EXPLORING THE POTENTIAL FOR ADAPTIVE DIFFERENTIATION IN THE GRANITE OUTCROP PLANT, *HELIANTHUS PORTERI*

by

SCOTT DAVID GEVAERT

(Under the Direction of Lisa A. Donovan)

ABSTRACT

Natural selection is a major evolutionary factor driving population differentiation and local adaptation. When plants are exposed to different levels of abiotic factors, such as water limitation, their ability to survive and reproduce may be affected. Continuous exposure to such factors can lead to adaptations to resist the stressor and potentially lead to adaptive differentiation. Identification of selective agents, such as drought, could help to determine if populations are adaptively differentiated and on what traits selection is acting. Granite outcrops of the southeastern US are rare, geographically isolated habitats harboring many endemic plant species. Additionally, they are subjected to water limitation due to their patchy shallow soils, high evaporation rates and severe run-off from the rock surface. *Helianthus porteri* (Compositae, A. Gray, Pruski) is an endemic annual sunflower found on these granite outcrops. It germinates in late March and begins flowering in late August, thus persisting through the harshest environmental conditions. Here we investigated the potential for adaptive differentiation to drought among populations of *H. porteri*. We found drought to be an important selective agent, leading to differential plant performance among three natural populations of *H. porteri* and significantly affecting plant survival. While we found that these same populations are genetically differentiated when grown in a common environment, the trait patterns observed were not consistent with local adaptation for drought. Investigation of population genetics of *H. porteri* revealed that there is little genetic structure among populations (F_{ST}), indicating they are genetically similar across the species range. Additionally, we found high levels of genetic diversity (H_e) within populations. Taken together, our results indicate that while drought is an important selective agent among populations of *H. porteri*, the high levels of genetic diversity indicate that gene flow may be counteracting the effects of selection. Thus there does not appear to be adaptive differentiation to drought among populations of *H. porteri*. Future studies which directly assess local adaptation, such as reciprocal transplants, are needed to assess if populations are local adapted to factors other than drought, which may be influencing population differentiation.

INDEX WORDS: population variation, drought stress, granite outcrop, sunflower, *Helianthus porteri*

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BS, Saint Louis University, 2006

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2011

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August 2011

ACKNOWLEDGEMENTS

I developed a love of ecology and evolutionary biology as an undergraduate at Saint Louis University. Two mentors in particular, Peter Bernhardt and Janet Barber, helped me to explore the principles of ecological and evolutionary research. Both encouraged me to develop that passion in graduate school, and for that I am grateful.

I am also incredibly grateful for the guidance, support and feedback I have received from my doctoral advisor, Lisa Donovan. Lisa has been incredibly helpful in helping me develop as a scientist, writer, and mentor. During my most difficult years, she provided the support necessary for me to work through personal and professional hardships. Her ability to assist me in seeing the big picture of my research, as well as to learn how best to manage my many interests, enabled me to pursue a career in higher education of which I am proud. I am very grateful for her support.

I also want to thank my advisory committee, John Burke, Shu-Mei Chang, Jim Hamrick and Becky Sharitz. They have provided critical feedback for research designs and implementation, and manuscripts. I especially want to thank John Burke for sharing with me his lab facilities and training which helped me complete my research. The combined experience of these committee members provided me with the tools, training and knowledge to continuously improve my research and dissertation.

There are numerous graduate students who also significantly helped me both professionally and personally. Five of these students especially helped me through challenges with experimental design, data analysis, and fieldwork: Cara Gormally, Beau Brouillette,

Rebecca Shirk, Michael McKain and Ethan Milton. Jennifer Mandel provided additional help with the genetic analyses and helped me to see how these results complemented the ecological research I performed. Several other students offered considerable feedback on manuscripts for this dissertation including Cecile Deen, Luanna Prevost, Alan Bowsher, and Elise Wygant. Additionally, critical to my field and greenhouse experiments, Tom Patrick and Nikki Castleberry of the Georgia Department of Natural Resources, and Malcolm Hodges of The Nature Conservancy, provided me with the necessary permits to perform my research.

Also, as a graduate student, I explored and developed a love of teaching. Many people helped me develop my passion, especially Brigitte Bruns in the Plant Biology Department. I had many conversations and discussions about teaching strategies and challenges with her, and she further helped me realize the greater significance of my teaching. I also wish to thank a number of people across the UGA campus that additionally supported and encouraged my development as a teacher including Peggy Brickman, Paul Quick, Denise Domizi, Sherry Clouser, and the other graduate student participants of the Future Faculty Program.

Finally, I thank my extended family and friends for the incredible support they provided me throughout my graduate career. I especially thank my parents: Charles and Linda Gevaert, who as teachers, provided me with the support of their many years of experience to help me through some of my greatest challenges as a student and teacher. Additionally, my sister, Kristin Dryden, and several friends provided me with continuous encouragement. I especially want to thank: Chris Allen, Mark Allen, Kathleen Carmichael, Chad & Elizabeth Cheely, Andrew Johnson, Jennifer Mandel, Breanna Mathis, Michael McKain, Ethan Milton, and Phillip Nowicki.

TABLE OF CONTENTS

CHAPTER

LIST OF TABLES

Page

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Selection is a major factor driving population differentiation and local adaptation, which can have important implications for conservation and management of plant species. Additional factors, such as genetic drift, can drive population differentiation as well. Adaptive differentiation can occur when a plant population is exposed to a variety of biotic or abiotic factors affecting its ability to survive and reproduce. Continuous exposure to limited resources, such as water or nutrients, can lead to adaptations to resist those factors that limit growth, survival and reproduction (Reich, Wright et al. 2003). Identification of selective agents in natural populations, such as drought or nutrient stress, can help to elucidate the factors driving adaptive differentiation among populations and the traits on which selection acts (Heschel and Riginos 2005). Studies that identify differences in native field populations are often complemented by common garden studies that compare plants grown from seed from the natural populations in a common environment, which minimizes environmental variation and thus allows the detection of genetically based population differences for traits (Mooney and Billings 1961; Donovan, Maherali et al. 2011). These common garden studies provide a starting point for investigating traits which may be under selection and can provide information on variation within and among populations (Ackerly, Dudley et al. 2000; Arntz and Delph 2001). A growing number of studies are focusing on the potential for adaptation to low resource environments, such as water and nutrient limitations (Dudley 1996; Sandquist and Ehleringer 1997; Arntz and Delph 2001; Sherrard and Maherali 2006; Easlon and Richards 2009; Brouillette and Donovan 2011;

Donovan, Maherali et al. 2011) as part of a broader effort to determine the prevalence of adaptive differentiation leading to local adaptation at both landscape and microhabitat scales (Antonovics and Bradshaw 1970; Hereford 2009).

Drought, as limited soil water availability, is a major limiting factor for plants. Plants are able to resist the effects of drought by three basic strategies: escape, avoidance or tolerance (Ludlow 1989; Kramer and Boyer 1995; McKay, Richards et al. 2003; Verslues, Agarwal et al. 2006). Plants are able to escape drought temporally, by adjusting phenology to minimize their exposure to soil water limitations. Plants can avoid drought and delay internal water deficit by maximizing water uptake, minimizing water loss, and using available water more efficiently. There are several traits generally thought to be associated with drought avoidance: inherently slower growth, greater root biomass and rooting depth, higher root:total biomass ratio, decreased stomatal conductance and higher water-use efficiency (WUE, ratio of carbon gain to water loss). Leaf level WUE can be assessed at two time scales: instantaneous leaf WUE as measured from the rate of photosynthesis to transpiration, and leaf WUE integrated over the lifetime of the leaf as estimated from leaf carbon isotope ratio ($\delta^{13}C$) (Farquhar, Ehleringer et al. 1989; Donovan and Ehleringer 1994). Water-use efficiency can also be assessed at the whole plant scale, as biomass gain per transpirational water loss, and system scale as biomass gained per evapotranspiration (Lambers, Chapin III et al. 1998). When low plant water status can’t be avoided, some plants are able to additionally tolerate low plant water status with the physiological process of osmotic adjustment, which is the maintenance of cell turgor by accumulating osmotically active compounds, enabling the plant to continue to acquire water from the soil and avoid losing turgor until a much more negative water potential than tolerated by non-acclimated plants (Rodriguez, Chaves et al. 1993).

When soil water is limiting, nutrients often become limiting as well because the delivery of nutrients to the plant also declines. This occurs because nutrients arrive at the root surface as a result of mass flow or diffusion (Lambers, Chapin III et al. 1998). When water is limiting, diffusion rates are reduced, lengthening the path from bulk flow and resulting in a reduction of nutrient uptake (Chapin III 1991; Wright, Reich et al. 2001). Thus greater drought resistance may also be associated with the ability to increase uptake and use acquired nutrients more efficiently. Plants can use nutrients more efficiently in a variety of ways including increasing nutrient-use efficiency, leaf resorption proficiency (ability to remove nutrients from a leaf before senescence), and maintaining a lower leaf N by maintaining a higher photosynthetic nitrogen-use efficiency (PNUE, the ratio of photosynthetic rate to leaf organic nitrogen content) (FIeld and Mooney 1986; Lambers and Poorter 1992; Aerts and Chapin 2000; Wright, Reich et al. 2001). The ability to use nutrients more efficiently can reduce the negative effects of nutrient limitation on fitness, which includes delayed flower initiation, and reduced pollen production and pollen viability (Marschner 2002).

Granite outcrops of the southeastern United States are rare and geographically isolated habitats which harbor many endemic plant species and are known to have resource limitations that affect plant growth, productivity, physiology and survival (Burbanck and Platt 1964; McCormick and Platt 1964; Shure and Ragsdale 1977; Baskin and Baskin 1988). They are found in the piedmont region, extending from eastern Alabama to North Carolina, with the majority occurring in Georgia. Outcrops occur when bedrock is exposed at the surface, usually as small islands in the forest expanse, though some can be larger exposures, occurring as mountains above the surrounding landscape (e.g., monadnocks). Plant communities are established on the outcrops after lichens cover the bare rock, eroding the rock and capturing soil particles, allowing other organisms to establish; first as mosses, then annuals and perennials later (McVaugh 1943; Burbanck and Platt 1964; McCormick and Platt 1964). These successional stages consist of four major plant communities, based on soil depth and characteristic vegetation present: *Diamorpha* community (2-6 cm), lichen-annual herb community (7-15 cm), annual-perennial herb community (16-39 cm), and perennial-shrub community (40-50 cm) (Burbanck and Platt 1964). Soil water availability is often limiting in these habitats because of shallow soils, high evaporation rates and run-off from the rock surface (Cumming 1969; Sharitz and McCormick 1973; McCormick, Lugo et al. 1974; Burbanck and Phillips 1983). Nutrient concentrations have been found to be potentially limiting since they are relatively low and vary among granite outcrops (Mellinger 1972; Shure and Ragsdale 1977). Resource limitation in these environments could be driving selection in native populations, which combined with limited gene flow expected due to the geographically isolated nature of populations, may result in adaptive differentiation or local adaptation for drought (McCormick and Platt 1964; Chapman and Jones 1975).

Helianthus porteri (A. Gray) Pruski (Pruski 1998) is an endemic annual sunflower on granite outcrops of the southeastern United States (Shelton 1963). It is found primarily in the lichen-annual herb and annual-perennial communities of granite outcrops, frequently occupying soils of 7-15 cm in depth (Burbanck and Platt 1964). It is found from eastern Alabama, through the piedmont to North Carolina. It grows in full sun and at forest edges, germinating in late March and flowering from late August to the first frost. It is one of the few granite outcrop species which does not complete reproduction prior to early summer, thus persisting through the driest and most environmentally limited seasons. It has been found to be endemic to granite outcrops mostly due to its inability to tolerate competition, but is also capable of exploiting resources when available for quick growth, and surviving under resource-limited conditions for short periods of time (Mellinger 1972; McCormick, Lugo et al. 1974; Baskin and Baskin 1988). Water potentials reported in the literature for this species are well within the range of mesic plants, suggesting it has the ability to avoid drought. Given the seasonal limitation which *H. porteri* is subjected to, it is an excellent species to study adaptive differentiation for the response of drought on these geographically isolated habitats.

I ask the following questions in order to understand the potential for adaptive differentiation to drought in *Helianthus porteri* on granite outcrops:

1. Is there differential performance among populations of *H. porteri* for drought resistance and is it indicative of avoidance or tolerance?
2. Are populations genetically differentiated for ecophysiological and growth traits related to drought resistance?
3. How much genetic diversity does *Helianthus porteri* harbor and how is it partitioned among populations of this endemic outcrop species?

To address these questions, I combined techniques from ecology, ecophysiology and population genetics to assess the effects of drought on population differentiation for *Helianthus porteri* spatially and temporally. Assessing differential performance over a larger time period in natural populations allows me to characterize the role of the environment in *H. porteri* growth, survival, and reproduction. By controlling the environment in a common garden study, I was able to separate the effects of environment and genetics and determine if there is an underlying genetic basis to population differences observed in the field. I also determined the potential for populations to be locally adapted if they are responding differently to controlled levels of water availability. Finally, I used population genetic techniques to determine how genetic diversity is partitioned among populations.

Taken as a whole, these studies were used to determine if drought is a selective agent driving population differentiation among populations of *Helianthus porteri*. The geographically isolated granite outcrops, with their known resource limitations, serve as an excellent study system to assess population divergence and the potential for local adaptation to resource limitation. Understanding the genetic structure of populations and levels of genetic diversity within populations adds to our understanding of population differentiation of *Helianthus porteri*. As a whole, this research functions to advance our understanding of adaptive differentiation in resource limited environments.

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CHAPTER 2

DROUGHT INDUCED MORTALITY FOR AN ANNUAL SUNFLOWER, *HELIANTHUS PORTERI* (COMPOSITAE)[1]

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[1] Gevaert, S.D. and L.A. Donovan. To be submitted to *American Journal of Botany*.

Abstract

Drought stress plays a major role in shaping community structure and driving species zonation in granite outcrop plant communities. Here we ask whether drought is an agent of selection that may additionally drive adaptive differentiation for geographically isolated populations on granite outcrops. We focus on *Helianthus porteri*, an endemic annual species that has to persist though hot dry summer conditions to reproduce. We followed plant performance traits in *H. porteri* for three years in three populations, and in relatively wet and dry habitats within each population. Population differences in plant growth and survival depended on year, with the smallest differences in survival during the wettest year (2009). During both relatively dry years, the Heggie's Rock population fared more poorly, with 0% survival to flowering in 2008. Panola Mountain growth and survival was better than that of Heggie's Rock during both of the drier years, and Camp Meeting Rock performance was intermediate. Within populations, dry habitats had lower growth and survival in years when habitats differed. Across populations and habitats, survival to flowering was correlated with soil water availability estimated by plant predawn water potentials. The range of water potentials was characteristic of mesic plants, suggesting drought resistance through avoidance rather than tolerance. Population differences in *H. porteri* performance related to water availability could drive adaptive differentiation in drought avoidance traits among the isolated populations. Common garden comparisons are needed to assess genetic differentiation among populations for traits related to drought avoidance.

Introduction

Drought, defined as limited soil water availability, has often been invoked as a selective agent expected to drive adaptive differentiation. Numerous common garden studies have demonstrated population differentiation for plant traits related to drought resistance that is suggestive of local adaptation to drought, but not conclusive evidence (Sandquist and Ehleringer 1997; Ackerly, Dudley et al. 2000; Arntz and Delph 2001; Berg, Becker et al. 2005). Fewer studies have demonstrated local adaptation that is likely due to drought or climate by using reciprocal transplant approaches (Knight, Vogel et al. 2006) and F_{ST}-Q_{ST} comparisons (Ramirez-Valiente, Lorenzo et al. 2009). However, it remains challenging to demonstrate the role of a putative selective agent such as drought in natural populations, because it is difficult to capture the extreme years when selection may be strongest, and it is difficult to capture the relationship between the selective agent and plant performance that differs consistently between populations (Conner and Hartl 2004). The broad objective of this study was to assess the effect of drought on natural populations over several years to determine its role as a potential selective agent driving adaptive differentiation of a sunflower species endemic to granite outcrop communities.

Plant communities occurring in shallow depressions and surrounded by exposed granite, often called “island” communities, are found on granite outcrops from Virginia to Alabama in the southeastern US (McVaugh 1943; Burbanck and Platt 1964). Although the regional climate is relatively mesic and generally supports temperate deciduous forests, the island community habitats are characterized by high light levels, shallow soils, and high evapotranspiration rates, resulting in frequent and extreme declines in soil moisture. Growth of *Pinus taeda* , which is common to both the deeper outcrop depressions and the adjacent non-outcrop habitats, indicates that the outcrop plants are more sensitive to climatic fluctuations, particularly to high temperatures and low precipitation in summer (Houle and Delwaide 1991). In shallower depressions that support only herbaceous vegetation, soil depth and soil moisture limitations affect competitive interactions and thus drive succession and the species zonation characteristic of these island communities (Cumming 1969; Sharitz and McCormick 1973; McCormick, Lugo et al. 1974; Shure and Ragsdale 1977; Burbanck and Phillips 1983). These granite outcrop island plant communities provide an excellent opportunity to study the potential role of drought in adaptive differentiation.

Helianthus porteri, formerly designated *Viguiera porteri* (Pruski 1998), is an annual sunflower species endemic to granite outcrops ranging from South Carolina to Alabama, but most prevalent in Georgia. It is dominant in the annual-perennial successional zone, germinating in the late winter and early spring and persisting through the drought prone summers to reproduce in the fall (Shelton 1963; Burbanck and Platt 1964). Previous field studies have documented that *H. porteri* has reduced growth and increased mortality in response to drought in both naturally occurring and artificially created populations (Cumming 1969; Mellinger 1972; Shure and Ragsdale 1977; Houle and Phillips 1989). If some populations experience more drought stress than others across many years, then this might drive adaptive differentiation over time, particularly given the expectation of limited gene flow between these geographically isolated populations. Other species from granite outcrops have shown evidence of population differentiation consistent with adaptive differentiation, although the agent of selection was not assessed in native populations (McCormick and Platt 1964). In addition, *H. porteri* native and transplant populations differ in biomass when grown in a common garden comparison, although the effects of intraspecific competition were not excluded (Mellinger 1972). We examine the performance of *H. porteri* in three populations spanning its geographic range in Georgia, in order to assess differential performance and relationship to drought.

The persistence of *H. porteri* through the hot dry summers, when many other annuals have completed their reproduction, has led to speculation that it may have a greater capacity to resist drought as compared to other outcrop and non-outcrop species (Shelton 1963; Burbanck and Platt 1964). This is of interest from a conservation perspective for this endemic species, and because of ongoing efforts to mine wild sunflowers for traits to improve productivity and stress resistance of the related commercial species *Helianthus annuus* (Seiler 1992; Seiler, Gulya et al. 2010). The data available to date are conflicting. Early work indicated that *H. porteri* wilted at higher (less stressful) soil water contents than non-outcrop species with a similar life history (Shelton 1963; Mellinger 1972). Additionally, *H. porteri* wilted before co-occurring vegetation in outcrop plant communities. However, *H. porteri* also survived in the wilted state for several weeks, recovered quickly after small precipitation events, and had greater survival than species that didn't wilt until later in a drought. The comparison of *H. porteri* water status (predawn water potentials) in native populations to the literature can provide insight as to whether *H. porteri* achieves drought resistance through either avoidance of or tolerance of low internal water status (Ritchie and Hinckley 1975; Monson, Smith et al. 1992; McKay, Richards et al. 2003).

Our broad objective was to determine the role of drought as a potential selective agent driving adaptive differentiation of a sunflower species endemic to granite outcrop communities. Our specific objectives were:

1) To determine whether drought is associated with differential performance of *H. porteri* populations from across its range.
2) To determine whether *H. porteri* water potentials in natural populations are suggestive of drought resistance through either avoidance or tolerance.

Materials and Methods

Study sites**.** We investigated three populations of *Helianthus porteri* (A. Gray) Pruski (Fig 2.1): Camp Meeting Rock (hereafter CMR), Heard Co., Georgia (33.2475 N, -85.1470 W), Panola Mountain State Park (hereafter PM), Henry Co., Georgia (33.6359 N, -84.1704 W), and Heggie's Rock (hereafter HR), Columbia Co., Georgia (33.5434 N, -82.2670 W) (McCormick and Platt 1964; Braun 1969; Mellinger 1972; Shure and Ragsdale 1977). These sites were chosen because they span the range of *H. porteri* in Georgia, are protected by state (PM) or non-profit groups (Nature Conservancy, CMR and HR) and were large enough to investigate wet and dry microhabitat differences. They are separated by at least 100 km, with intervening outcrops of various sizes occurring between (McVaugh 1943).

Precipitation data for each population were obtained from the nearest weather stations. For CMR, the long term average is from Carrollton, GA (station 091640, 5 km from the site) and the 2008-2010 data are from the Roopville station (GA Automated Environmental Monitoring Network, 24 km from the site). For PM, the long term average is from Jonesboro, GA (station 094700, 18 km from the site) and the 2008 -2010 data are an average of available data from station 094700 and a GA Automated Environmental Monitoring Network also in Jonesboro. For HR, the long term average and the 2008-2010 data are from the Appling, GA (station 090311, 3 km from the site). It is worth noting that weather stations located closer to the sites would have been preferable for estimating precipitation during the 2008-2010 growing seasons because summer precipitation is often patchy due to localized thunderstorms.

The experimental design consisted of the three populations (CMR, PM, HR), two habitats within each population (wet and dry) and six replicate plots (1 x 2 m each) established in each population and habitat type. Habitats were classified as relatively wet or dry in spring of 2008. Habitats were classified as "wet" if they had wetland vegetation and standing or flowing water in the spring of 2008 when plots were being established. Plant community assemblages varied within plots and populations, with all plots having the spikemoss, *Selaginella repestris*, and various lichens. Wet habitats often included some combination of *Packera tomentosa*, *Houstonia pusilla*, and *Arenaria uniflora*. Dry habitats included *Andropogon virginicus, Opuntia compressa, Yucca filamentosa* and *Hypericum gentianoides*. *Liatris microcephala* was found exclusively in CMR dry habitats, and *Oenothera fruticosa*, *Linaria canadensis*, and *Tradescantia hirsuticaulis* were found in both wet and dry habitats. Density of *H. porteri* varied, but during germination, at least 150 *H. porteri* seedlings were found in each plot. Each spring, 20 seedlings in each plot with 4-6 true leaves were randomly selected and tagged (28-29 March in 2008, 27-29 March in 2009, and April 17-19 in 2010). For all soil and plant traits, the experimental unit is the plot, and any multiple measurements within a plot were averaged before further analyses. Data were transformed as necessary to meet the assumptions of normality of residuals and homogeneity of variance for residuals for statistical analyses.

***Plant* measurements**. Tagged plants in the plots were assessed monthly May through September each year for height and survival, near the beginning of each month. Flowering phenology was assessed by recording the date of first flower for each surviving plant (assessed twice weekly starting September 6, 2008, September 5, 2009, and September 15, 2010). Flowers were counted as open when at least one ligule of the ray flowers in the inflorescence was fully expanded. Height at flowering was not assessed on all individuals as it was found to not differ on a subset measured each year. *Helianthus porteri* demonstrates an indeterminate growth pattern.

Survival to first flower, height of surviving plants in September, and date of first flower were analyzed with a three-way ANOVA (SAS Version 9.2, SAS Institute, Inc, Cary, NC, USA) for the effects of population (CMR, PM, HR), habitat type (wet, dry), and year (2008, 2009, 2010) and their interactions. September height and date of first flower were missing for HR in

2008 due to 0% survival to flowering. For 2008, the number of flowers was also recorded for each surviving plant.

Predawn leaf water potentials (Ψ_{pd}) were measured with a pressure chamber (PMS Instrument Company, OR, USA) in the interval 2-3 hours before sunrise to estimate soil water availability (Ritchie and Hinckley 1975; Donovan, Linton et al. 2001). Although nighttime transpiration and other processes can affect Ψ_{pd}, the extent is usually small for *Helianthus,* particularly when soil water is limiting (Donovan, Linton et al. 2001; Ludwig, Jewitt et al. 2006; Howard and Donovan 2007). Ψ_{pd} were measured for two individual plants located in or within 0.5 m of each plot, in May, July and September. For each month, it was not possible to sample all three populations on the same day, so they were sampled on three consecutive days during an interval with no precipitation. In 2008, HR Ψ_{pd} data for July and September are missing because of 0% survival. For each year, the most negative Ψ_{pd} value for May or July (the main growing season) was designated as MinΨ_{pd}. For data compiled from all three years, the correlation between % survival to first flower and Ψ_{pd} (May, July, Sept, and Minimum) was assessed with PROC CORR (SAS Institute, Inc, Cary, North Carolina, USA). Minimum Ψ_{pd} was analyzed with a three-way ANOVA for the effects of population (CMR, PM, HR), habitat type (wet, dry), and year (2008, 2009, 2010) and their interactions, with missing data for HR in July and Sept 2008.

Leaf N & δ^{13}C. The leaves measured for Ψ_{pd} were collected in May 2009 and May 2010 for analysis of leaf nitrogen, leaf carbon and carbon isotope. Leaves were bulked by plot, dried at 60°C, ground with a ball mill (SPEX Mixer/Mill 8000, SPEX SamplePrep, Metuchen, NJ, USA), and analyzed for leaf N, C and δ^{13}C (Micro-Dumas Combustion; δ^{13}C, Finnigan, continuous flow mass spectrometer, Bremen, Germany; at the Stable Isotope/Soil Biology

Laboratory, University of Georgia). Leaf data were analyzed with three-way ANOVA for the effects of population (CMR, PM, HR), habitat type (wet, dry), year (2009, 2010) and their interactions.

Soils. Soil depth was measured in March 2008 as depth from soil surface to bedrock. Three measurements taken within each plot were averaged before further analysis. In May 2010, three soil samples at a depth of 6-11 cm (dependent on plot depth) were collected and pooled into one sample from each plot at each population (N=36) for soil nutrient analysis. Soil samples were dried at 60°C, ground using a ball mill and analyzed for total N, C and $\delta^{13}C$ (Micro-Dumas Combustion, University of Georgia, Stable Isotope/Soil Biology Laboratory). Soils were additionally analyzed for pH, Ca, K, Mg, Mn, and P at the University of Georgia Agricultural and Environmental Services Laboratory (pH: LabFit AS-3000 pH Analyzer; nutrients: Mehlich-1 extraction method using an Inductively Coupled Plasma Spectrograph). Soil data were analyzed with a two-way ANOVA for the effects of population (CMR, PM, HR), habitat type (wet, dry), and their interaction. The correlation between soil depth and Minimum Ψ_{pd} was assessed for each year.

Results

Precipitation. The three population sites differ in long term average precipitation, forming a gradient of decreasing precipitation from west to east across the range of *H. porteri* in the Piedmont Region of Georgia (CMR>PM>HR) (Fig 2.2). However, these spatial differences in long term annual means are small compared to the variation in annual precipitation among the three study years, and variation within each year in relation to the summer growing season. In general, 2008 precipitation was below average, particularly for May through July, and part of a regional “extreme” drought. Precipitation in 2009 was above average with peaks in spring and fall. Precipitation in 2010 returned to below average for annual precipitation, but with the CMR and PM sites getting above average precipitation in May.

Plant Performance. Plant survival to first flower differed by population, habitat and year, with an interaction between population and year (Table 2.1). The driest year, 2008, was associated with the most dramatic effect on survival to first flower. There was 0% survival of *H. porteri* at HR past June in both the wet and dry habitats (Fig 2.3a). In that same year, survival declined more slowly at CMR and PM and was approximately 20% at first flower in late summer. Also, the steep drop between September survival and survival at first flower in 2008 was due in part because September survival was assessed on September 6, 2008, but most plants did not begin flowering until Sept. 19, at which time, many more individuals had died. In 2009, the wettest year, all of the populations and habitats had a similar seasonal profile of declining survival to 20-50% at first flower (Fig 2.3b). In 2010, PM maintained higher survival through the latter part of the growing season (40-60%) as compared to CMR and HR (15-30%, Fig 2.3c). When habitats within a population differed for survival, the wet habitats had higher survival than the dry habitats (Table 2.1, Fig 2.3a-c).

The surviving plants increased in height as the growing season progressed in all years and for all populations and habitats (Fig 2.3d-f). Plant height in September differed by population, habitat and year (Table 2.1). Across the years, PM plants tended to be tallest, and HR plants tended to be the shortest. Phenology of surviving plants, as indicated by date of first flower, differed by population, habitat and year, with an interaction between population and year (Table 2.1). The most prominent effect was that all three populations initiated flowering later in 2010 by ~ 10-15 days as compared to 2008 and 2009 (Fig 2.3a-c). For 2008, final plant height was positively correlated with the number of flowers produced based on data gathered during the

2008 season, when it was possible to obtain an accurate count of flowers per plant ($r = 0.49498$, $r^2 = 0.2450$, $p < 0.0001$).

Leaf N differed by population and year with an interaction between population, habitat and year (Table 2.1). The leaf N values ranged from 2.57-4.26%, and in general, were highest (>3.5%) in 2009 at CM wet, CM dry, and PM wet. In both years, HR tended to have the lowest leaf N values (2.69-3.09%). Integrated leaf level WUE (estimated by leaf $\delta^{13}C$) averaged -31.01‰ and did not differ by population, habitat or year.

Plant soil water availability and survival. The seasonal patterns of plant soil water availability, estimated with Ψ_{pd}, differed by population and year (Fig 2.3g-i). In 2008, the HR Ψ_{pd} was already relatively low in May, and this was followed by 0% survival to July sampling. MinΨ_{pd}, which reflects the more negative value from the main growth interval (May through July) differed by population, habitat and year, with an interaction between population and year (Table 2.1). Using data from all three years, MinΨ_{pd} is correlated with survival to first flowering ($r = 0.33$, $p < 0.001$, $n = 108$, Fig 2.4). The relationship between July Ψ_{pd} and survival to first flowering was similar, although it did not include HR plots for 2008 because there were no surviving plants for July Ψ_{pd} sampling ($r = 0.32$, $p = 0.002$, $n = 96$, data not presented).

Soil. Soil depth averaged 10.75 cm (± 0.42) and did not differ by population or habitat within population, although there was a trend ($p = 0.08$) for soils to be deeper in wet habitats (Table 2.2). Soil nutrient availability differed among populations, with PM having a higher organic content and overall nutrient availability as compared to CMR and HR, as indicated by higher soil percent C and N (Table 2.2). For soil P, K and Mg, the PM population again had among the highest values, but soil P was also high for HR, and soil K and Mg were also high for CMR. The populations did not differ for soil Ca or Mn. Soil pH is the only characteristic that differed by population and habitat, with a significant interaction between population and habitat. PM wet and dry plots had soil pH values intermediate to the range for the other populations.

Discussion

Drought has already been established as an important component of the community ecology of these island communities surrounded by bare granite (Cumming 1969; Sharitz and McCormick 1973; McCormick, Lugo et al. 1974). Soil depth and resulting soil moisture play a large role in determining which species can survive and outcompete other species, and thus drive community structure and species zonation. The shallower soils support a lichen-annual community generally dominated by winter annuals that largely escape effects of drought by completing reproduction before summer. *Helianthus porteri* dominates in relatively deeper soils of the annual-perennial zone, and has to persist through the stressful summer to reproduce in late summer and early fall (Shelton 1963; Burbanck and Platt 1964; Mellinger 1972; McCormick, Lugo et al. 1974). Here we complement the community ecology focus with an evolutionary focus looking at differential performance of *H. porteri* populations over time. Comparing the performance for individual *H. porteri* populations across years, we found decreased growth and survival associated with periods of reduced precipitation, consistent with previous reports (Mellinger 1972; Shure and Ragsdale 1977; Houle and Phillips 1989). This variation is more closely associated with intervals of low precipitation and high evapotranspiration in late spring and summer growing seasons than with annual precipitation. This is expected because soil moisture is depleted relatively quickly after rainfall events unless there are additional moisture inputs such as water draining from soils or seeps at higher elevations (Shelton 1963; Cumming 1969; Houle and Phillips 1989). However, previous studies have not compared multiple *H. porteri* populations for their relative performance across multiple years.

Comparing *H. porteri* populations across three years of variable precipitation, the populations differed least for survival in 2009, when annual precipitation was above average, and differed most for survival in 2008, when annual precipitation was well below average. PM generally performed the best, with among the highest rates of survival in both of the relatively dry years (2008 and 2010), and the tallest plants in the relatively wet year (2009). HR generally performed the poorest, with no survival past June in one of the dry years and shortest plants during the wettest year. CMR performance was intermediate, more similar to PM in one dry year, but more similar to HR in the other dry year. When habitats within a population differed, wet habitats had higher growth and survival, but among population differences were generally larger than habitat differences within populations. The relative consistency in ranking of population performance across years could be due to differences in current environmental factors that could act as selective agents, such as drought, or genetic differences, adaptive or otherwise.

The environments do differ among the *H. porteri* populations. Although soil depth did not differ, PM had a higher soil organic component, consistent with previous studies (Braun 1969; Shure and Ragsdale 1977). The higher soil organic content at PM was associated with higher MinΨ_{pd} in drier years, as expected since the organic matter has a greater water holding capacity. If drought has a larger effect on survival and thus fitness in HR as compared to PM in most dry years, then drought could be a major selective agent driving population differentiation in traits related to drought resistance. This expectation is supported by the correlation between MinΨ_{pd} during the main growth interval (May, July) and survival to first flower. Thus, we hypothesize that drought is driving adaptive differentiation for drought resistance traits. This would additionally require that there is heritable variation in drought resistant traits for selection to act on, and that there are no genetic constraints limiting the response to selection (Conner and

Hartl 2004; Agrawal, Erwin et al. 2008). There are, of course, many other environmental factors that may differ among the populations that could additionally be selective agents, such as light levels, nutrients and other aspects of soil chemistry, and biotic interactions. Field environment manipulations would be needed to separate the effects of drought and other factors on relative performance of populations through years differing in amount and timing of precipitation.

Our results suggest that *H. porteri* may be differentially adapted to drought. Partial support for this hypothesis is provided by a previous study that compared *H. porteri* populations from near PM (Mt. Arabia, GA) to HR and a population near CMR (Almond, AL) in a common garden study in NC (Mellinger 1972). In that study, Mt Arabia plants were taller and had greater biomass than HR and Mt Almond plants, demonstrating genetic differentiation. Additionally, common garden studies found that ecotypes of *Diamorpha cymosa* collected from 16 outcrops from Alabama up to NC differed in their responses to light intensity, drought, and temperature treatments (McCormick and Platt 1964). Tests of local adaptation would require reciprocal transplants among populations that demonstrated a “home site advantage.” Strong inference for drought as the selective agent would be provided by an alleviation of home site advantage by water supplements in reciprocal transplant experiments. Additional support would be provided by common garden studies demonstrating that populations from drier sites perform better under drought conditions and have traits expected to be related to drought resistance. Knowledge of whether not *H. porteri* is locally adapted will have implications for the potential use of transplants in the conservation of this rare species endemic to granite outcrops in the southeastern US.

The predawn water potentials of *H. porteri* in these natural populations are well within the range for most mesic-adapted plants (i.e. generally less than -1.5 MPa (Kramer and Boyer

1995)), and provide no support for the suggestion that *H. porteri* survives through the summer droughts by tolerating more negative water potentials. This suggests that *H. porteri* has traits allowing it to avoid or delay lower water potentials by maximizing water uptake and minimizing water losses when faced with declines in soil moisture. This would be consistent with the observations that it can remain wilted at relatively higher water potentials for extended periods of time and then recover after small precipitation events (Shelton 1963; Mellinger 1972). It has also been suggested that *H. porteri* may be able to take up moisture from fog or dew, which is common in the summer in these habitats (Lugo 1969). Water acquisition from fog or dew has been well documented in *Sequoia sempervirens* (coastal redwood), which is able to acquire water from its roots (dripped off the crown) and through its leaves (Dawson 1998). Other species in the coastal redwood ecosystem, including shrubs and ferns, are also capable of foliar water uptake, especially during periods of drought (Limm, Simonin et al. 2009). The apparent ability of *H. porteri* to resist drought should be further investigated due to the potential for improving stress resistance of the commercial species *H. annuus.*

Acknowledgments

We would like to thank Natasha Sherman and Michael McKain for assistance with data collection during the first year of the study (2008). We also want to thank Malcolm Hodges with the Nature Conservancy and Tom Patrick and Nikki Castleberry for the Georgia Department of Natural Resources for help in acquiring and maintaining permission to use these field sites for research purposes.

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Table 2.1. ANOVA statistics for *H. porteri* plant traits sampled across 3 years, except leaf traits which were sampled in 2 years (2009 & 2010). Leaf N was square-root transformed for statistical analyses. Survival FF, dfn(2,1,2,2,4,2,4), ddf 90; Sept Height, Day FF dfn(2,1,2,2,3,2,3), ddf 776-8; Leaf N & δ^{13}C dfn(2,1,1,2,2,1,2), ddf 59 (May 2009 and 2010). **p* < 0.05, ***p* < 0.01, ****p* < 0.001

Plant traits	Pop	Habitat	Year	Pop*Habitat	Pop*Year	Habitat*Year	Pop*Habitat*year
Survival to First Flower	36.6***	19.1***	25.6***	0.2	14.2***	2.9	0.2
Sept Height	9.1***	20.3***	5.7**	2.1	0.8	1.1	0.2
Min PD WP1	65.6***	102.8***	3.3*	6.2**	3.6**	0.2	1.2
First Flower (Julian date)	46.6***	24.8***	24.5***	2.2	8.6***	0.2	0.7
Leaf %N	11.4***	1.9	39.6***	3.8*	8.3***	1.1	5.8**
Leaf δ^{13}C	1.4	0.1	0.9	0.6	1.8	1.5	0.3

Table 2.2. Soil depth, N, C, P, K, Ca, Mg, Mn and pH means (±SE) and F (ndf(2,1,2) ddf 30) from sampling of *H. porteri* study sites in 2010. For statistical analyses, C, Ca, Mg, pH and K were log-transformed. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, †$p < 0.1$.

Soil traits	CMR wet	CMR dry	PM wet	PM dry	HR wet	HR dry	*F* Pop	*F* Habi-tat	*F* Pop* Habitat
Soil depth (cm)	11.3±0.9	9.9±1.0	12.4±1.1	9.5±1.0	10.8±0.5	10.6±1.4	0.1	3.2†	0.9
Soil N (%)	0.56±0.16^{c}	0.63±0.16^{bc}	1.19±0.14^{a}	0.92±0.14^{ab}	0.63±0.10^{bc}	0.47±0.1^{c}	7.8**	1.1	0.8
Soil C (%)	11.2±4.4^{b}	13.1±3.4^{ab}	16.2±0.8^{a}	15.8±2.8^{a}	7.8±1.3^{b}	6.8±1.4^{b}	6.6**	0.1	0.7
P (lbs/acre)	12.5±1.1^{b}	15.3±2.7^{b}	26.2±4.7^{a}	26.7±2.8^{a}	26.8±5.6^{a}	30.1±3.3^{a}	9.2***	0.5	0.1
K (lbs/acre)	46.0±5.5^{abc}	47.1±10.4^{abc}	60.0±7.3^{a}	49.9±3.4^{ab}	35.6±1.0^{bc}	36.4±5.5^{c}	5.8**	0.5	0.2
Ca (lbs/acre)	261.1±104.4	83.2±19.7	130.7±27.8	132.4±22.4	87.8±19.5	58.4±11.3	2.5	2.3	0.8
Mg (lbs/acre)	24.5±6.5^{ab}	19.7±5.5^{abc}	26.5±4.6^{a}	18.4±1.5^{abc}	14.9±1.3^{bc}	12.6±2.2^{c}	3.6*	2.4	0.1
Mn (lbs/acre)	38.6±16.5	7.2±2.9	31.2±12.4	17.5±5.1	19.2±6.8	33.1±8.9	0.1	1.7	2.7
pH	4.8±0.2^{a}	4.1±0.1^{c}	4.4±0.1^{b}	4.4±0.1 ^{b}	4.8±0.1^{a}	4.8±0.1^{a}	9.3***	6.2*	8.6**

Figure 2.1. Regional map of *Helianthus porteri* study sites.

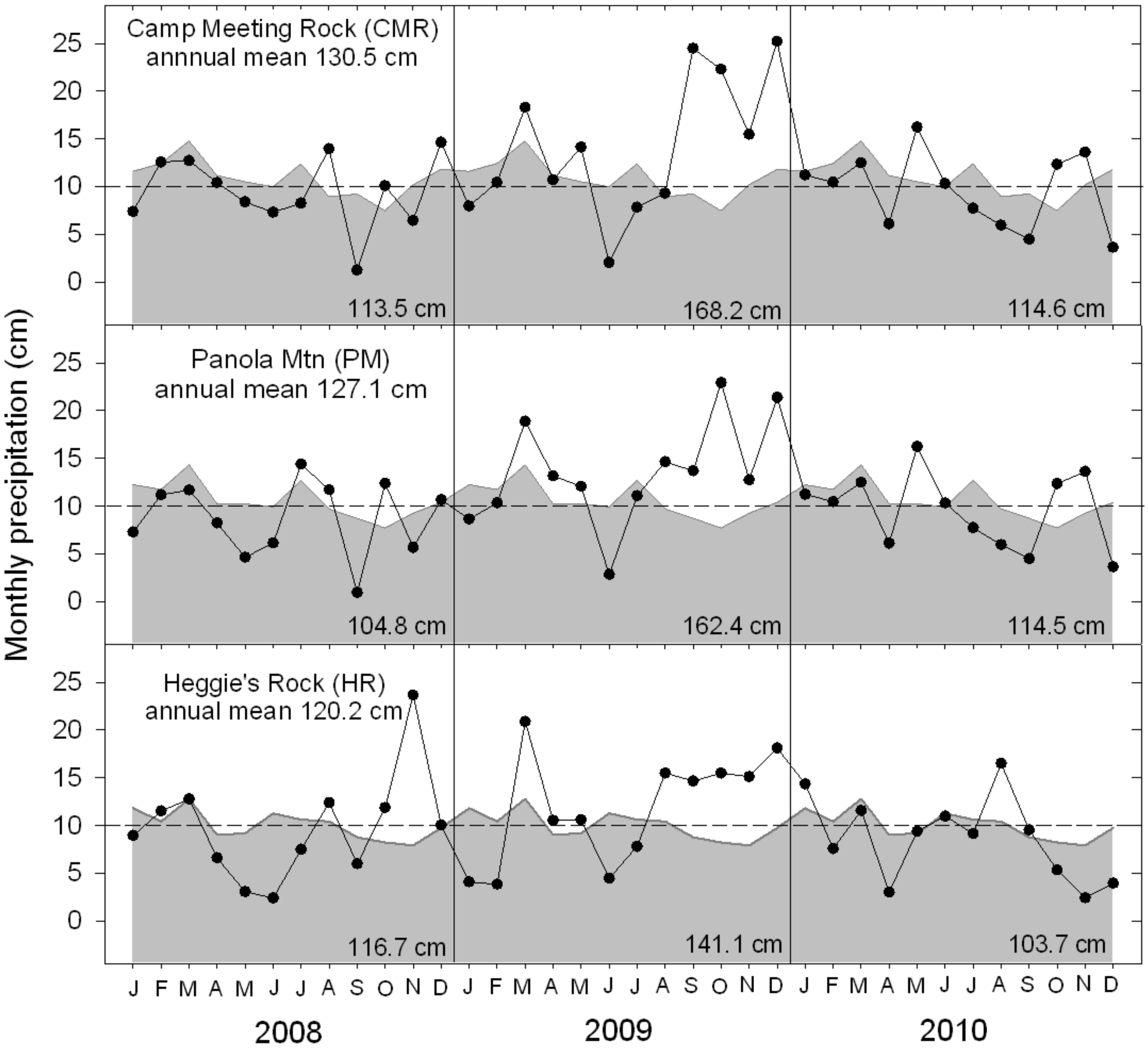

Figure 2.2. Precipitation at the three *Helianthus porteri* granite outcrop study sites (CMR, PM, HR) in Georgia, 2008-2010 (black line) in relation to long term mean (gray area).

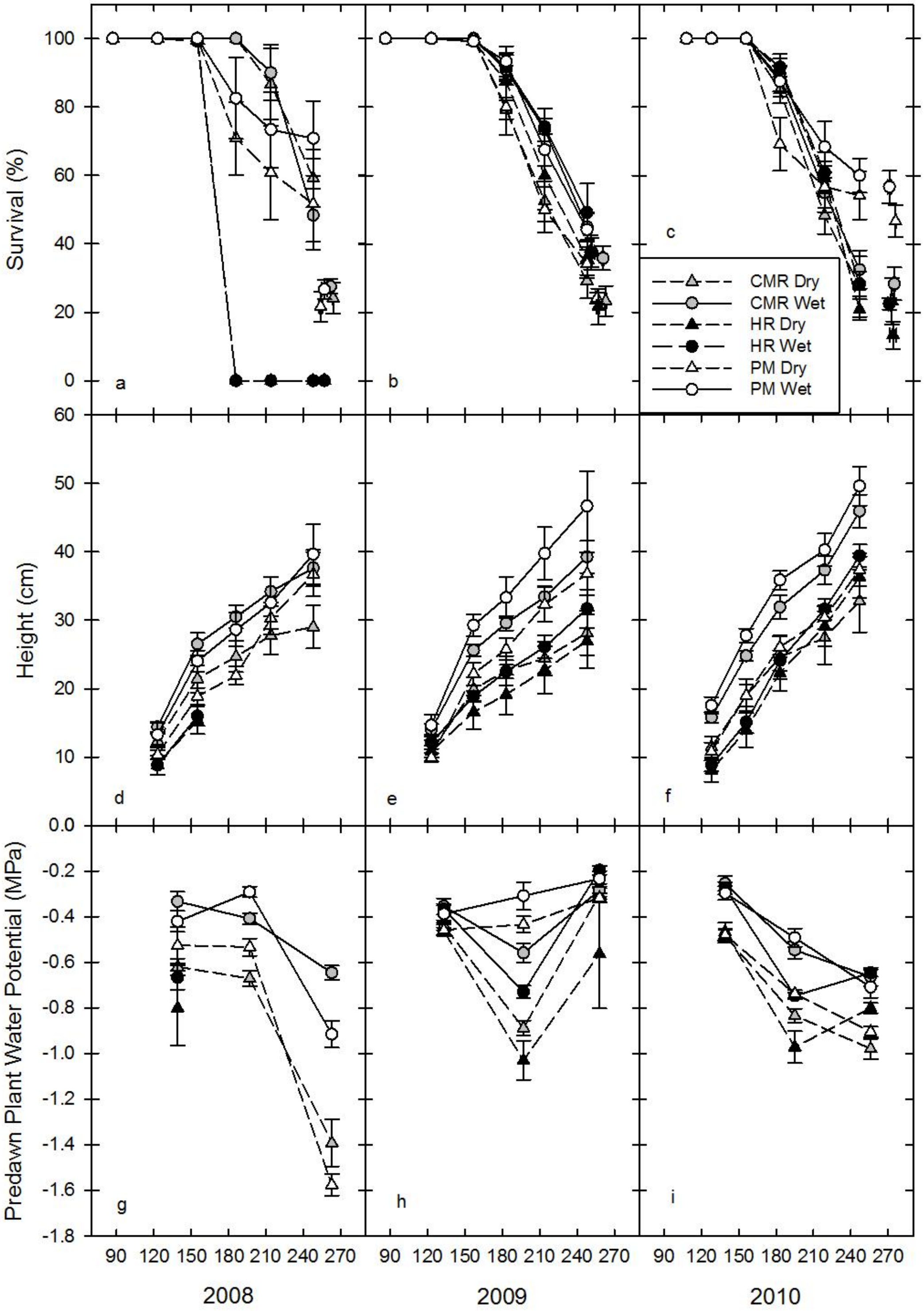

Figure 2.3. *Helianthus porteri* survival (a-c), plant height (d-f) and predawn water potential (g-i) at the three study sites (CMR, PM, HR) in Georgia, 2008-2010. In figures a-c the detached points show survival at flowering. See legend in 3c.

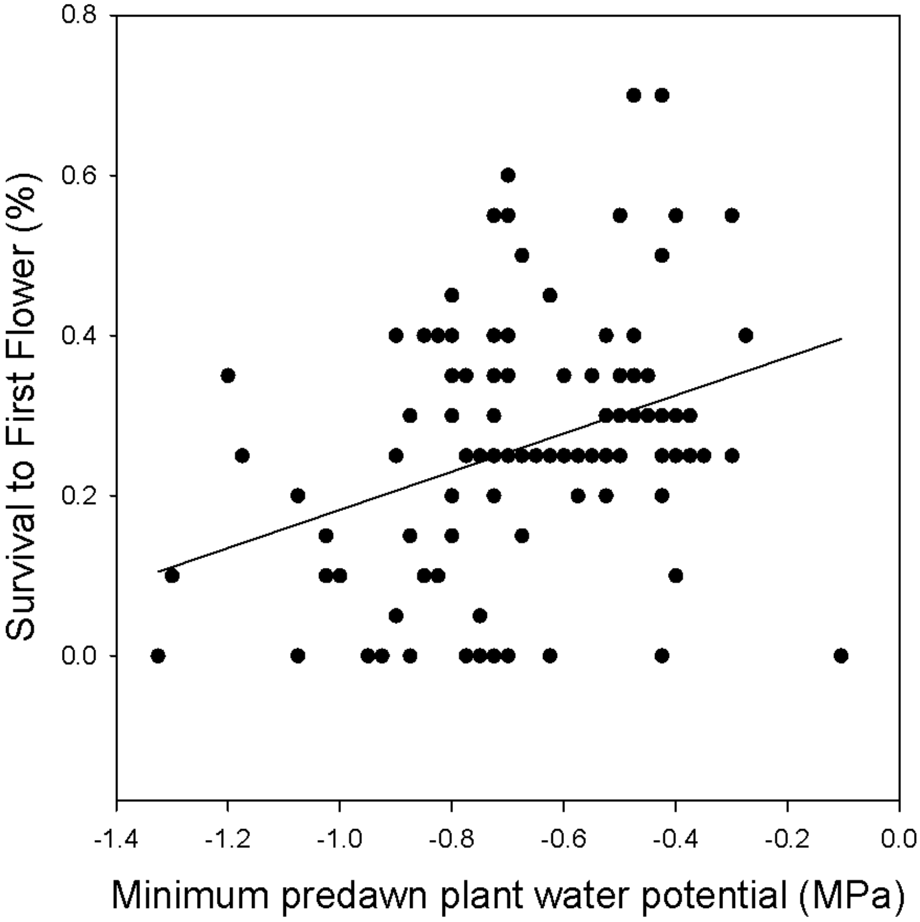

Figure 2.4. *Helianthus porteri* survival to first flower as a function of MinΨ_{pd} (minimum predawn plant water potential from May and July).

CHAPTER 3

POPULATION DIFFERENTIATION OF *HELIANTHUS PORTERI* PROVIDES NO EVIDENCE FOR ADAPTIVE DIFFERENTIATION IN RESPONSE TO DROUGHT[1]

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[1] Gevaert, S.D. and L.A. Donovan. To be submitted to *American Journal of Botany*.

Abstract

Granite outcrop plants are prone to drought stress due to extensive exposed rock, patchy shallow soils and high summer temperatures. For *Helianthus porteri*, an endemic annual sunflower, we previously demonstrated that three populations differed consistently for extent of summer water stress across three years, and hypothesized that they are locally adapted to drought. Here we test whether they are genetically differentiated for growth and ecophysiological traits in a manner consistent with local adaption to drought. In a greenhouse common garden study, we compared the populations for seedling maximum relative growth rate (RGRmax) and mature plant growth and ecophysiological responses to water and nutrient limitation. The populations are genetically differentiated for growth and ecophysiological traits. As expected, the population that generally experiences the least stressful water potentials and drought induced mortality in its native habitat has the largest mature plants (height, stem diameter and biomass) under optimal resource conditions. However, this population did not have higher seedling RGRmax or other traits (e.g., higher gas exchange rates, lower water-use efficiency) thought to be associated with greater growth and lower drought resistance. Additionally, there were no interactions between populations and resource treatment (water limitation, nutrient limitation, or both) suggesting differential ability to resist water and associated nutrient limitations. The populations are genetically differentiated but the ecophysiological trait patterns provide no support for the hypothesis that the populations are locally adapted to drought. Future studies, such as reciprocal transplants among the three populations, are needed to determine whether or not populations are locally adapted in response to other selective agents.

Introduction

Granite outcrops of the southeastern United States are geographically isolated habitats that have many endemic plant species as well as resource limitations that affect plant productivity, physiology and survival (Burbanck and Platt 1964; McCormick and Platt 1964; Shure and Ragsdale 1977; Antonovics, Ellstrand et al. 1988; Baskin and Baskin 1988). Soil water availability in these habitats is often limiting due to high evaporation rates and run-off from the rock surface, as well as shallow soils (Cumming 1969; Sharitz and McCormick 1973; McCormick, Lugo et al. 1974; Shure and Ragsdale 1977; Burbanck and Phillips 1983). Nutrient concentrations have also been found to be relatively low and may also be limiting (Braun 1969; Mellinger 1972; Shure and Ragsdale 1977). Studies which assess limitations to resources on granite outcrops could elucidate the potential selection in native populations. One such study, a 3-year field study of natural populations of *H. porteri*, an annual sunflower endemic to granite outcrops of the southeastern US, investigated whether drought is a selective agent among geographically isolated granite outcrops (Gevaert & Donovan, in prep). The ranking of populations for drought and the correlation between drought stress and mortality demonstrated the potential for drought to be a selective agent driving population differentiation for this species. Additionally, granite outcrops are highly geographically isolated habitats scattered throughout the piedmont forest, creating natural barriers for gene flow and the potential for plant populations to be locally adapted(McCormick and Platt 1964; Chapman and Jones 1975; Antonovics, Ellstrand et al. 1988). Here we test whether populations of *H. porteri* are genetically differentiated for growth and ecophysiological traits in a manner consistent with local adaption to drought and nutrient stress.

Drought is characterized by limited soil water availability. Plants can resist drought by three basic strategies: escape, avoidance or tolerance, although the terminology differs slightly among authors (Ludlow 1989; Kramer and Boyer 1995; McKay, Richards et al. 2003; Verslues, Agarwal et al. 2006). Plants can escape drought in time by adjusting their phenology to minimize the exposure to soil water limitations. Plants can avoid or slow down the onset of internal plant water deficit by maximizing water uptake, minimizing water loss, and using available water more efficiently. Traits often associated with drought avoidance are inherently slower growth, higher root:total biomass ratio, greater root biomass and rooting depth, decreased stomatal conductance, and higher water use efficiency (WUE, ratio of carbon gain to water loss). Leaf level WUE can be assessed at two time scales: instantaneous leaf WUE as measured from the ratio of photosynthesis to transpiration, and leaf WUE integrated over the lifetime of the leaf as estimated with leaf carbon isotope ratio ($\delta^{13}C$) (Farquhar, Ehleringer et al. 1989; Donovan and Ehleringer 1994). When low plant water status can't be avoided, then some plants can additionally tolerate low plant water status with the physiological processes such as osmotic adjustment. For *H. porteri*, the water potentials reported for field populations are well within the range of mesic plant species, suggesting that the ability to persist through summers on granite outcrops is achieved through avoidance mechanisms that maximize water uptake and/or minimize water loss (Gevaert & Donovan, in prep).

When soil water is limiting, this is often accompanied by nutrient limitation because the delivery of nutrients to the plant in the soil water declines (Wright et al. 2002). This can have effects on fitness through multiple routes, including delayed flower initiation, and reduced pollen production and pollen viability (Marschner 2002). Thus greater drought resistance may also be associated with the ability to increase uptake and use acquired nutrients more efficiently. Plants can do this by maintaining a higher photosynthetic nitrogen-use efficiency (PNUE, the ratio of photosynthetic rate to leaf organic nitrogen content), thereby maintaining a lower leaf N and increasing nutrient-use efficiency (Field and Mooney 1986; Lambers and Poorter 1992; Aerts and Chapin 2000).

Common garden studies are highly informative in ecological research because they can be used to determine a genetic basis for differences observed in natural populations, through a controlled environment (Ackerly, Dudley et al. 2000; Arntz and Delph 2001). Common garden studies have been used to consider the effects of water and nutrient limitation in a variety of plant species. Sambatti & Rice (2007) used a common garden to investigate the ecotypic differentiation in riparian and serpentine populations of *Helianthus exilis* for traits that would infer an advantage to those habitats. They found habitat differentiation with regard to integrated WUE, biomass, and leaf micronutrient concentrations. However, while it was known that these populations are locally adapted, they found that only one trait, average dry weight, was consistent with a locally adapted existence. Previous work with *H. porteri* in a common garden environment showed that native and transplant populations differed in plant biomass, demonstrating genetic differentiation (Mellinger 1972). However, ecophysiological traits related to water and nutrient limitation were not assessed.

Here we investigate population differentiation for several ecophysiological and growth traits among three populations of *H. porteri* that differ in their severity of drought in a greenhouse common garden experiment. We hypothesized that these populations of *H. porteri* would be genetically differentiated for growth and ecophysiological traits associated with drought resistance in a manner consistent with local adaption to drought.

Materials and Methods

Study species. *Helianthus porteri* (A. Gray) Pruski (Pruski 1998), Compositae, is an annual, self-incompatible sunflower endemic to granite outcrops, found from eastern Alabama through the piedmont to North Carolina. It grows in shallow soil pools (7-15 cm deep), in full sun, germinating in late March and flowering from late August through to the first frost. It is capable of exploiting resources when available for quick growth, but also surviving under resource-limited conditions for short intervals of time (Mellinger 1972; McCormick, Lugo et al. 1974). Like many outcrop endemics, it has an extensive seed bank, though it is uncertain how long the seeds remain viable (Houle and Phillips 1988).

We compared three populations that span the geographic range of *H. porteri* in Georgia (Fig 3.1): Camp Meeting Rock, Heard Co., Georgia (CMR; 33.2475 N, -85.1470 W), Panola Mountain State Park, Henry Co., Georgia (PM; 33.6359 N, -84.1704 W), and Heggie's Rock, Columbia Co., Georgia (HR; 33.5434 N, -82.2670 W). While each of these outcrops is separated by at least 100 km, there are intervening outcrops of various sizes between them. All three populations are relatively large with more than 1000 individuals. Seeds were collected from HR, PM and CMR in 2009 and stored at 4°C until use. Ten seeds per family (maternal individual) were weighed and averaged to obtain an estimate of individual seed weight to account for maternal effects during statistical analyses. Seeds were germinated in Petri dishes on moist filter paper. The blunt end of each seed was excised after 24 hrs in the dark and seed coats were removed after 48 hours in the dark. When the hypocotyl had formed and root hairs were present, the Petri dishes were placed under florescent light (80-90 µmol m^{-2} s^{-1}) with a 12-hour photoperiod. After 7 days (October 7, 2009), 40 seedlings per population (N=120) were transplanted into 1-gallon pots of 3:1 sand-to-Turface (Profile Products, Buffalo Grove, IL) in the UGA Plant Biology Greenhouses. Seedlings were misted twice daily for one week. At study initiation on October 14, 2009, seedlings were watered to field capacity with nutrient solution (Peter's Plant Starter 9-45-15, 100 ppm, Scotts Company, Marysville OH) three times each week. Supplemental greenhouse lighting was provided to simulate the progression of photoperiod experienced by native populations.

Seedling maximum relative growth rate (RGRmax). At 2 weeks and 5 weeks after study initiation (October 28, and November 18, respectively), 8 randomly-selected plants from each population were harvested from each block (N=44) for determination of relative growth rate. Due to seedling mortality of the CMR population, harvest one comprised 7 CMR individuals and harvest two comprised 5 individuals. At each harvest, biomass was separated into roots, stems, leaves, and cotyledons, if present. Leaf area (LI-3100, Licor, Lincoln, NE) was measured. Whole plant leaf area and leaf dry biomass were used to calculate whole plant specific leaf area (SLA, cm^2/g) for each harvest. All biomass components were then dried at 60°C and weighed. Instantaneous measures of RGRmax were calculated according to Hunt (Hunt 1990). Population differences in seedling RGRmax were evaluated as the populations by time interaction in an ANOVA with ln plant biomass as the dependent variable (Poorter and Lewis 1986; Poorter 1989).

Plant growth responses to different resource treatments. Following the second relative growth rate harvest (November 18), the remaining plants were randomly assigned to one of four resource treatments (5-6 plants per treatment per population). The well-watered high nutrient (H_WH_N) and well-watered low nutrient (H_WL_N) treatment plants received 20 grams or 1g of slow-release fertilizer (Osmocote Classic, 8-9 month release, Everris US Ltd, Marysville, OH, USA), respectively, and were irrigated daily to field capacity (35%) soil moisture. The water-limited high nutrient (L_WH_N) and water-limited low nutrient (L_WL_N) treatment plants received 20 grams or 1 g of slow-release fertilizer, respectively, and had water withheld until more than half of the measured pots reached 15% soil moisture. Soil moisture was measured daily with a soil moisture probe (ML2x ThetaProbe soil moisture sensor, Dynamax, Houston, TX, USA) for all pots in the low water treatments initially, then half after the first 6 weeks of treatment conditions. During the weeks 1-5, 6-9, 10-13 and 14-15, the dry down of soil moisture from field capacity to 15% occurred over 5, 4, 3, and 2 days, respectively. Population differences in dry down were not observed until the final weeks of treatment prior to harvest. At the very last 2-day period of dry down, plants at PM had a lower soil moisture (8.59% ± 0.32; LS-Means ± 1 SE) than HR (9.83% ± 0.32), though neither differed from CMR (9.05% ± 0.35).

From December 20-21, plants were measured for gas exchange and leaf traits, just prior to re-watering for the water limited treatments. Maximum photosynthetic rate (A_{max}), stomatal conductance (g), and instantaneous water-use efficiency (A/g) were measured with a Li6400 (Licor Biosciences, Lincoln, NE) on the most recently fully expanded leaf, which was produced after the initiation of the resource treatments. Chamber conditions were 1500 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR), 380 ppm CO2, with block temperature and relative humidity adjusted to reflect ambient conditions on the date measurements were taken (27°C, 52-57%, respectively). The gas exchange leaf was scanned and analyzed for leaf area inside the chamber (for adjusting photosynthetic rates) and total leaf area and then dried at 60°C. Leaf tissue was analyzed for leaf nitrogen and leaf carbon isotope (leaf δ^{13}C) (leaf N, Micro-Dumas Combustion, Finnigan continuous flow mass spectrometer, Bremen, Germany; at the Stable Isotope/Soil Biology Laboratory, University of Georgia). Photosynthetic nitrogen-use efficiency (PNUE) was calculated after Field & Mooney (1986). During the course of the resource treatments, date to first bud and date to first flower were recorded. Leaf senescence was monitored every other day and recorded when a marked leaf reached 25% yellow as a measure for leaf lifetime.

Final harvest occurred March 5-9, 2010, approximately 4 weeks following the initiation of flowering of the last plant in the experiment. Reproductive biomass was measured as the combined weight of buds, flowers and heads. Additionally, biomass was sorted into aboveground (leaves and stems), roots, and reproductive material, and a subset of senesced leaves was collected. All biomass components were dried at 60°C and weighed.

Statistical Analyses. Two-way analyses of variance (ANOVAs) were performed for each trait with population (CMR, PM, HR), treatment (H_WH_N, H_WL_N, L_WH_N, L_WL_N) and block as fixed effects, and a population x treatment interaction effect in the analyses (Proc GLM, SAS version 9.2, SAS Institute, Cary, NC, USA). Analyses investigating water and nutrient treatments individually were also performed, but did not yield informative results as access to water directly impacts the ability to acquire nutrients. Maternal effects were accounted for by initial seed weight which was run as a covariate in analyses. However, no effect of seed weight was found in any analysis and it was subsequently removed as a factor. Traits were transformed as necessary to meet the assumption of normality of residuals and homogeneity of variance for residuals.

Results

Seedling RGRmax. Under optimal water and nutrient conditions, populations did not differ for maximum seedling relative growth rate (RGRmax) as indicated by a lack of significant population by time interaction effect ($F_{2,43} = 0.05$) and thus no difference in slope (Fig 3.2). The mean RGRmax was 0.156 $g \cdot g^{-1} \cdot day^{-1}$. The populations did differ for biomass at each harvest, with PM seedlings being the largest ($F_{2,43} = 4.55$, $p < 0.05$), and seedlings in populations increased in biomass with time as expected ($F_{1,43} = 332.17$, $p < 0.001$). At this seedling stage, PM whole plant specific leaf area (SLA) calculated from RGR (total leaf area per leaf biomass) was also significantly greater at both harvests than both HR and CMR (population $F_{2,43} = 6.73$, $p < 0.01$; time $F_{1,43} = 44.23$, $p < 0.001$; time x population $F_{2,43} = 4.81$, $p < 0.05$).

Population differences among mature plants. Populations differed in growth and ecophysiological traits at plant maturity. In the high resource (H_WH_N) treatment, PM plants were larger (greater height and stem diameter) and had greater biomass than HR, with no significant population x treatment interactions (Table 3.1, Fig 3.3). PM and HR also had greater root:total biomass ratio than CMR. For the measured gas exchange leaf, the SLA of PM and CMR was greater than HR (Table 3.1, Fig 3.4). Plants at PM also had a lower PNUE than those at HR. Unexpectedly, the greater growth of PM in the H_WH_N treatment was associated with a lower leaf photosynthetic rate (A_{max}) on an area or mass basis, and leaf nitrogen (leaf N). For traits related to water use, PM plants had a lower *g* and higher integrated WUE (as estimated by leaf $\delta^{13}C$), but did not differ from the other populations for instantaneous WUE (*A*/*g*). No significant differences among the populations were observed for time to first bud, time to first flower, or leaf lifetime.

Population response to resource limitations. As expected, plants in all three populations responded to the resource limitation treatments with reduced size (height, stem diameter) and biomass that was similar across all three populations (Table 3.1, Fig 3.3). Water limitation decreased g and increased instantaneous WUE (Figs 3.4b, 3.4e). Decreasing nitrogen reduced leaf N and generally increased PNUE of plants in those treatments (Figs 3.4c, 3.4d). In addition, no significant population by treatment interactions were found for any trait, except time to first bud. However, that effect does not appear to have biological meaning as this interaction did not hold for time to first flower.

Discussion

Based on the results from the 3-year field study with these same populations of *H. porteri* (Gevaert & Donovan, in prep), we expected the PM and HR populations to differ in plant responses and adaptation. In that study, plants at PM consistently had greater growth and survival to reproduction across years varying in precipitation, and experienced less water stress. In contrast, plants in the HR population experienced greater water limitation, with the greatest extreme in 2008 in which mortality reached 100% prior to reproduction. Thus, we hypothesized that drought was a selective agent driving local adaptation in *H. porteri* and that there should be greater resistance to drought for HR. Under optimal resource conditions, we expected the populations to differ for traits related to growth and drought resistance, with PM plants having higher seedling RGRmax, and larger mature plants with higher SLA, A_{max}, *g* and leaf N, and lower root:total biomass, instantaneous WUE, integrated WUE, and PNUE (Grime and Hunt 1975; Grime 1977; Ludlow 1989; Lambers and Poorter 1992; McKay, Richards et al. 2003).

At the seedling stage under optimal resource conditions, we did not find the expected inherent differences in RGRmax. Plants from the PM population did not have a faster seedling RGRmax than HR, though PM did have larger seedlings at each harvest, and a higher SLA indicating allocation differences. At plant maturity in the optimal resource treatment, differences in plant size were maintained, with PM having a larger size (height, stem diameter) and biomass at final harvest. Additionally, PM plants had a higher SLA, indicating allocation differences.

Plants at the PM population also did not meet our expectations for the traits under optimal conditions that would have indicated the population is adapted to higher resources.

Where we would have expected plants at PM to have higher A_{max}, *g*, and leaf N, and lower WUE and PNUE under optimal conditions, we found no population differences or the opposite results. While previous studies have suggested that PM may not experience resource limitation because soils there have greater soil moisture content and soil organic content (Burbanck and Platt 1964; Braun 1969; Mellinger 1972; Shure and Ragsdale 1977), we did not find the responses expected with such a regime (reduced WUE and PNUE under resource limitation). Additionally, the populations responded similarly to resource limitations, indicating no difference in ability to tolerate declining resource levels. Taken as a whole, the results do not support the hypothesis that PM and HR are locally adapted to the different drought regimes documented in the field populations. Much of the variation we observed in the field is likely plasticity, altering their phenotype in response to spatial and temporal variation in resources (Mellinger 1972; Lugo and McCormick 1981).

The population responses of instantaneous leaf WUE (measured as *A*/*g*) and time integrated leaf WUE (estimated from $\delta^{13}C$; Figure 3.4 e-f) to resource limitations were not consistent. While plants responded to resource limitation as expected for instantaneous WUE (plants in well-watered treatments, H_WH_N and H_WL_N, had lower instantaneous WUE than those in water-limited treatments, L_WH_N and L_WL_N), the same was not observed for integrated WUE. Measurements for both instantaneous and integrated WUE were taken on the most recently fully expanded leaf (MRFEL) which was produced after treatment conditions had been initiated. However, the gas exchange measurements were taken on day 3 of a dry down cycle, when stomata were closing in response to the water limitation, resulting in higher instantaneous WUE (Fig 3.4b). Because $\delta^{13}C$ is an integrated measure of WUE, it accounts for the life-time of the leaf at time of harvest (Farquhar, Ehleringer et al. 1989). The lack of a resource limitation treatment effect for leaf $\delta^{13}C$ may reflect that most of the CO_2 assimilation was taking place when soils were near field capacity and stomata were relatively open. The response of *H. porteri* stomata to declining water potentials is worth further investigation.

Given the differences in drought induced mortality that we previously found for field populations, and the geographical isolation of these populations, we expected to find evidence of local adaptation. However, here we find no evidence that these *H. porteri* are locally adapted to drought. Alternative explanations are that that the populations may be locally adapted to selective agents other than drought, that there is a more complex interaction among abiotic factors, or that there may be no local adaptation. In the case of the latter, genetic drift could have contributed to the genetic differentiation, although this would be relatively unexpected for the large populations in our study (1000's of individuals). While significant gene flow may limit the effects of selection in some environments (Lenormand 2002), if the selection pressure is great enough, plants may still adapt despite significant gene flow (Antonovics 1971). Although the current populations of *H. porteri* are isolated from each other, there were likely many more interspersed populations as recently as 50-60 years ago, which have since become extinct from environmental or human activity (e.g. quarrying, recreation; (Loehle 2006); S. Gevaert, pers. obs.). These intermediate populations may have facilitated historical gene flow among populations, thus causing a homogenizing effect of populations, limiting the potential for local adaptation.

Future studies are needed to assess what forces are driving genetic differentiation among these populations and if local adaptation is occurring, by what factors. A previous study with *Diamorpha smallii*, a winter annual endemic to granite outcrops of the southeastern U.S., inferred that populations are locally adapted to their isolated populations along a clinal gradient for numerous physiological and phenological traits related to light, temperature and water stress (McCormick and Platt 1964; Chapman and Jones 1975). Other abiotic factors, such as light and temperature, should be considered in attempts to determine what selective agents, if any, are influencing *H. porteri* differentiation, and reciprocal transplants would be necessary to find these differences (Hereford 2009).

Acknowledgments

We would like to thank numerous people who assisted during the final harvest, including Ethan Milton, Chase Mason, Kelly Bettinger, Rebecca Shirk, Cecile Deen, Cara Gormally, Britnie Foltz, Rachel Rogers, Joel Rabanal, Christopher Allen, and Philip Nowicki. Without their generous assistance, this research would not have been possible. We would also like to thank the University of Georgia Plant Biology greenhouse staff for assistance in plant germination and keeping plants alive during the experiment. Additional thanks also to University of Georgia Plant Biology department and National Science Foundation grant (0614737 to Lisa A. Donovan) for funds which supported this work.

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Table 3.1. ANOVA results for resource limitation study for *Helianthus porteri* with block as a fixed effect. Populations included CMR, PM, and HR. Treatments were H_WH_N, H_WL_N, L_WH_N, and L_WL_N. Type III sums of squares were used to calculate F values. **p* < 0.05, ***p* < 0.01, ***p< 0.001. Numerator degrees of freedom were 2, 3, and 6 for population, treatment and population x treatment, respectively. Denomination degrees of freedom were 67 for all traits.

	Population	***Treatment***	***Pop*Trt***
Effects	***F***	***F***	***F***
Plant Height	12.16***	11.72***	1.69
Stem Diameter	24.11***	12.17***	1.62
Aboveground Biomass	7.69**	27.92***	1.06
Reproductive Biomass	9.52***	36.49***	0.39
Root Biomass	9.87***	3.92*	0.98
Root:Total Biomass	4.77*	8.41***	0.49
Total Biomass	8.41***	29.19***	0.80
Time to First Bud	1.17	7.43***	2.47*
Time to first flower	1.28	7.40***	2.16
A (area basis)	23.05***	4.39**	0.88
A (mass basis)	4.41*	1.35	0.82
g	3.63*	4.77**	0.77
SLA (MRFEL)	5.42**	0.57	0.74
Leaf N	4.94*	25.41***	1.93
PNUE	4.21*	4.42**	0.79
Instantaneous WUE (A/g)	0.32	7.71***	0.59
δ^{13}C (Integrated WUE)	3.23*	0.58	0.52
Leaf Lifetime	0.61	40.78***	0.68

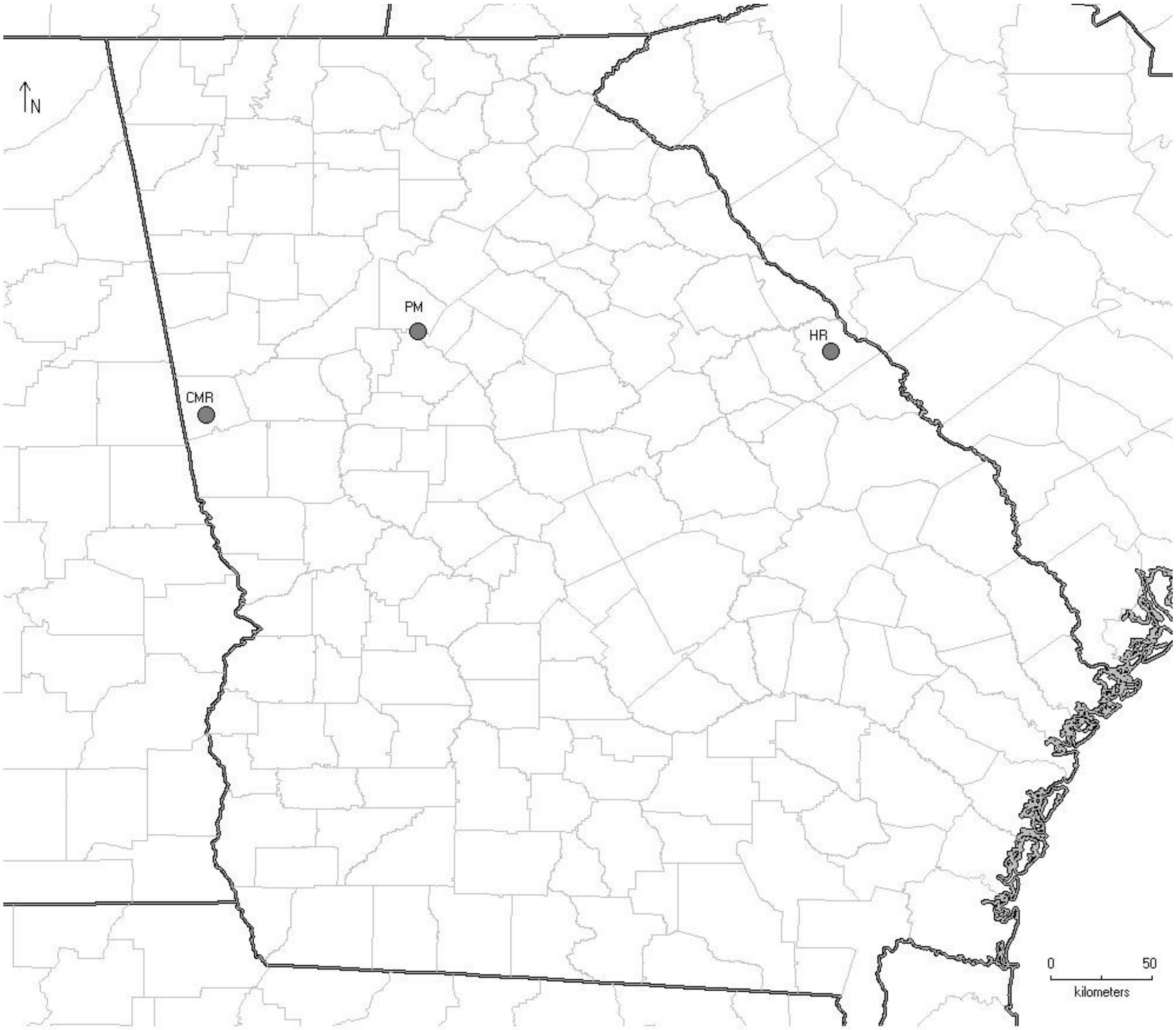

Figure 3.1. Regional map of *Helianthus porteri* study sites in Georgia.

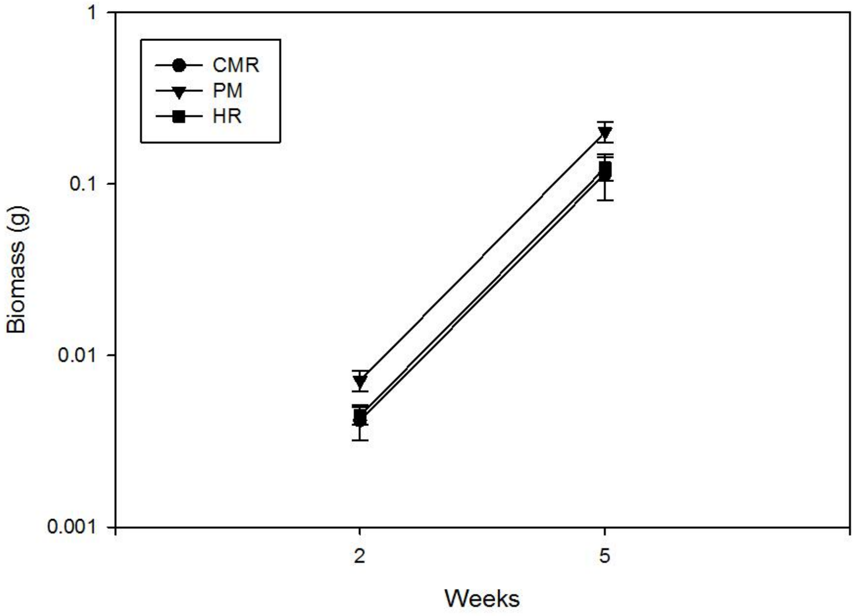

Figure 3.2. Biomass (LS Means ± SE) of seedlings for three populations of *Helianthus porteri* (CMR, PM, HR) over two harvests: the slopes indicate relative growth rate (RGRmax).

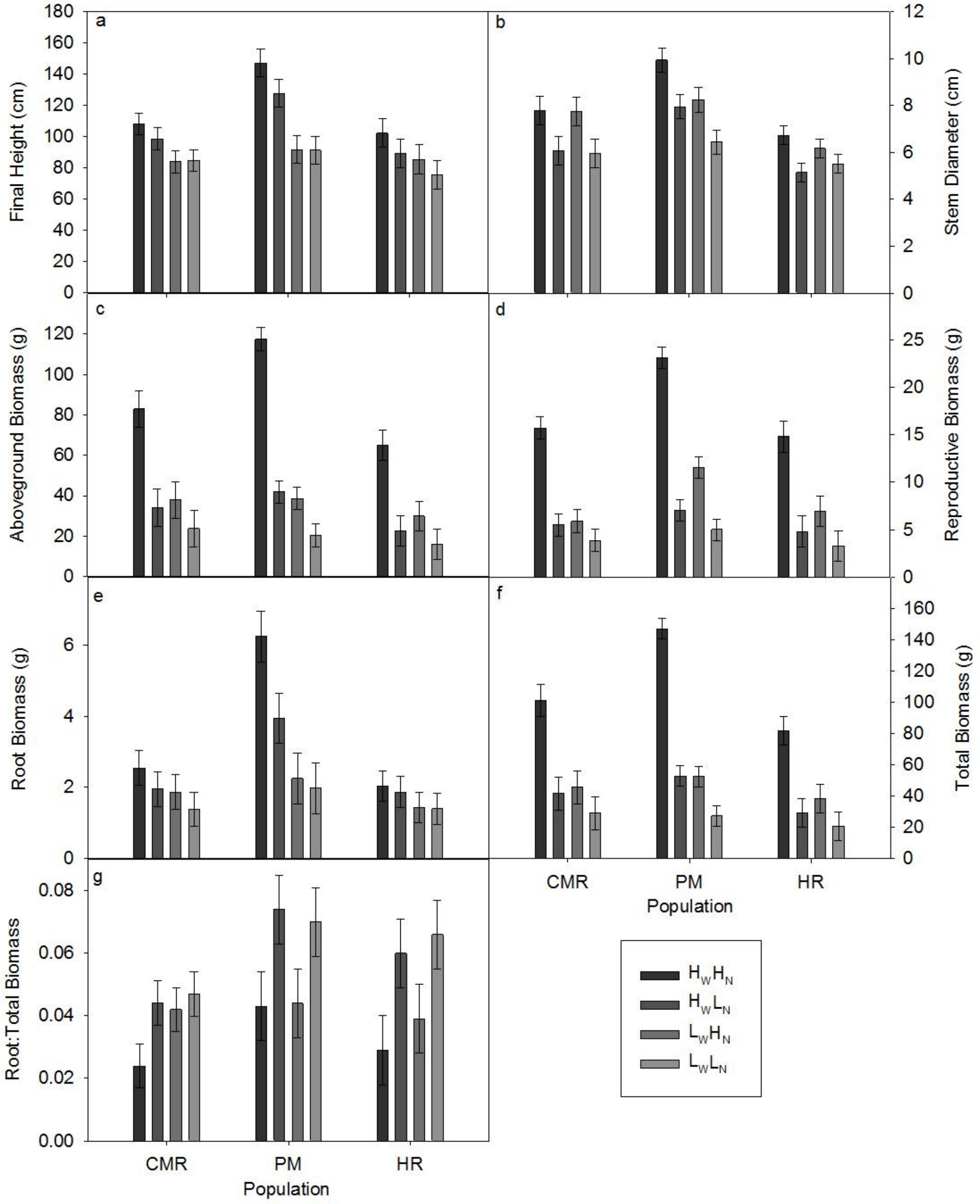

Figure 3.3. Plant growth and biomass traits at the final harvest for three populations of *Helianthus porteri* (CMR, PM, HR) growing in 4 resource treatments (H_WH_N, H_WL_N, L_WH_N, and L_WL_N, see legend in 3b): final plant height, stem diameter, aboveground biomass, reproductive biomass, root biomass, total biomass, root:total biomass ratio. LS-Means ± 1 SE for all traits.

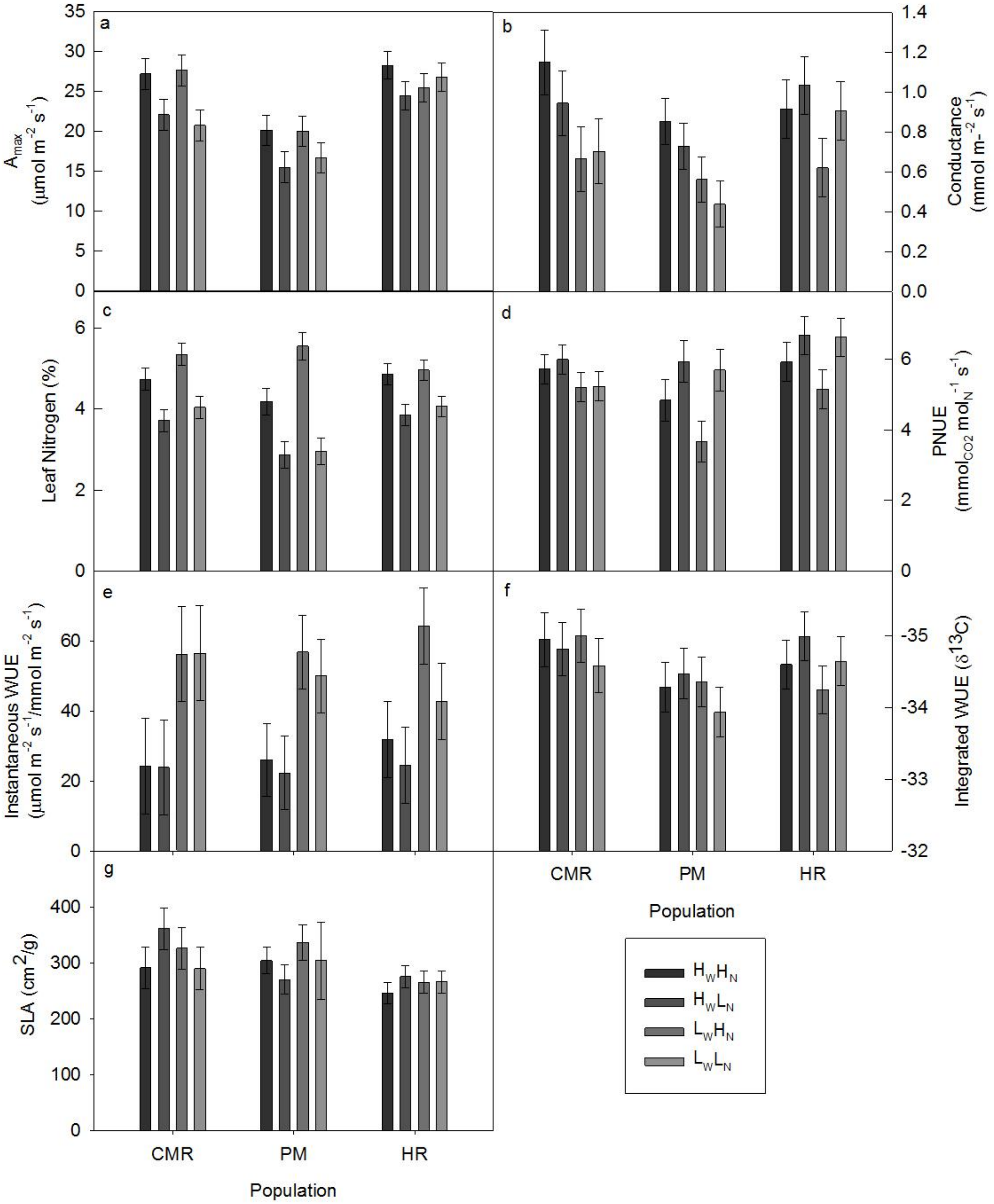

Figure 3.4**.** Plant ecophysiological traits measured once during the experiment for three populations of *Helianthus porteri* (CMR, PM, HR) and its 4 resource treatments (H_WH_N, H_WL_N, L_WH_N, and L_WL_N, see legend in 3.3b): maximum photosynthetic rate on an area-basis (A_{max}),

conductance (*g*), leaf nitrogen, photosynthetic nitrogen-use efficiency (PNUE), instantaneous water-use efficiency (*A*/*g*), integrated water-use efficiency (WUE as δ^{13}C), and specific leaf area (SLA). Populations are shown from left to right as their orientation from west to east in the state of Georgia (CMR, PM, HR). LS-Means ± 1 SE for all traits.

CHAPTER 4

POPULATION GENETICS OF *HELIANTHUS PORTERI*, AN ENDEMIC ANNUAL SUNFLOWER OF SOUTHEASTERN U.S. GRANITE OUTCROPS[1]

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[1] Gevaert, S.D., J.R. Mandel, J.M. Burke and L.A. Donovan. To be submitted to *Molecular Ecology*.

Abstract

Granite outcrops harbor many rare and endemic plant species. These plant species are frequently subjected to severe resource limitations, especially drought, and are also facing habitat loss as the result of human activity (e.g., quarrying, recreation). For *Helianthus porteri*, an endemic annual sunflower of granite outcrops, we have previously shown it to be severely limited by water availability, though there does not appear to be evidence for populations to be locally adapted for drought resistance. Here we investigate the level of genetic diversity and structure among populations of *H. porteri*. We used 18 EST-derived microsatellites from *H. annuus* to determine genetic diversity and structure within and among 12 populations of *H. porteri* across its range in the southeastern US. There is low genetic structure among populations (F_{ST}=0.084), but high genetic diversity within populations of *H. porteri*. Genetic diversity, measured as expected heterozygosity (H_e), ranged from 0.547-0.702, with a mean of 0.601 (± 0.015, SE) across loci. Compared to other species of *Helianthus*, including both perennials and annuals, these values of F_{ST} and H_e are similar. Despite geographic isolation of populations, we found no evidence for isolation by distance. A STRUCTURE analysis (K = 2) indicated that two populations are very similar to each other but very different from the other 10 populations. Populations of this endemic outcrop species have high levels of genetic diversity and low genetic differentiation among populations. These results suggest there may have been historically high levels of gene flow among populations or that presently there are more intermediate populations of *H. porteri* than are currently known. We also found that the populations on the western and eastern margins of the distribution had lower genetic diversity than those centrally located, which could indicate the need to conserve the peripheral populations. However, while there were some differences, the low level of genetic structure among populations is important for conservation

research since this suggests that the species can be relocated without the worry of local adaptation.

Introduction

Conservation genetics has important implications for the survival of a plant species as well as the potential for that species to be preserved. For example, the amount and partitioning of genetic diversity within a species influences the ability for its populations to respond to environmental changes. Intraspecific genetic variation is now a widely accepted parameter for determining population prioritization for protection (Petit, El Mousadik et al. 1998; Frankham, Ballou et al. 2002). Additionally, endemic species tend to have less diversity (both population and species level diversity) and greater genetic structure (Nybom 2004). Furthermore, annuals have greater species-level diversity than short-lived perennials, and outcrossing plant species (either animal or wind) have greater population-level diversity than selfing plant species (Nybom 2004).

Granite outcrops are geographically isolated habitats that occur as islands in the surrounding piedmont forest of the southeastern United States. These outcrops harbor many endemic and rare plant species, which grow in a resource limited environment (Burbanck and Platt 1964; McCormick and Platt 1964; Baskin and Baskin 1988). Species that occur in habitats which are geographically isolated likely have less gene flow. A reduction in gene flow can lead to population differentiation due to genetic drift or local adaptation. Thus, granite outcrop endemics should have high genetic structure among populations. Previous studies have investigated the genetic structure and diversity of plant species which occur on granite outcrops of the southeast, as well as other outcrop habitats (e.g. limestone). Results are mixed with some species having higher than expected levels of genetic diversity (*Tradescantia hirsuticaulis*)

(Godt and Hamrick 1993) while others have low genetic diversity and high structure (*Arenaria uniflora*) (Wyatt, Evans et al. 1992) as would be expected.

Helianthus porteri is an endemic annual sunflower of granite outcrops of the southeastern US. It is found in the shallow soil pools of the annual-perennial zone. Unlike most outcrop plant species, it must persist through the hottest, driest part of the season to reproduce in fall, making it especially susceptible to environmental limitations. Additionally, *Helianthus porteri* has been determined to be "at risk" based on a floristic quality assessment index (FQAI). The FQAI classifies plant communities based on the traditional ecological concepts of richness and diversity, but further factors in a plant species' tolerance to disturbance or fidelity to a specific habitat (Swink and Wilhelm 1979; Andreas, Mack et al. 2004). The FQAI uses a coefficient of conservatism (C of C, scale of 0-10), which is subjectively assigned by trained botanists familiar with the geographic range, narrowness or breadth of ecological tolerance of a plant species. From preliminary data, *H. porteri* has recently been assigned a C of C value of 10 (indicating highest potential risk), due to its narrow ecological tolerance and its endemism to granite outcrops (Zomlefer, W., pers. comm.). While *H. porteri* is not listed as threatened or rare in any US state, the high C of C value indicates that with environmental and continued human disturbance, it would quickly become so.

Using expressed sequence tag-derived simple sequence repeat markers (EST-SSRs) to evaluate population genetics has been shown to be quite successful due to their ease of transfer among different, but related species within a taxonomic range (Ellis and Burke 2007). In one of the first studies to demonstrate the potential for this transferability SSRs were designed from *H. annuus* (the common sunflower) ESTs and were tested in two other *Helianthus* species, *H. verticillatus*, a rare perennial, and *H. angustifolius*, a widespread perennial sunflower (Pashley, Ellis et al. 2006). They found that EST-SSRs were more transferable across these species than anonymous SSRs and that these loci provide the possibility for use in other, related sunflower species. A study of *H. verticillatus*, and two closely related species, *H. angustifolius*, and *H. grosseratus* also successfully used these EST-SSRs to demonstrate that *H. verticillatus* is not of hybrid origin (proposed from the two other species) and that this rare sunflower has higher levels of genetic diversity than *H. angustifolius* (Ellis, Pashley et al. 2006).

Here we propose to use the aforementioned EST-SSRs in *H. porteri*, an endemic annual sunflower of southeastern US granite outcrops. We will investigate the genetic diversity and population structure of *H. porteri* and determine its potential as a species of interest for conservation efforts in the southeastern US. Additionally, we will determine how comparable these values are to other *Helianthus* species, which have been assessed using the same suite of EST-SSRs.

Materials and Methods

Study Species. *Helianthus porteri* (A. Gray) Pruski (Pruski 1998) is an annual, diploid (n=17), self-incompatible sunflower endemic to granite outcrops of the southeastern United States. Its natural range occurs from eastern Alabama, through the piedmont of Georgia and South Carolina, and it has been introduced to North Carolina (Shelton 1963; Mellinger 1972). It germinates in late March and flowers from late August to the first hard freeze. It has been shown to be capable of utilizing resources for quick growth and surviving under drought conditions for short intervals of time (Mellinger 1972; Lugo and McCormick 1981)(Gevaert and Donovan, in prep). It is found primarily in the annual-perennial zone of vegetation on granite outcrops, of which it comes to dominate in the fall as one of the few species not completing reproduction by early summer (McVaugh 1943; Burbanck and Platt 1964). Putative pollinators are primarily bees

(S. Gevaert, pers. obs.) and seeds are primarily gravity dispersed, though can be moved short distances through water run-off (Houle and Phillips 1988).

Collection of plant material and DNA extraction. Leaf material was collected from 10 native populations of *H. porteri* throughout the range of the species in Georgia in 2009 (Figure 4.1). Additionally, we sampled from one introduced population in eastern Georgia (PL) and from one introduced population in North Carolina (NC) in 2009. Harvested leaves were frozen at -80 C until DNA could be extracted. Total genomic DNA was isolated using the CTAB method (Doyle and J.L. 1987) from 24 individuals at each of the 12 populations. In the smallest populations (COM, PL) individuals were at least 0.5 meters apart, while in the largest populations, one individual was sampled from each soil island across the outcrop. All DNA samples were quantified using a NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA).

Selection of loci, PCR protocols, and genotyping. Eighteen of 22 EST-SSR loci developed for *Helianthus annuus* and found to be cross-trasnferable to *H. porteri* were chosen as genetic markers for this study. These loci were previously determined to amplify successfully in *H. verticillatus*, *H. angustifolius*, and *H. grosseratus* (Ellis, Pashley et al. 2006; Pashley, Ellis et al. 2006).

SSR genotyping was performed using a modified version of the fluorescent labeling protocol of (Schuelke 2000), and further detailed in Wills et al. (2005). Polymerase chain reaction (PCR) was performed in a total volume of 15 µL containing 10 ng of DNA for *H. porteri*, 30 mM Tricine pH 8.4-KOH, 50 mM KCl, 2 mM MgCl2, 100 µM each of dNTP, 0.1 µM M13 forward (-29) sequencing primer labeled with either HEX, FAM, or NED, 0.1 µM reverse primer, 0.01 µM forward primer and one unit of *Taq* DNA polymerase. The PCR conditions were as follows: 3 min at 95°C; 10 cycles of 30 s at 94°C, 30 s at 65°C, and 45 s at

72°C, annealing temperature decreasing to 55°C by 1°C per cycle; followed by 30 cycles of 30 s at 94°C, 30 s at 55°C, 45 s at 72°C, followed by 20 min at 72°C.

PCR products were diluted 1:50 and visualized on an ABI 3730xl DNA sequencer (Applied Biosystems) with MapMarker 1000 ROX size standard (BioVentures) included in each lane to allow for accurate fragment size determination. GeneMarker (v. 1.70; SoftGenetics) was used to call allele sizes for all individuals.

Data Analysis. Using the SSR data, descriptive population genetics statistics were calculated using GenAlEx (Version 6.2)(Peakall and Smouse 2006). These statistics included percentage of polymorphic loci, mean number of alleles per locus, and gene diversity (calculated as Nei's (1987) unbiased expected heterozygosity, H_e). Relationships among populations were graphically assessed via principal coordinate analysis (PCO; using GenAlEx) using pairwise genetic distances among all individuals in all 12 populations of *H. porteri* using the covariance standardized method. Population structure in *H. porteri* was investigated using the Bayesian clustering program STRUCTURE (Version 2.3.3)(Pritchard, Stephens et al. 2000). We used the admixture model and correlated allele frequencies parameter. For each analysis, K = 1-12 population genetic clusters were evaluated with 5 runs per K value, and the probability values were averaged across runs for each cluster. The initial burn-in period was set to 50,000 replicates with 10^6 MCMC iterations. This analysis was repeated and the results were found to be consistent across five runs. Population structure was also examined using analysis of molecular variation (AMOVA)(Excoffier, Smouse et al. 1992), as implemented in GenAlEx, which was used to hierarchically partition genetic variation and estimate F_{ST} (Wright 1951).

We compared genetic structure (F_{ST}) of *H. porteri* to two perennial sunflowers (*H. verticillatus* and *H. angustifolius*) and species-level diversity (H_e) of *H. porteri* to three perennial sunflowers (*H. verticillatus*, *H. angustifolius,* and *H. grosseratus*) using two-way analysis of variance (ANOVA; SAS version 9.2; SAS Institute, Cary, NC, USA) with species and locus as fixed effects. Data from the three perennial species was collected by Ellis et al. (2006) and used the same set of EST-SSRs as we did with with *H. porteri*. For an ANOVA analysis of F_{ST}, we used 11 shared loci, and F_{ST} was log-transformed to meet the assumption of normality of residuals and homogeneity of variance for residuals. For an ANOVA analysis of H_e, we used 10 shared loci, and H_e was arcsine-transformed.

Results

Genetic Diversity. All 18 EST-SSR loci were polymorphic in at least one population, though locus BL0018 was monomorphic in all populations except PL. The percentage of polymorphic loci varied from 88.89% (CMR) to 100% (PL), with a mean of 94.44%. Gene diversity per population ranged from 0.547 to 0.702 (as H_e; (Nei 1987) while species-level (pooled) H_e was 0.685 (± 0.055 SE). The mean number of alleles per locus was 6.565 ± 0.205 (mean ± SE) for the species, with an effective number of alleles of 3.385. Estimates of genetic diversity, as number of alleles per locus (A), observed heterozygosity (H_o), expected heterozygosity (H_e), and the inbreeding coefficient (F) were made for each population (Table 4.1). Values for A, H_o, and H_e were all higher than values reported for plant species of similar strategies (annual and endemic; (Nybom 2004) . Analyses of variance were performed to determine population differences for A, H_o, H_e, and F. Locus was significant in all analyses, but significant population effects were only found for A and H_e (Figure 4.2), with populations on the western and eastern margins having lower A and H_e than centralized populations. All populations had at least 4 private alleles, of which, SM (19), MA (15), and COM (13) had the most, and WG (5) and HR (4) the fewest. Private alleles were found for all 18 loci, with BL-10 (16) and BL-27

(14) having the most and BL-2, BL-18, and BL-22 having one each. Nine private alleles occurred at a frequency of 0.075 or greater.

Population Structure. The *H. porteri* populations demonstrated a low to moderate level of population structure (F_{ST} = 0.084, $p < 0.001$), though less so than expected given the geographically isolated nature of populations. This value is also lower than expected as compared to other values reported for plant species of similar strategy (annual, out-crossing, and endemic)(Nybom 2004). Pairwise population values were all significantly different from zero (Table 4.2, $p < 0.01$) with the greatest structure between CMR and PL (F_{ST} = 0.144), CW and PL (F_{ST} = 0.139), and WG and PL (F_{ST} = 0.130). The results of a principal coordinates analysis (PCO) plotting individuals within populations revealed that PL and HR are most different from the other 12 populations, but most similar to each other, separating along PCO1 (PCO1: 43.09%, PCO2: 13.80%; Figure 4.3). A Mantel test was performed to check for isolation by distance, however, none was found (Rxy = 0.054, p = 0.28). The STRUCTURE analysis with K = 2 indicated that HR and PL were very similar, as were CMR and CW, and both pairs were considerably different from all other populations of *H. porteri* (Fig 4.4). Values of ΔK confirm that K = 2 was the best K value (per Evanno et al 2005).

Several loci in each of the populations were found to be significantly out of Hardy-Weinberg equilibrium. Six loci were consistently out of Hardy-Weinberg equilibrium: BL0002, BL0003, BL0004, BL0020, BL0023, and BL0025. All values of F_{IS} were greater than zero. Biparental inbreeding is unlikely given that *H. porteri* is self-incompatible. Instead this may be attributable to both null alleles at loci and/or the Wahlund Effect. In the case of the latter, the smallest populations (in size and area), COM and PL, had the lowest F_{IS} values (0.186 and 0.183, respectively).

Genetic Diversity and Structure for Natural Populations. We also removed the introduced populations (PL, NC) from analyses to determine what effect they might have on the genetic diversity and structure observed. We found very little difference in the diversity parameters. Values for H_e and A increased slightly, though they were not significant ($H_e = 0.604 \pm 0.017$; $A = 6.617 \pm 0.233$). We also found F_{IS} increased slightly (0.251 ± 0.022), but they were not different from analyses with all 12 populations. We also found that genetic structure decreased ($F_{ST} = 0.077$) but this was not significantly different from the structure observed with all 12 populations ($F_{ST} = 0.084$).

Species Comparisons. We compared *H. porteri* to three perennial sunflowers, *H. angustifolius*, *H.verticillatus*, and *H. grosseratus* for H_e (pooled species value) and F_{ST}. We found no species differences for F_{ST} (population effect: $F_{2,34} = 0.36$, locus effect: $F_{12,34} = 1.36$). However there was a significant species effect for H_e, with *H. porteri* having a significantly greater H_e than *H. angustifolius* (Table 4.3; $F_{3,73} = 4.39$, $p < 0.01$; locus effect: $F_{19,73} = 3.06$, $p < 0.001$). Measures of gene diversity reported in Ellis et al. (2006) for the annual, wild *H. annuus* (range-wide) are 0.57 ± 0.02 and are comparable to those for *H. porteri*, indicating that the measures of diversity in annual sunflowers are comparable with these markers.

Discussion

Given what is known about the geographic isolation of populations and relative rarity of *H. porteri*, we expected to find lower genetic diversity within populations and greater genetic structure among populations. Instead, we found the opposite. Levels of genetic diversity (species as pooled and averaged across populations; H_e; see Table 4.1), were higher than species of similar life history (annual and endemic; (Nybom 2004) , with *H. porteri* ranging from 0.547 to 0.702. There was no isolation by distance found, which is consistent with the low genetic structure observed among populations, in that populations are more similar than expected based on geographic isolation. The maintenance of genetic diversity in this species may be a related to its ecology since it is self-incompatible, which functions to slow the loss of diversity. Additionally, it is known that *H. porteri* has an extensive seed bank (Houle and Phillips 1988), which indeed may function to harbor additional genetic diversity and explain the results seen here for higher than expected levels of genetic diversity. Most notably, the HR population suffered an extensive drought in 2008, which caused 100% mortality of all *H. porteri* individuals prior to flowering (Gevaert & Donovan, in prep). The following season, *H. porteri* regenerated with no obvious decline in population size from previous years, indicating that the seed bank is indeed vast and still remarkably viable.

Contrary to expectations for species on geographically isolated granite outcrops, it appears that significant gene flow is occurring among populations of *H. porteri*. Gene flow would account for the low genetic structure among populations, indicating that populations are similar genetically, and would also account for the high genetic diversity within populations. We also found that two populations, PL and NC, which were introduced from the MA population (early 1960's), did not share the same level of genetic diversity as their parent population (Table 4.1). In addition, the PL population appears to be more similar to the neighboring HR population, as was found from the principle coordinates analysis (Fig 4.3), STRUCTURE analysis (Fig 4.4) and pairwise F_{ST} (0.024; Table 4.2). These populations (PL, HR) are separated by ~1.5 km, and it appears that gene flow is high between the two populations. It is known that *H. porteri* is self-incompatible and numerous pollinators have been observed visiting flowers including small bees, moths and butterflies (S. Gevaert, pers. obs.). Pollinators have been known to move pollen long distances in other plant species (Broyles, Schnabel et al. 1994), indicating the potential for long distance gene flow among populations. Additionally, the data suggest that there may be additional unknown populations further contributing to gene flow among populations.

This pattern of gene flow may also contribute to the differences in genetic diversity between peripheral populations (CMR, CW, PL, HR) and centralized populations (Fig 4.1). We found peripheral populations to have lower H_e as well as a lower A. Theoretical studies indicate that A is more likely to be reduced due to stochastic events, such as those that occur at range limits, than H_e (Nei, Maruyama et al. 1975). Rare alleles have a greater influence on A than on H_e, and are more likely to be lost during population bottlenecks and fluctuations in population size, which are more common at range limits (Durka 1999; Eckert, Samis et al. 2008). Eckert et al. (2008) report that either estimate (H_e or A) when reported for central and peripheral populations indicate similar trends and thus are both accurate estimates for the reduction of genetic diversity at range limits. Furthermore, reduced genetic diversity at the edge of a species distribution may not be consistent with increased genetic structure (Eckert et al., 2008), as was observed with *Helianthus porteri*. The differences in genetic diversity at the periphery may be a function of greater isolation from the range center, potentially resulting in more limited gene flow, selection or maladaptation.

The results of the STRUCTURE analysis further indicate that all populations are fairly similar with two exceptions: PL (introduced from MA, early 1960's; (Mellinger 1972) and HR. This is consistent with the pairwise F_{ST}, which showed PL and HR ($F_{ST} = 0.024$) to be most similar, and least similar to all other populations (Table 4.2). The CMR and CW populations appear to share a similar amount variation to each other, compared to other populations, but not as obviously so as PL and HR given the CMR-CW pairwise F_{ST} was higher (0.081). This may be due in part to the greater geographic distance between CMR and CW (~12 km, compared with

~1.5 km for PL and HR) and because there are fewer intermediate populations of *H. porteri* not sampled in this study between CMR and CW to increase gene flow between the populations. While seeds are gravity dispersed, it is possible that birds may be able to disperse seeds between granite outcrops, contributing to a small amount of gene flow via seed dispersal.

Another possibility regarding the lack of genetic structure observed is recent habitat loss. Within the last 50-100 years, quarrying activity has continued to occur in the southeastern United States, especially in the state of Georgia. This activity has caused the extinction of some granite outcrop plant communities. Other human activities, including recreational-use (as in large parks near Atlanta), trash dumps, and covering of exposed granite by land-owners, have also caused the extinction of some intermediate populations. These intermediate populations likely contributed to historical gene flow among populations, accounting for the lower levels of genetic structure observed. Since these extinctions are relatively recent events, it is possible that not enough time has passed for genetic drift to have affected diversity. It is important to note that 5 of the 12 populations are of large size (1000's of individuals) and due to the relatively large size, the effects of drift at these populations may be lessened (Ellstrand and Elam 1993).

Conservation of granite outcrop species is of great importance in the southeastern United States. As granite outcrops continue to fall to human impacts, loss of species diversity becomes a greater issue. Having found reduced genetic diversity at the periphery of the *H. porteri* distribution, it is promising that two of these populations are already conserved (CMR, HR; Nature Conservancy). Additionally, having found lower levels of genetic structure than expected, it would be possible to attempt to re-colonize granite outcrops which have already lost *H. porteri*, or current populations which may suffer random stochastic events resulting in a loss of diversity.

Acknowledgments

We wish to thank Rebecca Shirk, Jeremy Rentsch, and Tyler Kartzinel for assistance in collecting and analyzing microsatellite data. Special additional thanks to Rebecca Shirk for help in creating a population map, as well as providing valuable feedback on early drafts of this manuscript, with Cecile Deen, Alan Bowsher, and Ethan Milton contributing to manuscript review as well. Tom Patrick and Nikki Castleberry of the Georgia Department of Natural Resources, Malcolm Hodges of the Nature Conservancy, and Jim Allison provided additional assistance in either locating populations of *Helianthus porteri* or providing permits for leaf tissue collection. National Science Foundation grant (0614737 to Lisa A. Donovan) provided funds for this research.

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Table 4.1. Mean ± 1 SE for 12 populations of *Helianthus porteri* for 18 loci, and the species grand mean. Values are averaged over all loci in each population. *P*, is the percent polymorphic loci; *A*, mean number of alleles per locus; H_O, mean observed heterozygosity; H_E, mean expected heterozygosity; *F*, within population coefficient of inbreeding.

Population (Arranged west to east)	***P***	***A***	H_O	H_E	***F***
CMR	88.89%	5.944 (0.716)	0.427 (0.054)	0.547 (0.060)	0.205 (0.059)
CW	94.44%	5.333 (0.505)	0.411 (0.062)	0.554 (0.053)	0.225 (0.088)
PM	94.44%	6.889 (0.646)	0.408 (0.059)	0.630 (0.053)	0.346 (0.073)
MA	94.44%	8.056 (0.777)	0.523 (0.063)	0.702 (0.048)	0.270 (0.064)
SM	94.44%	7.889 (0.893)	0.535 (0.067)	0.641 (0.057)	0.214 (0.072)
IBR	94.44%	6.389 (0.622)	0.477 (0.063)	0.608 (0.055)	0.223 (0.075)
COM	94.44%	6.889 (0.918)	0.477 (0.052)	0.598 (0.059)	0.186 (0.052)
WG	94.44%	6.611 (0.611)	0.413 (0.047)	0.631 (0.050)	0.329 (0.061)
RS	94.44%	6.611 (0.719)	0.415 (0.056)	0.576 (0.052)	0.252 (0.073)
PL	100.0%	5.667 (0.572)	0.481 (0.057)	0.558 (0.047)	0.183 (0.081)
HR	94.44%	5.556 (0.776)	0.422 (0.057)	0.550 (0.055)	0.262 (0.081)
NC	94.44%	6.944 (0.508)	0.483 (0.048)	0.616 (0.052)	0.208 (0.045)
Grand Mean (averaged across populations)	94.44%	6.565 (0.205)	0.456 (0.016)	0.601 (0.015)	0.242 (0.020)
Pooled species level	100.0%	18.889 (2.278)	0.459 (0.047)	0.685 (0.055)	0.365 (0.058)

Table 4.2. Pairwise population F_{ST} values from the analysis of molecular variance and 999 permutations for *Helianthus porteri*. Both natural and introduced populations are included. Population abbreviations are shown in the first row and last column, with populations following a west to east geographical gradient. All values are significant at $p < 0.001$, unless noted as $^*p < 0.01$.

CMR	**CW**	**PM**	**MA**	**SM**	**IBR**	**COM**	**WG**	**RS**	**PL**	**HR**	**NC**	
0.000												**CMR**
0.081	0.000											**CW**
0.105	0.104	0.000										**PM**
0.095	0.093	0.037	0.000									**MA**
0.082	0.058	0.044	0.047	0.000								**SM**
0.073	0.064	0.063	0.071	0.017*	0.000							**IBR**
0.067	0.063	0.053	0.046	0.029*	0.033	0.000						**COM**
0.110	0.108	0.053	0.035	0.065	0.084	0.059	0.000					**WG**
0.126	0.110	0.079	0.074	0.078	0.083	0.077	0.075	0.000				**RS**
0.144	0.139	0.104	0.090	0.093	0.105	0.087	0.130	0.114	0.000			**PL**
0.127	0.125	0.098	0.096	0.076	0.083	0.073	0.124	0.112	0.024*	0.000		**HR**
0.112	0.110	0.098	0.090	0.075	0.085	0.066	0.110	0.093	0.117	0.103	0.000	**NC**

Table 4.3. Species comparisons for genetic diversity (H_e) and genetic structure (F_{ST}; mean (± SE)) for the annual sunflower *Helianthus porteri* and three perennial sunflowers, *H. angustifolius*, *H. verticillatus*, and *H. grosseratus*. Data for *H.grosseratus* was not available for F_{ST}. Only 11 shared loci (for all species) were used to calculate the values. The F_{ST} presented in the text for *H. porteri* (0.084) accounts for all 18 loci used with that species.

Species	***Genetic Diversity (H_e)***	***Genetic Structure (F_{ST})***
H. porteri	0.605 (0.061)	0.109 (0.044)
H. angustifolius	0.343 (0.061)	0.170 (0.044)
H. verticillatus	0.486 (0.061)	0.112 (0.044)
H. grosseratus	0.444 (0.063)	N/A

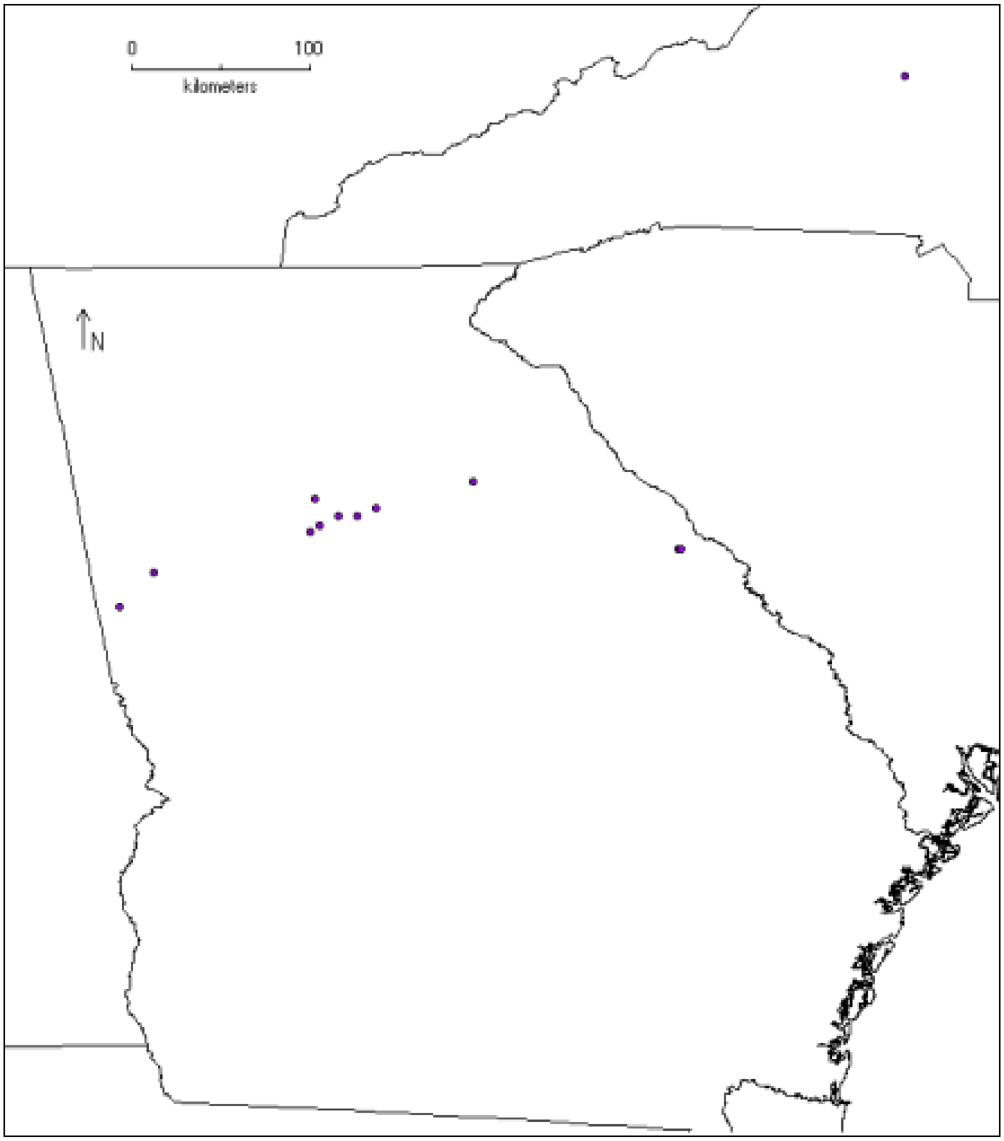

Figure 4.1. Collected populations of *Helianthus porteri*. Each dot indicates the location of one population.

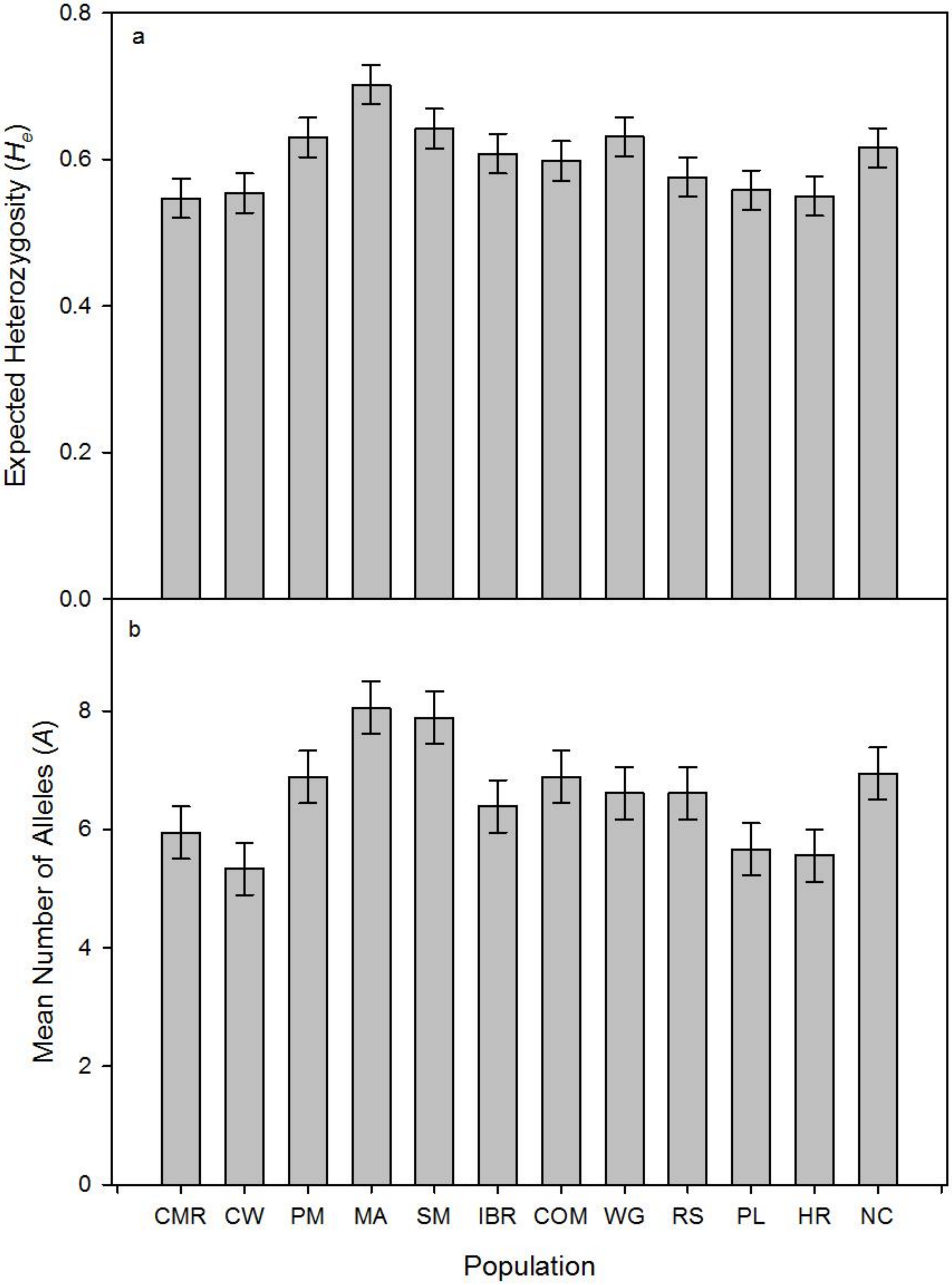

Figure 4.2. Population comparisons of expected heterozygosity (H_e) and mean alleles per locus (A) for *Helianthus porteri*.

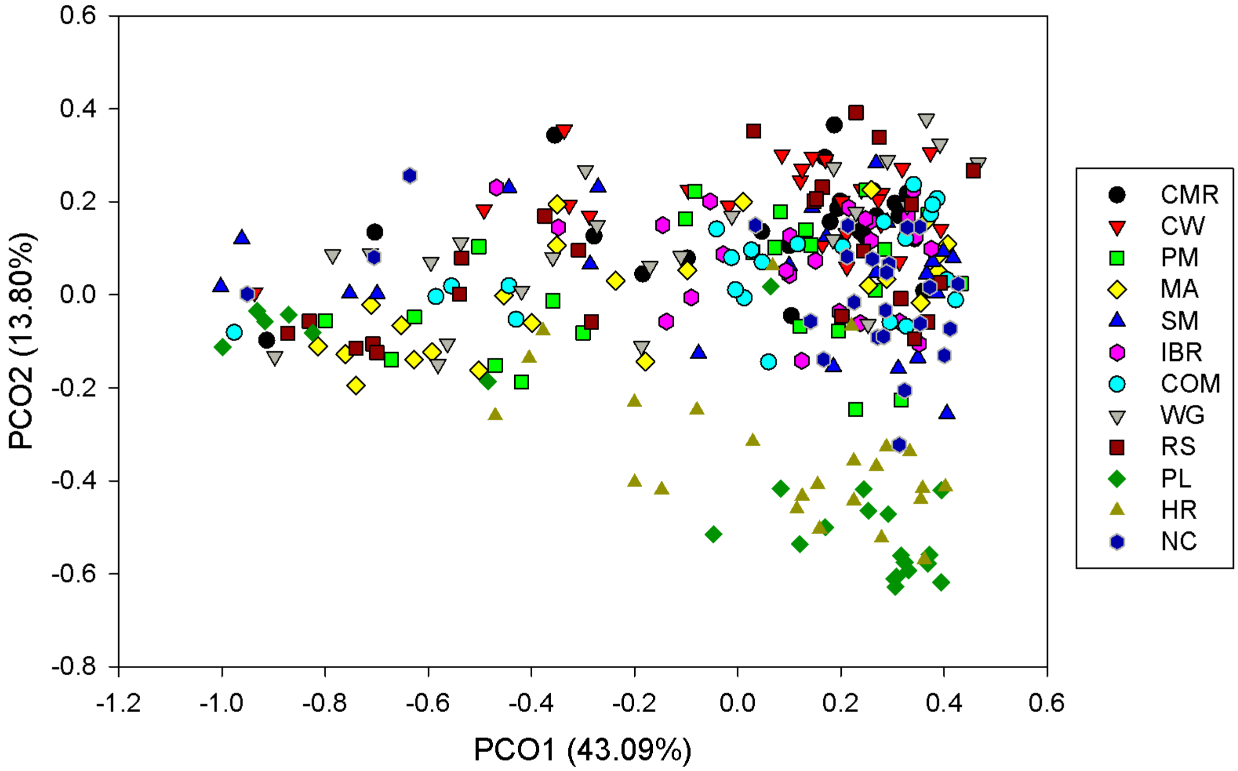

Figure 4.3. Principal coordinate analysis (PCO; using GenAlEx) representing relationships among individuals in all 12 populations of *H. porteri* using genetic distances in the covariance standardized method. Populations are listed in the legend according to a west to east gradient (CMR: western-most, NC: eastern-most populations).

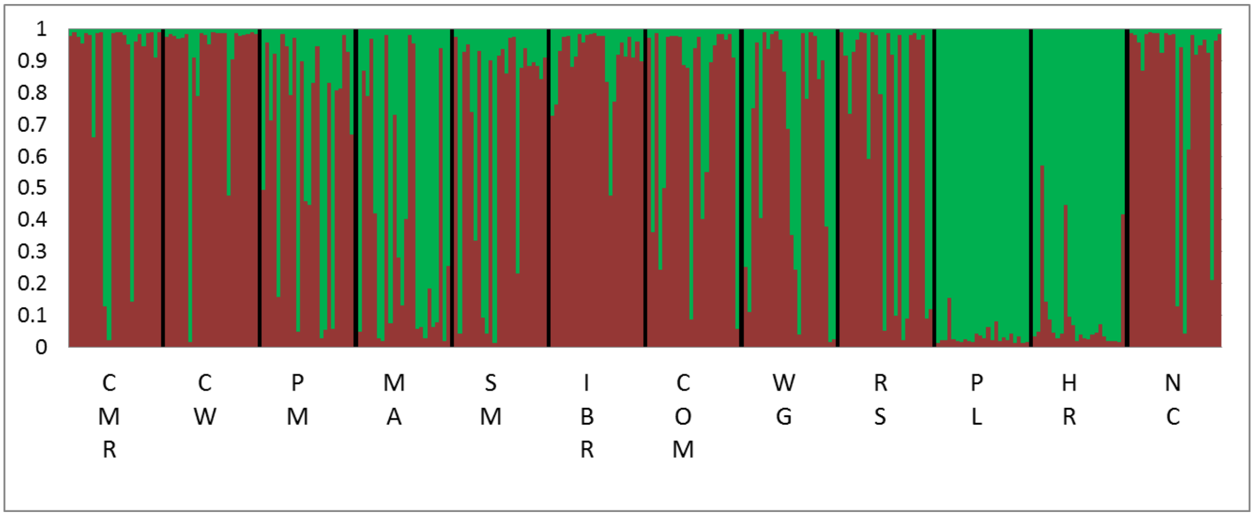

Figure 4.4. Results of the STRUCTURE analysis with $K = 2$ corresponding to 12 populations of *Helianthus porteri*. Bars for each individual indicate the average result across five independent iterations. Populations are presented in a west to east geographical gradient.

CHAPTER 5

CONCLUSIONS

Here I sought to determine the potential for adaptive differentiation in response to drought in *Helianthus porteri*. Studies focusing on the potential for adaptation to low resource environments are being used to determine the prevalence of adaptive differentiation which may lead to local adaptation on multiple scales (Hereford 2009; Donovan, Maherali et al. 2011). *Helianthus porteri* is an endemic annual sunflower on geographically isolated granite outcrops of the southeastern United States, which are known to be resource limited (Shelton 1963) and therefore serve as an excellent study system to assess population differentiation. By combining field and lab techniques I was able to determine that drought was a selective agent driving population differentiation in *H. porteri*. However, while I found that populations are differentiated for traits related to drought, I did not find any evidence to suggest that populations of *H. porteri* may be locally adapted in their response to drought. Additionally, there were low levels of genetic structure among populations, and considerably high levels of genetic diversity within populations. Taken together, this suggests that while drought is a selection agent on *Helianthus porteri* populations, gene flow may be counteracting the effects of selection among populations. Future studies, such as reciprocal transplants, are needed to specifically test for local adaptation to drought or any other selective agents. In the first chapter, I posed a series of questions that were addressed in this dissertation. Here I revisit those questions and summarize my findings.

Is there differential performance among populations of H. porteri for drought resistance and is it indicative of avoidance or tolerance?

I followed *H. porteri* performance in three populations for three years, and in relatively wet and dry habitats within each population for traits related to growth, plant water status and survival. I found population differences in plant growth and survival were greatest in the driest years (2008 and 2010), with no differences in 2009 when there was average precipitation. Panola Mountain (PM) maintained greater growth and survival than Heggie's Rock (HR) during both dry years, and dry habitats were additionally reduced in growth and survival during dry years. Survival to flowering was found to be correlated with soil water availability across all populations and habitats. I conclude that drought stress is a selective agent that could drive adaptive differentiation of populations of *H. porteri*, with HR likely to have the greatest response through drought avoidance and PM adapted to less water limited conditions.

Are populations genetically differentiated for ecophysiological and growth traits for drought resistance?

I examined the same three populations of *H. porteri* as in the field study to determine the genetic basis for population differences observed in the field with regard to drought stress as a potential selective agent. I found that the populations are differentiated for growth and ecophysiological traits under optimal resource conditions (well watered, optimal nutrients). Mature plants from PM had greater growth than those of HR, which I expected. However, with the expectation of adaptive differentiation for drought stress, I expected plants from HR (greatest drought-induced mortality in dry years) to have a slower seedling maximum relative growth rate (RGRmax), but I found no population differences for seeding RGRmax. Additionally, I found PM to have lower maximum photosynthetic rate, stomatal conductance and to be more water-use efficient, against expectations for a population adapted to greater resource availability. Where significant interaction effects of population by treatment would have indicated differential response of populations to water or nutrient stress, I found none. Therefore, I conclude that while populations are differentiated, the patterns observed provide no support for adaptive differentiation to drought. Reciprocal transplants among the three populations are needed to determine if populations are locally adapted in response to drought or other selective agents.

How much genetic diversity does Helianthus porteri harbor and how is it partitioned among populations of this endemic outcrop species?

I expected to find relatively high genetic structure among populations based on the geographic isolation of granite outcrops and the endemism of *H. porteri* to outcrops (Shelton 1963; Nybom 2004). Instead I found low to moderate structure ($F_{ST} = 0.084$) among populations and relatively high levels of genetic diversity ($H_e = 0.601$, species level). I also found no evidence of isolation by distance. I did, however, find that populations on the periphery of the species range had lower levels of genetic diversity (as expected heterozygosity and mean number of alleles) compared to central populations, though they did not differ in genetic structure. This suggests that gene flow, while much higher than expected across the range of *H. porteri*, is more limited at the periphery, potentially causing a reduction in diversity there. I conclude that populations of *H. porteri* are more similar genetically than was expected from geographic isolation. This lower level of genetic structure among populations of *H. porteri*, while found with neutral markers, may indicate that local adaptation is less likely to occur..

Future Directions

While little evidence was found to suggest that populations are locally adapted in their response to drought from common garden studies, and that there is little genetic structure among populations to suggest that the underlying genetics of the species supports local adaptation, no direct tests of local adaptation were performed. Tests of local adaptation, such as reciprocal transplants (Knight, Vogel et al. 2006; Hereford 2009) and F_{ST}-Q_{ST} studies are needed to determine if populations are locally adapted to any factor, not just drought (Kawecki and Ebert 2004). The likelihood of populations being locally adapted as a result of drought is lessened given the results found in my common garden study. Having observed lower genetic diversity at the periphery of the *H. porteri* distribution, it suggests that these populations may be more likely to be locally adapted and should be considered in any future test of local adaptation (Eckert, Samis et al. 2008) (Hereford 2009). The potential for populations to be locally adapted has important implications for conservation and management of the species. If populations are locally adapted to any factor, re-introductions may be less likely to succeed in “foreign” habitats (Frankham, Ballou et al. 2002).

There has been on-going interest to mine wild sunflowers for traits to improve stress resistance of the related commercial sunflower, *Helianthus annuus (Seiler 1992)*. In the field study, I reported plant water status (as predawn water potential) to be similar to other mesic plants, indicating that the response to drought most likely occurs as avoidance. Since this avoidance is similar to other mesic plant species *H. porteri* is unlikely to harbor genes to improve drought resistance in commercial sunflower. However, I propose that future studies assess response to nutrient limitation in *H. porteri*. In my field study, I reported that populations of *H. porteri* vary considerably in soil nutrient composition, and previous studies have shown granite outcrops to be reduced in soil nutrient availability compared to surrounding ecosystems (Shure and Ragsdale 1977). This has the potential to be a source for improvement of cultivated sunflower as climate change leads to increased marginalization of growing environments (IPCC, 2001).

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