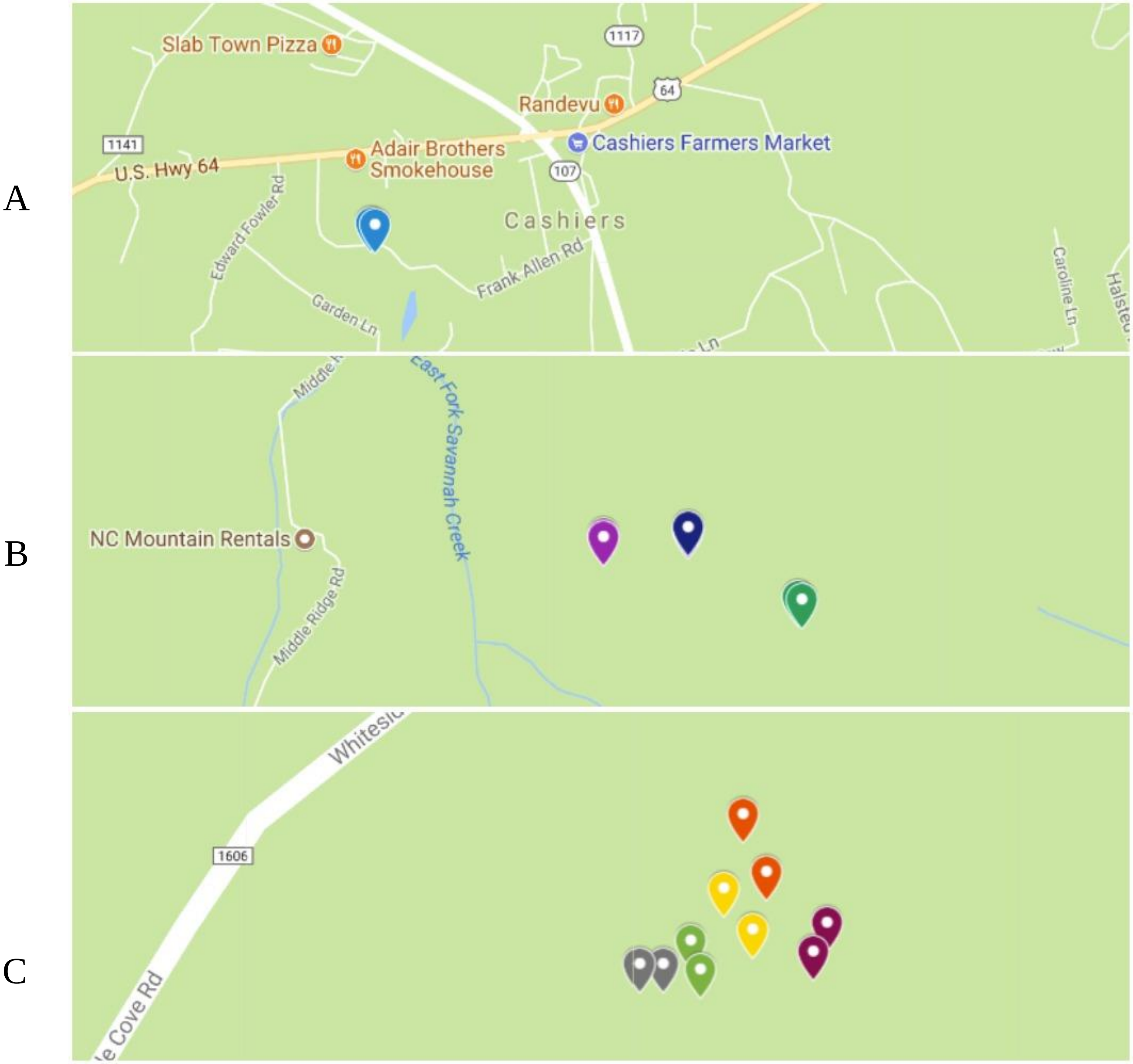

Figure 4. Map of high-elevation *Solenopsis invicta* colony pairs observed in the winter of 2017-2018 for site-selection study. Nine pairs of colonies are represented in this map at three localities: one pair is at the Cashiers Recreation Center (A; N35.110823 W-83.104809), three pairs are at East Fork Road trail (B; N35.301692 W-83.248668), and five pairs are at Chattooga Narrows trail (C; N35.038167 W-83.137133). ©2017 Google LLC, used with permission. Google and the Google logo are registered trademarks of Google LLC.

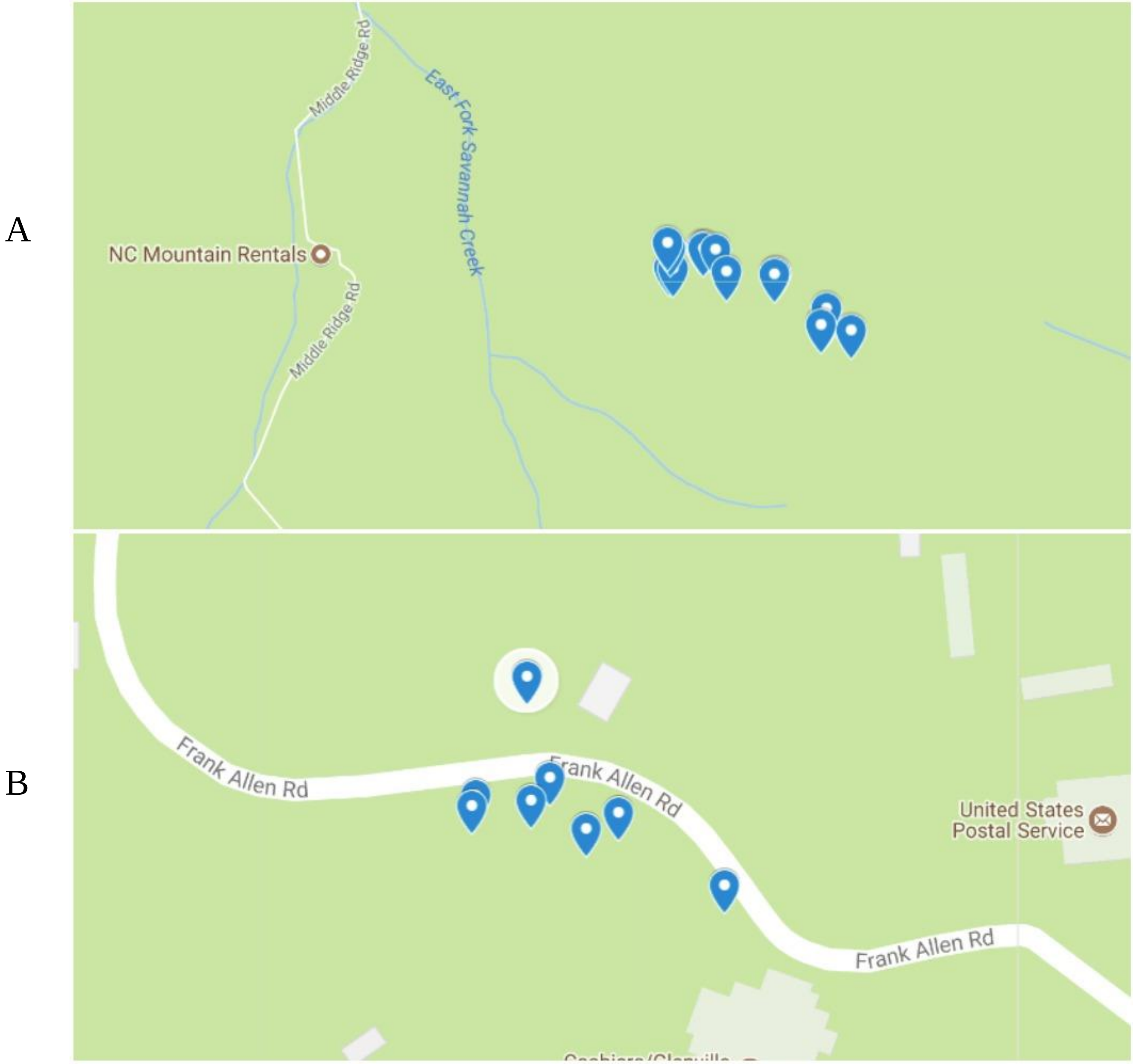

Figure 5. Map of high-elevation *Solenopsis invicta* colonies observed in the winter of 2017-2018. Thirty-four colonies are represented in this map. The first locality is at East Fork Road trail (A; N35.301692 W-83.248668) and the second locality is at the Cashiers Recreation Center (B; N35.110823 W-83.104809). ©2017 Google LLC, used with permission. Google and the Google logo are registered trademarks of Google LLC.

Comparative Thermal Tolerance Limits

Thermal tolerance testing occurred in the lab (Highlands Biological Station) no more than twenty-four hours after field collection. Lower and upper thermal tolerance tests were performed to determine physiological limits to temperature extremes for two ant species: *S. invicta* and the native *A. picea* for comparison. I collected 30 individual ants from each of 42 *S. invicta* colonies, and only the largest (major) and the smallest (minim) workers in each *S. invicta* colony were tested because they are polymorphic, whereas *A. picea* is not. Following the method of Warren et al. (2016), ants from each colony (15 majors and 15 minims) were aspirated out of their respective bag and placed individually in a 16mm labeled glass vial plugged with cotton.

Only worker ants were tested in this assay, which represented the colony genetically, as they are closely related (coefficient of relatedness, r=0.75) due to haplodiploid sex determination (Johnstone et al. 2011). Early in the trials, *S. invicta* frequently succumbed to desiccation (data for these trials were not used), so to prevent this, filter paper seven millimeters in diameter was saturated with water and placed in each test tube. Ten ants from each colony were kept in control test tubes at room temperature (around 24°C) for the duration of the test on the laboratory counter. At the conclusion of both the minimum and maximum thermal tolerance test, the control ants were checked for their righting response.

Five minims and five majors (ten total) from each *S. invicta* colony were used for the cold tolerance test. Once the ants were secure in the test tubes, they were placed in a pre-cooled (20°C) Thermo Fisher™ ARCTIC A40 refrigerated water bath designed for water temperature regulation. Ants acclimated to the water bath for ten minutes at 20°C and temperatures were subsequently decreased by 1°C min^{-1}. Exactly one minute after the water bath reached the desired temperature, the ants, in their glass tubes, were observed. If an ant was moving, it was placed back into the water bath where it remained for another round (i.e. another decrease of 1°C). If an ant appeared to be immobile, its test tube was flipped over or flicked until the ant was situated on its back. Critical thermal minimum was measured as the temperature at which the ants displayed a loss of motor control and were unable to right themselves. The temperature at which the ant could no longer right itself was recorded and represented the ant's critical thermal minimum limit. Next, ten ants from each colony were tested for heat tolerance following similar methodology. Ants were acclimated to the water bath at 30°C for ten minutes before temperatures were increased by 1.0°C min^{-1} and the remainder of the procedures for the cold tolerance test were followed. *Aphaenogaster picea* were collected in proximity to each site where *S. invicta* was collected. I collected 15 individual worker ants from each of the 21 colonies. Five workers were used in each part of the thermal tolerance test (minimum, maximum and control) since workers are not polymorphic, and the same procedures applied to thermal tolerance in *S. invicta* were applied for *A. picea*.

Critical thermal minimum limits and critical thermal maximum limits were calculated by averaging together each individual ant selected from each colony. Differences between sizes of ants was tested using a Welch's t-test. Differences among colonies along an elevational gradient in CT_{min}, CT_{max}, and range (CT_{max} - CT_{min}) were assessed with general linear models, again following the method of Warren et al. (2016). The statistical package R (R Core Team 2016) was used for all statistical analyses.

At the conclusion of this assay, all live fire ants were disposed of by freeze-killing them. The ants were stored in labeled reusable plastic bags and kept on a shelf in a -80°C freezer for at least three days. Although only worker ants were collected and cannot establish new colonies, freeze-killing the fire ants abated any risk of spreading the species to the Highlands Biological

Station campus where these assays were conducted.

Lipid Analysis

Several hundred ants from each colony (42 colonies in total) were collected with a shop vacuum, stored in plastic bags, and then stored on ice to transport back to Highlands Biological Station (HBS), as described above. At HBS, they were freeze-killed by storing them in a -80°C freezer for at least three days. Two hundred ants from each colony were then counted out haphazardly and placed in a labelled container in a drying oven at 60°C for 48 hours until completely dried. The 200 ants were weighed with an analytical balance and the dry mass recorded.

Following the protocol of Smith and Tschinkel (2009), lipid was removed using a Soxhlet extractor. Lipids were extracted by filling a capsule with exactly 200 dried ants from each colony and running ether through the capsule for twenty-four hours. All extractions were conducted in a fume hood. The same ants were placed in the drying oven for another 48 hours and weighed on an analytical scale. Fat content of ants from each colony was calculated by measuring the collective biomass of all two hundred ants before and after lipid removal. Statistical analysis followed the same methodology as that used for thermal tolerance.

Thermal Effects of Nest Site Selection

Colonies used for soil temperature monitoring were selected in the fall of 2017. Three main sites contained the colony pairs used for this portion of the study. The first site was a privately owned logging road located in Franklin, North Carolina off East Fork Road (N35.301692 W-83.248668) and is part of a conservation easement with the Highlands-Cashiers

Land Trust. The second site was at Chattooga Narrows Trail owned by the U.S. Forest Service in Highlands, North Carolina, on the Macon-Jackson County line (N35.038167 W-83.137133). The third site was at the Cashiers Recreation Center in Jackson County, North Carolina (N35.110823 W-83.104809).

Potential thermal buffers included concrete curbs, rocks, logs, live trees, and tree stumps. The size and number of potential thermal sources were recorded for each colony and location was recorded with a GPS to ensure the colonies were identified correctly in future assessments. Soil temperatures of these colonies were monitored in the winter of 2017-2018 starting 19 December 2017 and concluding 24 January 2018. The diameter of the ant mound was measured with a ruler and iButtons® were placed directly in the center of the mound approximately 15 cm deep into the soil in two layers of plastic bags attached to a flag (Maxim Integrated 2017). A trowel with a 15 cm blade was used to dig a small hole and insert the iButtons® to keep consistency with the placement and depth. Two iButtons® were used to monitor soil temperature of each colony inside the nest and recorded temperature data every 255 minutes (six times per day) for forty days.

Each colony was photographed, described in detail, recorded with GPS, and mapped in Google Maps™ (Google Maps 2018). The temperature data for both iButtons® in one colony were averaged together and then organized by week (Maxim Integrated 2017). Mean weekly minimum soil temperatures were calculated for each colony over the course of five weeks. Differences in soil temperatures between colony pairs (near a potential thermal source and away from a potential thermal source) were tested with a two-sample t-test in R (R Core Team 2016).

Finally, to gather additional information on the site selection tendencies of high elevation *S. invicta* colonies through the winter, I mapped 34 colonies and described their immediate surroundings. I noted whether the colonies were on top of or surrounded by potential thermal sources and tallied the quantity of potential thermal sources. Lastly, I recorded all colonies with GPS and mapped them in Google Maps™ to aid in future eradication of *S. invicta* from these sites (Google Maps 2018).

CHAPTER THREE: RESULTS

Comparative Thermal Tolerance Limits: $\mathbf{CT_{min}}$, $\mathbf{CT_{max}}$ and $\mathbf{CT_{range}}$

There was no difference in thermal tolerance temperatures for minim or major worker ants for CT_{max} (df = 393.74, t-value = 1.6094, *P-value* = 0.1083; Table 1) or CT_{min} (df = 415.88, t-value = 1.3727, *P-value* = 0.1706; Table 2). Therefore, I did not separate the two size classes of worker ants in further analyses.

Both species' CT_{min} decreased as elevation increased (SE = 0.0004, t-value = -8.443, *P-value* < 0.001; Fig. 7A). The heat tolerance (CT_{max}) for both species also decreased as elevation increased (SE = 0.0002, t-value = -3.627, *P-value* < 0.001; Fig. 7B). Ants at higher elevations had a higher range of thermal tolerances (CT_{max} - CT_{min}) than ants at lower elevations (SE = 0.0004, t-value = 6.092, *P-value* < 0.001; Fig. 7C), with *S. invicta* exhibiting a larger range than *A.* picea (SE = 0.352, t-value = -5.284, *P-value* < 0.001; Fig. 7C). There was not a significant interaction term between species and elevation (*P-value* = 0.472) indicating that both species responded to the effects of elevation.

Although both species' thermal tolerances changed as elevations increased, minimum thermal tolerances (CT_{min}) differed significantly between species (SE = 0.3183, t-value = -4.15, *P-value* < 0.001; Fig. 8A) with *A. picea* having a lower cold tolerance than *S. invicta*. Maximum thermal tolerances (CT_{max}) differed significantly between species with almost no overlapping data points (SE = 0.15, t-value = -21.19, *P-value* < 0.001; Fig. 8B) with *S. invicta* exhibiting higher heat tolerance than *A. picea*. Lastly, the range of thermal tolerances differed between the two ant species (SE = 0.3518, t-value = -5.284, *P-value* < 0.001) with *S. invicta* having a wider range and being more variable than *A. picea*.

Lipid Assay

Lipid content was analyzed for forty of the forty-two colonies collected over the summer of 2017. The two colonies that were not analyzed were 'L4' and 'M5', the former having been degraded in the freezer and the latter having been dropped in the drying oven.

Across the elevational gradient, there was no detectable difference in lipid content of *S. invicta* colonies (Coef. = 2.21 X 10^{-5}, SE = 1 X 10^{-5}, t-value = -1.925, *P-value* = 0.684). The regression has essentially a slope of zero with colony lipid content varying widely across all elevations (Fig. 9).

Thermal Effects of Nest Site Selection

In the winter of 2017-2018 I monitored soil temperatures of nine pairs of colonies for five weeks. I explored possible differences of weekly minimum soil temperatures for all colonies near and away from a potential thermal source. Minimum soil temperatures were overall higher away from a potential heat source (out in the open) than colonies near a potential heat source (Fig. 10; *P-value* = 0.03796; Table 3).

I also recorded observations of 34 additional high elevation *S. invicta* colonies in the winter of 2017-2018 that were not a part of the soil temperature study. Of the 34 colonies observed, eight (24%), were situated completely in the open and exposed to direct sunlight, eleven (32%), were situated among grasses or small plants, and fifteen (44%), were situated on rocks, concrete, or tree stumps (Table 4). I found that contrary to my prediction, most colonies were not sited on or near a potential thermal buffer.

Figures and Tables

Table 1. Effect of worker size of *S. invicta* on maximum temperature tolerances.

t	df	p	Conf. low	Conf. high
1.609	393.74	0.108	-0.046	0.462

Table 2. Effect of worker size of *S. invicta* on minimum temperature tolerances.

t	df	p	Conf. low	Conf. high
1.373	415.88	0.171	-0.132	0.741

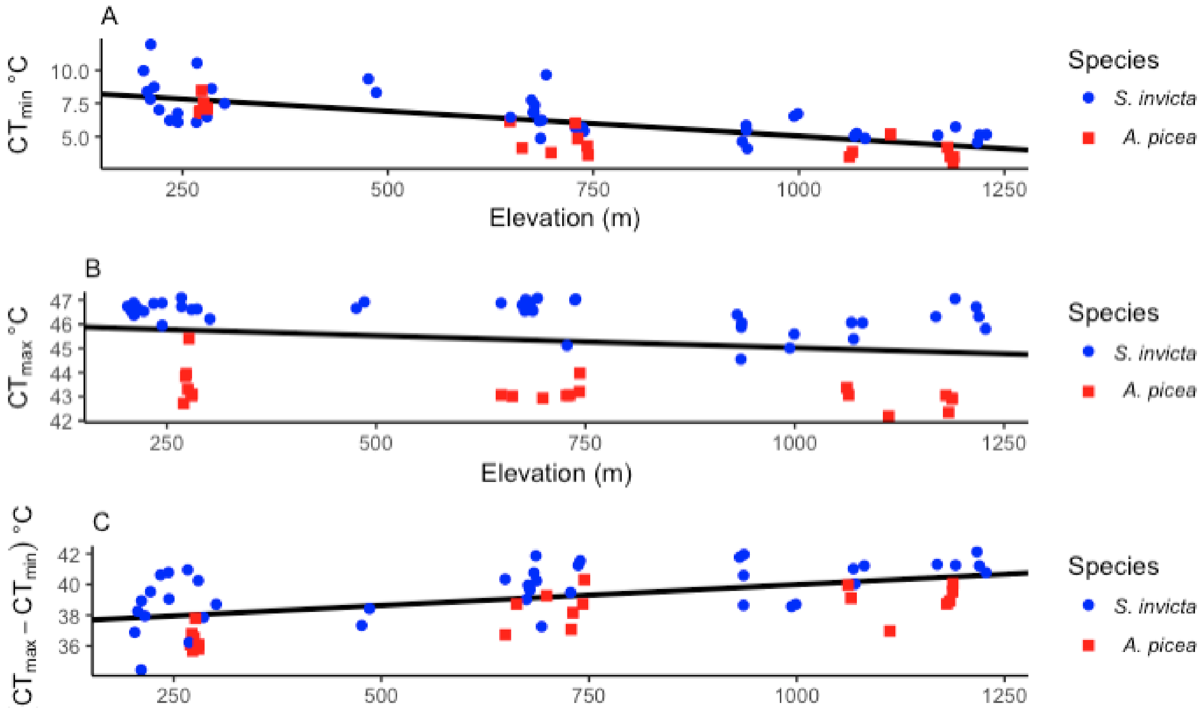

Figure 6. Regression plots showing *Aphaenogaster picea* and *Solenopsis invicta* cold tolerance (CT_{min}) with elevation in meters (7A), heat tolerance (CT_{max}) with elevation (7B), and range of tolerances ($CT_{range} = CT_{max} - CT_{min}$) with elevation (7C).

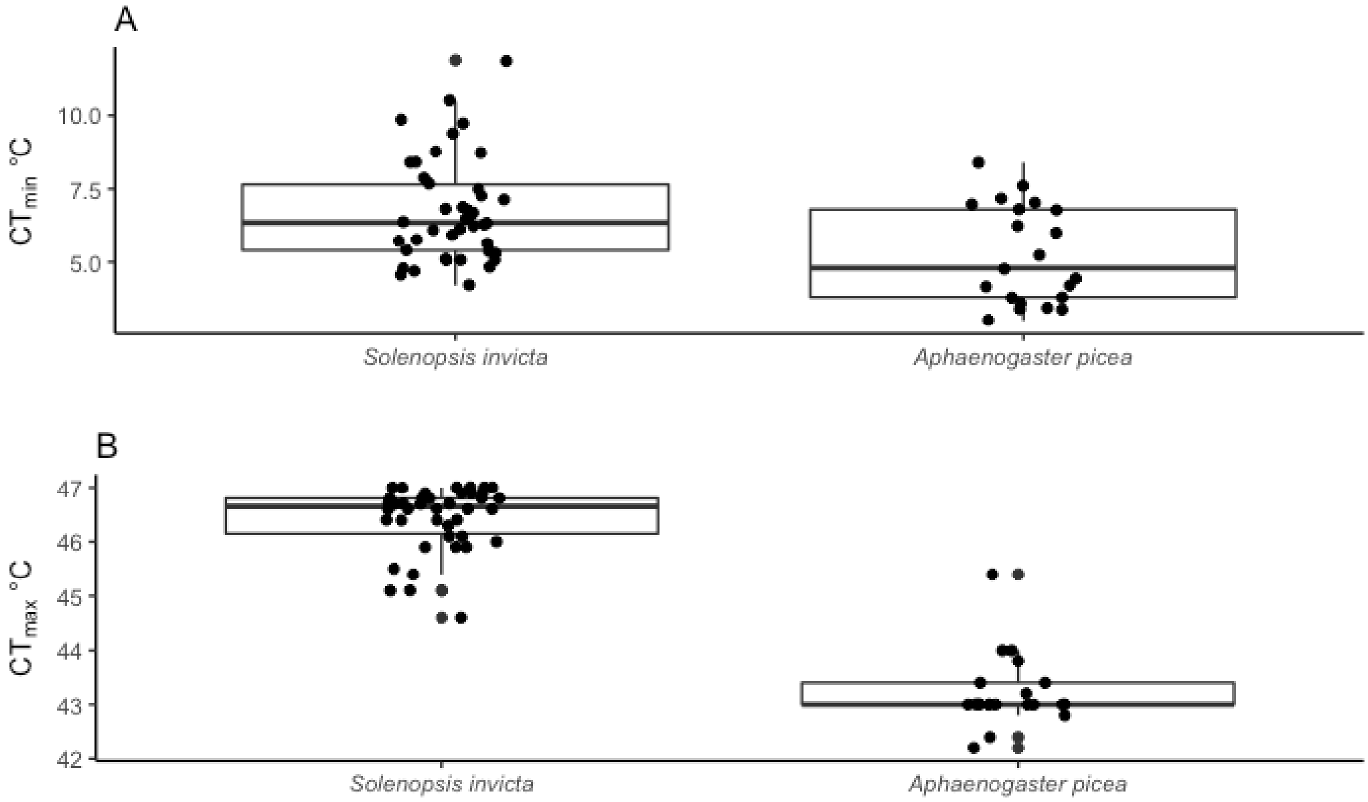

Figure 7. Boxplots displaying thermal tolerances for *Aphaenogaster picea* and *Solenopsis invicta*; cold tolerance (CT_{min}) for each species (A), and heat tolerance (CT_{max}) for each species (B). Each includes the median (solid line), and upper and lower bars indicating 25th and 75th percentiles with outliers beyond the bars.

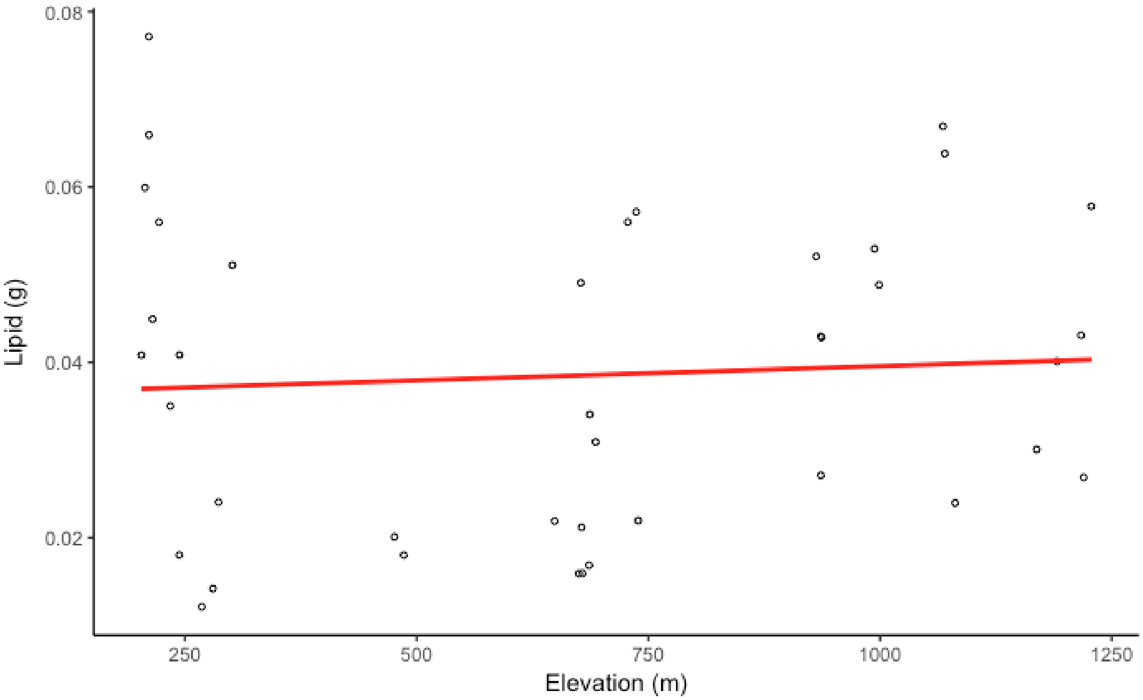

Figure 8. Regression of lipid content (g) of *S. invicta* colonies (200 worker ants) and elevation (m). Slope < 0.001; *p-value* = 0.684

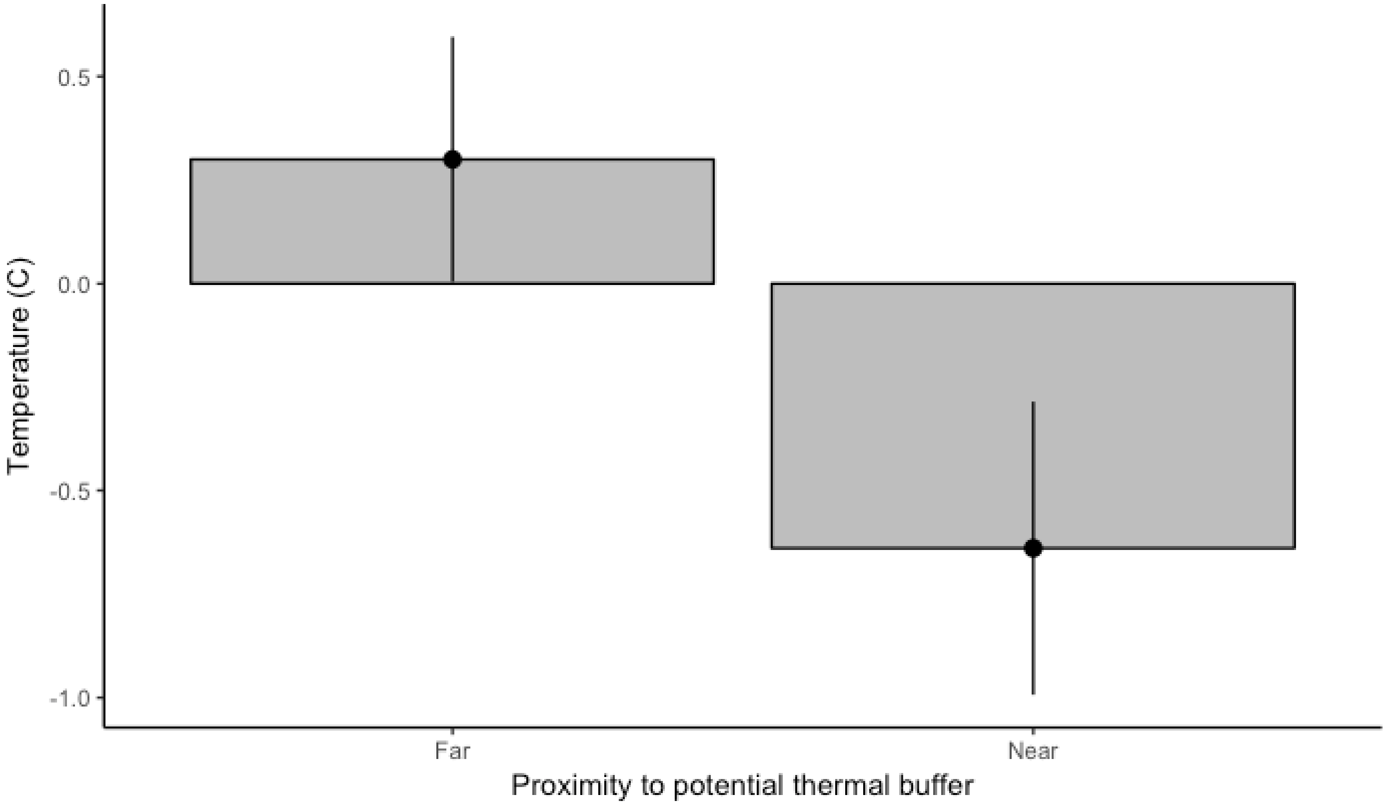

Figure 9. Boxplot of mean weekly minimum soil temperatures of all colonies near or away from a potential thermal buffer.

Table 3. Paired t-test for average minimum weekly soil temperatures of all colony pairs.

t	df	p	Mean diff.	Conf. low	Conf. high
2.482	8	0.03796	0.9389	0.06674	1.811

CHAPTER 4: DISCUSSION

My data show that *Solenopsis invicta* is able to acclimate or adapt to cold temperatures at high elevations in the Blue Ridge Escarpment. Both the cold and heat tolerance thresholds of *S. invicta* decrease as elevations increases, and the shift in thermal tolerance paralleling that of the native ant *A. picea*. *Solenopsis invicta* body fat content did not vary with elevation in this study, nor do high elevation colonies appear to ameliorate cold winter temperatures by siting their nests near a thermal buffer. This suggests that *S. invicta* may be able to achieve similar foraging activity at lowered temperatures than low-elevation colonies via acclimation to high elevation (and therefore high latitude) environments via stabilization at the molecular or cellular level.

Comparative Thermal Tolerance Limits

The marked reduction in minimum thermal tolerance thresholds exhibited by *S. invicta* at higher elevations in this study is consistent with other studies pertaining to minimum thermal tolerance shifts in ants in response to elevation. Bishop et al. (2017) found that along a 1500 m elevational gradient, minimum thermal tolerance limits (CT_{min}) decreased with decreasing temperatures at higher elevations for 28 southern African ant species. These researchers found more geographic variation in minimum thermal tolerance than maximum thermal tolerances, and that foraging behavior and phenology were influenced by CT_{min}, so that ants at high elevations with lower CT_{min} were able to forage at similar times as the low elevations ants. They also found "greater geographic variation in lower than in upper thermal tolerance limits," which supports the findings in my study (Bishop et al. 2017 pg. 110).

In my study, there was a shift in heat tolerance on an elevational gradient as well; ants at higher elevations exhibited lower heat tolerances (H1c) and *S. invicta* had an overall higher heat tolerance than did *A. picea* (H1d), both as predicted. The climate of southern Brazil and northern Argentina, specifically the floodplains surrounding the Paraguay River, is classified as subtropical with more stable yearly temperatures than the southern Appalachian mountains, so it was expected that *S. invicta* would be able to tolerate higher temperatures. The shift in heat tolerance was, accordingly, not as dramatic as that of the cold tolerance shift, but this is consistent with Brett's Rule which states that there is "less geographic variation in upper than in lower thermal tolerances" (Brett 1956; Bishop et al. 2017 pg. 106).

The slight increase in thermal tolerance ranges ($CT_{max} – CT_{min}$) observed in *S. invicta* can be attributed to the greater tolerance to cold temperatures (Addo-Bediako et al. 2000), as these temperatures influence the range more strongly than changes at the upper end of the range (heat tolerance). This is consistent with Janzen's Rule (Janzen 1967) which states that "greater variation in environmental temperatures is matched by a greater range in organismal thermal tolerances," and is consistent with other thermal tolerance studies on insects (Gaston and Chown 1999; Bishop et al. 2017 pg. 106).

Solenopsis invicta exhibited greater variance in thermal tolerance than native *A. picea*. This can perhaps be explained by the contrasting habitats that these two species prefer; *A. picea* is a woodland ant that favors shaded environments, whereas *S. invicta* is a thermophilic species that thrives in highly disturbed environments with full-sun. These open disturbed habitats experience a wider range of temperature fluctuations throughout the day, making thermoregulation under varying temperatures, especially heat, a necessity for survival.

If *S. invicta*'s acclimation to cooler, high elevation environments is an evolutionary response, it occurred on a rapid timescale, as this species was not known to be present in the southern Appalachians prior to about the mid-2000s (although there are anecdotal reports of *S. invicta* in the southern Appalachians in the late 1990s). Heat shock proteins (Hsps) may play a role in the rapid development of cold tolerance in high-elevation *S. invicta* populations. These proteins, specifically Hsp70, are present in all organisms assessed to date and contribute to the cold tolerance of insects by binding to other proteins to lessen detrimental effects of misfolding during unfavorable conditions such as extremely cold temperatures during winter (Rinehart et al. 2007; Kostal and Tollarova-Borovanska 2009). An up-regulation response of these proteins can be induced by several environmental factors, such as exposure to extreme but non-lethal low temperatures, and is usually triggered by shorter and colder days so insects are able to go into diapause during the winter. Although researchers have demonstrated up-regulation of Hsps in response to cold temperatures in several insect species, mainly flies (Diptera), true bugs (Heteroptera), and beetles (Coleoptera) (Kostal and Tollarova-Borovanska 2009), this physiological response has not been tested in Hymenopteran insects such as *Solenopsis invicta*.

Rinehart et al. (2007) found direct evidence that Hsps play a positive role in the cold tolerance of insects. These researchers observed the effects of injecting two Hsp suppressants (*hsp23* and *hsp70*) into larvae of the fly *Sarcophaga crassipalpis* before diapause. Their findings are consistent with the predictions of the roles of Hsps in cold tolerance: larvae injected with these double-stranded RNAs experienced a reduced cold tolerance and Hsps mRNA levels, but the insects' ability to enter into diapause was unaffected (Rinehart et al. 2007). It is important to note that up-regulation of Hsps could reflect a plastic thermoregulatory response which does not necessarily reflect a novel genetic adaptation.

Thermal Effects of Nest Site Selection

High-elevation *S. invicta* colonies in this study were not found to be disproportionally sited near potential thermal buffers such as rocks or logs. The hypothesis that such objects may conduct thermal energy below-ground and therefore provide a thermal buffer during the winter was not borne out: in most cases minimum soil temperatures in nests in proximity to a potential thermal source were lower than soil temperatures of nests out in the open. This is not the only behavioral adaptation for thermoregulation that *S. invicta* might manifest, however. Fire ant queens choose nest sites based on “openness” as well, and shade avoidance may be a more important factor in microhabitat selection than availability of a prospective heat source. High elevation areas in the southern Appalachian mountains are predominantly rural, with *S. invicta* colonies establishing on recently disturbed logging sites and trails rather than large parking lots and roadsides (personal observation). Factors such as sun exposure, shade, and canopy cover likely played a more important role in nest site selection for *S. invicta* queens than proximity of potential thermal buffers.

It is possible that the mound itself is such a fine-tuned adaptation for microclimatic regulation that *S. invicta* does not need to compensate in any other way (behaviorally) in order to survive colder winters. In this niche construction scenario, fire ant mounds serve as a means to regulate colony temperature; workers can bring brood up or down depending on the temperature fluctuations because brood are not fixed to single cells like some other social insect brood (Wilson 1971; Tschinkel 2006). Worker ants build chambers at varying depths in their mound, allowing a wide selection of temperatures throughout the day that are ideal for incubating brood, and researchers have reported that temperatures are substantively more stable than the ambient temperatures outside of the mound once a depth of about 20-30 cm is reached (Wilson 1971).

Implications for Conservation

Global climate change may facilitate the colonization of ants at higher elevations as temperatures warm, as has been shown for several other groups of animals (Sunday et al. 2012). Warming temperatures allow some species to expand their ranges at different rates and it is well known that competitive species often have a strong dispersal ability (Urban et al. 2012; Warren et al. 2016). Some native North American ant species in the *A. picea* complex have exhibited climate-induced range expansion, where a competitive low elevation species is able to replace high elevation species by means of habitat selection or competition (Warren et al. 2016). Warren and Chick (2013) investigated the relationship between thermal tolerance limits and distribution shifts in two woodland ants, *Aphaenogaster picea* and *A. rudis*, and tested CT_{max} and CT_{min} for both species in order to describe patterns such as distribution shifts as a result of climate change. The lower elevation *A. rudis* consistently exhibited temperature tolerance limits 2°C higher than that of the higher elevation species *A. picea* in both minimum and maximum thermal tolerance testing. This phenomenon has serious implications in the context of climate change, because as temperatures rise, other ant species like *S. invicta* may be able to shift their distribution into higher elevation regions where they were once unable to persist.

This potential for further range expansion via climate warming could be amplified by *S. invicta*'s ability to rapidly acclimate to high elevation habitats. The absolute eradication of *S. invicta* from the United States is most likely impossible, and several failed attempts of eradication using pesticides such as Heptachlor and Mirex have made many people cautious to undertake another large-scale extermination (Tschinkel 2006). Instead, I suggest a thorough survey of *S. invicta*'s northern range. If the exact latitudinal range limits are established, then conservation groups could have a better chance of eliminating colonies on a case-by-case basis.

For example, *S. invicta* is established in parts of Jackson and Macon county, NC, but have not been observed yet in Haywood or Buncombe county, NC. There is an abundance of land in Haywood and Buncombe count that suit the climatic and elevational requirements for *S. invicta,* so it is possible to prevent the spread of this invasive species to these areas if adequate measures are taken.

Future Studies

1. Common garden experimental approach

I found that *S. invicta* appears to be acclimating to high elevation regions of the Blue Ridge Escarpment both physiologically and, perhaps, behaviorally, but future studies on whether thermal tolerance in this species reflects an evolutionary adaptation rather than acclimatization or phenotypic plasticity would be useful. Diamond et al. (2017a) conducted a common garden experiment to answer a similar question using the ant *Temnothorax curvispinosus* on an urban-to-rural temperature gradient. These authors aimed to disentangle the contributions of evolutionary change versus plasticity in regard to phenotypic shifts across an urban temperature cline, which had not previously been a point of interest in studies researching thermal tolerance (see Angilletta et al. 2007; Diamond et al. 2015). Differences in critical thermal maxima of insects is thought to be a plastic response to slight changes in temperature (Diamond et al. 2015), but minimum thermal tolerances are primarily responsible for broadening thermal tolerance ranges (Addo-Bediako et al. 2000; Diamond et al. 2015, 2017a).

Urban ants experience warmer temperatures than rural ants, averaging 2°C higher on a yearly average (Imhoff et al. 2010) due to the urban heat island effect, where rural ants experience lower temperatures the farther they are from the urban area. The acorn ant, *T.*

curvispinosus, exhibited both a plastic and an evolutionary response to temperature changes on an urban to rural gradient, suggesting that both of these mechanisms can contribute to shifts in thermal tolerance in this ant species (Diamond et al. 2017a). Diamond's data show that urban ants continued to exhibit a greater heat tolerance regardless of the temperatures they were reared at (Diamond et al. 2017a). However, critical thermal maximum (CT_{max}) was higher in urban ants reared in warm temperatures versus cold temperature rearing, suggesting that phenotypic plasticity was partially responsible in thermal tolerance limits. This study (Diamond et al. 2017a) has implications for tolerance limits on an elevational gradient as well. Just as temperatures warm on a rural to urban gradient, temperatures increase with decreasing elevation. Implementing similar methods of this common garden experiment for *S. invicta* would be helpful in generating future predictive range maps of the species, and would give scientists a better understanding of their potential range.

2. *S. invicta – S. richteri* hybridization

The red imported fire ant, *Solenopsis invicta*, is not the only invasive fire ant in the United States. Shortly before *S. invicta* was inadvertently brought to the port of Mobile, Alabama, a closely related species established itself in the southern United States: the black imported fire ant, *Solenopsis richteri* (Tschinkel 2006). *Solenopsis richteri* is the same size as *S. invicta* and exhibits some reddish coloring in certain populations, making it almost impossible to differentiate the two species in the field. In fact, it was not until 1972 that a scientist described them as separate species rather than a color variant (Buren 1972), partially owing to the fact that these two species can hybridize easily. *Solenopsis richteri* has since spread throughout the southern U.S., albeit not as aggressively or as prolifically as *S. invicta* has (Tschinkel 2006; Chen et al. 2014).

Solenopsis richteri has not transformed landscapes and ecosystems in the same manner as *S. invicta*, which is attributable to its lowered competitiveness and susceptibility to heat and desiccation stress (Chen et al. 2014). However, this species is able to tolerate higher latitudes and elevations due to its greater cold tolerance (James et al. 2002; Tschinkel 2006). These introduced species can hybridize (Tschinkel 2006), raising the possibility of greater cold tolerance in *S. richteri* - *S. invicta* hybrids. Researchers studying hybridization between these two species have discerned at least two distinct hybrid zones in the United States. One hybrid zone occurs in Mississippi and Alabama, and extends into western Georgia (Shoemaker et al. 1996; Tschinkel 2006). The second known hybrid zone occurs in eastern Tennessee and western North Carolina. The exact location of the hybrid zone is still largely unknown in this region, so entomologists must rely on genetic markers to distinguish the two phenotypically similar species (Shoemaker 2016). More research is needed to delineate the hybrid zone and map the extent of interbreeding. It would be interesting to note whether there is a difference in critical thermal minimum temperatures of *S. invicta* compared to *S. richteri* or *invicta* x *richteri* hybrids. Future studies should include genetically identifying high-elevation populations of fire ants in western North Carolina and eastern Tennessee using the methods established by Shoemaker et al. (1996) and Shoemaker (2016).

3. *S. invicta* Heat-shock proteins

Lastly, a study on heat shock proteins of *S. invicta* would contribute to the understanding of the physiological mechanism of different cold tolerance levels of these insects on an elevational gradient. An investigation into the importance of these proteins to the cold tolerance of fire ants at high elevations would help determine whether this physiological response is an evolutionary or plastic response. It would be interesting to note which Hsps, if any, are up-regulated in *S. invicta* with the onset of winter at high elevations.

CHAPTER 5: CONCLUSION

Solenopsis invicta is able to tolerate cold temperatures at high elevations in the Blue Ridge Escarpment. Populations of *S. invicta* at 915 m elevation and above differed significantly in their cold tolerance threshold relative to conspecifics at low elevations. This suggests that these ants are experiencing a physiological change on an elevational gradient, although it is unknown whether this is an evolutionary response or a result of phenotypic plasticity.

It is likely that *S. invicta* will persist in the Blue Ridge Escarpment region and will continue their migration to increasing latitudes and altitudes. Climate change could facilitate the spread of this species to higher latitudes and altitudes where there is abundant open, highly disturbed habitat. Minimum temperatures are rising at twice the rate of maximum temperatures as the climate warms (Walther et al. 2002), and this could have serious implications for *S. invicta*, as they seem to be partially limited by minimum temperatures. Furthermore, this species can acclimate to colder temperatures, so it is likely that they have not reached their northern range limits.

Solenopsis invicta is currently unable to persist in forested areas of the southern Appalachian mountains that experience substantial shade and canopy cover. However, the destruction of some tree species due to infestations such as the invasive hemlock wooly adelgid (HWA), *Adelges tsugae* result in the opening of the canopy which lets in light (Spaulding and Rieske 2010; Ford et al. 2011). *Solenopsis invicta* queens can disperse several hundred meters after a mating flight (Tschinkel 2006), so if there is a viable population within a suitable distance to a stand of infested hemlock, there is an opportunity to invade this new region after the tree dies and loses its leaves or falls to the forest floor, opening up the canopy for more light.

Solenopsis invicta thrives in open disturbed habitat

The rapid high-elevation acclimation and/or adaption of *S. invicta* could allow them to spread to new counties, and high-elevation grassy balds or rocky summits are good candidates for future invasions. Open areas that are frequented by humans have a huge potential for *S. invicta* invasion if they are within the current elevational range limits of this species. For example, a single colony of this species was observed on the rocky summit of Satulah Mountain, indicating that humans probably facilitated the spread of a gravid queen to this suitable habitat. Perhaps if conservationists and land owners are aware of the potential for *S. invicta* infestation in these habitats that were once thought to be uninhabitable, then their spread can be prevented.

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APPENDIX

A1. Site descriptions of 34 high-elevation (915 meters and above) colonies.

ID	Latitude	Longitude	Elevation (m)	Site Name	Description	Date
1	35.111533	-83.105117	1061	Cashiers Recreation	The colony is situated beneath bars of a gate but not actually placed on the gate.	18-Dec-18
2	35.110917	-83.1044	1060	Cashiers Recreation	On the curb. About a third of the colony is situated on the cement.	18-Dec-18
3	35.111133	-83.104783	1060	Cashiers Recreation	At the base of a tree on mulch.	18-Dec-18
4	35.111083	-83.1049	1061	Cashiers Recreation	On a cement curb.	18-Dec-18
5	35.111233	-83.105033	1057	Cashiers Recreation	At the base of a tree on mulch. The mound touches the tree.	18-Dec-18
6	35.111167	-83.1051	1059	Cashiers Recreation	At the base of a tree on mulch. The mound touches the tree.	18-Dec-18
7	35.111183	-84.1053	1060	Cashiers Recreation	On a cement curb and overflowing to the concrete. A few inches away from a gutter.	18-Dec-18
8	35.11115	-84.105317	1061	Cashiers Recreation	On a cement curb and overflowing to the concrete. A few inches away from a stop sign.	18-Dec-18
9	35.28265	-84.231633	1082	East Fork	Against a cut stump and a rock.	19-Dec-18
10	35.282433	-84.231433	1087	East Fork	On open ground.	19-Dec-18
11	35.28235	-84.231317	1088	East Fork	Mostly open, on small rock 21 cm and ~10 rocks all less than 10 cm.	19-Dec-18
12	35.2813	-84.23075	1103	East Fork	On open ground with direct sunlight.	19-Dec-18
13	35.281233	-84.230833	1101	East Fork	On open ground except grass that is starting to grow tall and shadow it.	19-Dec-18
14	35.281083	-84.23065	1105	East Fork	South facing. Built on top of 17 cm rock and near some long grass.	19-Dec-18
15	35.281217	-84.229917	1121	East Fork	Exposed to full sun; 2 rocks 20 cm and 21 cm. Also supported by large tufts of grass.	19-Dec-18
16	35.2812	-84.229767	1123	East Fork	Supported by long grass.	19-Dec-18
17	35.281217	-84.2297	1123	East Fork	On open ground.	19-Dec-18
18	35.281233	-84.229633	1123	East Fork	In the middle of the trail and completely built up on large tufts of grass.	19-Dec-18
19	35.281167	-83.2294	1126	East Fork	On open ground.	19-Dec-18
20	35.28115	-83.2293	1127	East Fork	Built up on large rock 0.6 meter long.	19-Dec-18
21	35.281617	-83.22935	1153	East Fork	Seven small rocks <10 cm surround the colony.	19-Dec-18
22	35.281733	-83.229433	1153	East Fork	Built up on two large rocks each about 0.5 meters.	19-Dec-18
23	35.281633	-83.22845	1175	East Fork	On open ground.	19-Dec-18
24	35.28165	-83.228383	1175	East Fork	Supported by long grass. Just below a rock 23 m at longest length.	19-Dec-18
25	35.2816	-83.228433	1175	East Fork	Surrounded by small plants that are starting to overtake the colony. About 25% shade.	19-Dec-18
26	35.281567	-83.228083	1177	East Fork	On open ground.	19-Dec-18
27	35.281067	-83.227767	1180	East Fork	On open ground.	19-Dec-18
28	35.28105	-83.22635	1190	East Fork	On open ground.	19-Dec-18
29	35.281017	-83.226383	1190	East Fork	Surrounded by thorny shrubs in about 15% shade.	19-Dec-18
30	35.2799	-83.225	1208	East Fork	Supported by long grass.	19-Dec-18
31	35.2799	-83.225033	1208	East Fork	Supported by long grass.	19-Dec-18
32	35.279717	-83.224217	1207	East Fork	Supported by long grass, surrounded by a dozen small rocks <10 cm.	19-Dec-18
33	35.280217	-83.224917	1217	East Fork	In the middle of the trail and completely supported by large tufts of grass.	19-Dec-18
					Extremely active; and colony had eggs present when nest was disturbed.	
34	35.27985	-83.22505	1205	East Fork	Supported by long grass. Tall and active.	19-Dec-18