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Winkler, Daniel E.

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UNIVERSITY OF CALIFORNIA,
IRVINE

Effects of climate change on protected and invasive plant species

DISSERTATION

Submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Ecology and Evolutionary Biology

by

Daniel Edward Winkler

Dissertation Committee:
Professor Travis E. Huxman, Chair
Professor Brandon S. Gaut
Associate Professor Kailen A. Mooney
Assistant Professor Valerie A. Olson

2017

DEDICATION



Photo caption: A saguaro cactus (*Carnegiea gigantea*) rises above mesquite trees (*Prosopis velutina*) in Saguaro National Park, Tucson, Arizona. Photo by D. E. Winkler.

“...what their relations may be to the physical environment...to the air in the soil and its temperature, and what to the varying amounts of available water of the soil, as well as to the root-systems of adjacent plants, and the influence of these and other factors on the presence of plants in their peculiar habitats are among the most pressing problems of desert botany that await studious inquiry.”

-V. M. Spalding, *Distribution and movements of desert plants*, 1909

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CURRICULUM VITAE

Daniel Edward Winkler

Education

- Sept 2017 PhD, Ecology and Evolutionary Biology. Department of Ecology and Evolutionary Biology, University of California, Irvine. Advisor: Dr. Travis Huxman. Dissertation: Climate change effects on protected and invasive plant species.
- May 2013 MS, Environmental Systems. Environmental Systems Graduate Group, University of California, Merced. Advisor: Dr. Lara M. Kueppers. Thesis: A multi-level approach to assessing alpine productivity responses to climate change. [link](#)
- May 2008 BA, Biological Anthropology, cum laude. Department of Anthropology. New York University.

Peer-Reviewed Publications

5. **Winkler DE**, JL Conner, TE Huxman, DE Swann. *accepted*. The interaction of drought and habitat explain space-time patterns of establishment in saguaro (*Carnegiea gigantea*). *Ecology*
4. Conner, JL, T Foley, **DE Winkler**, DE Swann. 2017. Demographic changes over > 70 yr in a population of saguaro cacti (*Carnegiea gigantea*) in the northern Sonoran Desert. *Journal of Arid Environments* 139:41–48. doi: 10.1016/j.jaridenv.2016.12.008 [link](#)
3. Kudo G, Y Kawai, Y Amagai, **DE Winkler**. 2017. Degradation and recovery of an alpine plant community: experimental removal of an encroaching dwarf bamboo. *Alpine Botany* 127:75–83. doi: 10.1007/s00035-016-0178-2 [link](#)
2. **Winkler DE**, Y Amagai, TE Huxman, M Kaneko, G Kudo. 2016. Seasonal dry-down rates and high stress tolerance promote bamboo invasion above and below treeline. *Plant Ecology* 217(10): 1219–1234. doi: 10.1007/s11258-016-0649-y [link](#)
1. **Winkler DE**, KJ Chapin, LM Kueppers. 2016. Soil moisture mediates alpine life form and community productivity responses to warming. *Ecology* 97(6): 1553–1563. doi: 10.1890/15-1197.1 [link](#)

Other Publications

- 2016 **Winkler DE**. 16 August 2016. Improving earth systems models through research in the tropics. PLOS Ecology Community Blog. [link](#)
- 2016 CM MacKenzie and **DE Winkler**. 15 August 2016. Common gardens for all your climate change needs. PLOS Ecology Community Blog. [link](#)
- 2016 **Winkler DE**. 12 August 2016. Redundancy in response to global change: ecosystem processes buffered by functional diversity. PLOS Ecology Community Blog. [link](#)
- 2016 **Winkler DE**. Forget the supervolcano, let's talk buckwheat! In: *The Wonder of It All: 100 Stories from the National Park Service*. pp. 8–10. San Francisco, Yosemite Conservancy.
- 2015 **Winkler DE**. Spring 2015. Citizen scientists and drought: expected and unexpected measures curtailing the invasive Sahara mustard. In: Wildflowers! newsletter. pp. 3–4. University of California Cooperative Extension.

- 2015 **Winkler DE**. 16 January 2015. When water chokes the desert: finding an unseen benefit in California's drought stricken deserts. Water UCI Graduate Team Blog. [link](#)

Invited Talks

- 2017 **Winkler DE**. A changing Sonoran Desert: the iconic saguaro cactus and the invasive Sahara mustard. Anza-Borrego Desert State Park. Borrego Springs, California.
- 2016 **Winkler DE**. Desert "extremes" and plant communities out of balance. Anza-Borrego Desert State Park Botany Society. Anza-Borrego Desert State Park. Borrego Springs, California.
- 2016 **Winkler DE**, Chapin KJ, Garmon D, Huxman TE. Mustard mayhem! Pinning the origin and pathways of a highly invasive species. Mathias Symposium 2016. Oral Presentation. Bodega Marine Laboratory. Bodega Bay, California.
- 2015 **Winkler DE**, Filippone CL, Hubbard JA, Huxman TE, Swann DE. With climate change, where does it all go? "Historic" plant species and seasonal water sources. National Park Service Young Leaders in Climate Change Professional Development Symposium. Oral Presentation. Department of the Interior. Washington, D.C.
- 2015 **Winkler DE**, Chapin KJ, Huxman TE, Garmon D. Determining the origins of the invasive Sahara mustard (*Brassica tournefortii*) in North America. San Diego Botanic Garden. Oral Presentation. Encinitas, California.
- 2015 Aguilar L, Berry M, Brauch J, Mata Lara M, Ogletree S, Fitzgerald Weber K, **Winkler DE**, Wally Wright C, Young D. Integration of watershed monitoring and citizen science at San Juan Island National Historical Park. Oral Presentation. San Juan Island, Washington.
- 2015 **Winkler DE**, Chapin KJ, Huxman TE, Garmon D. Enabling long term solutions for biocontrol of Sahara mustard (*Brassica tournefortii*) invasion in North American Deserts. The Biodiversity and Invasive Species Network. Oral Presentation. Borrego Springs, California.
- 2014 Aldred J, Bendana S, Bijak A, Devantier J, Fillippone C, Haserodt M, Lancaster H, Luna Golya G, Schook D, **Winkler DE**. Dynamics of rare and sensitive waters of Saguaro National Park. George Wright Society Park Break: Saguaro National Park. Oral Presentation. Tucson, Arizona.

Contributed Presentations

- 2017 **Winkler DE**, Conner JL, Huxman TE, Swann DE. Hydrologic refugia for establishment of the keystone saguaro cactus during drought. Ecological Society of America 102nd Annual Meeting. Oral Presentation. Portland, Oregon.
- 2017 **Winkler DE**, Chapin KJ, Kao M, Garmon D, Huxman TE. Local adaptation during the rapid expansion of the invasive Sahara mustard in the southwest. Ecological Society of America 102nd Annual Meeting. Oral Presentation. Portland, Oregon.
- 2017 Chapin KJ, **Winkler DE**, Agnarsson I. Island biogeography and ecological modeling of the amblypygid *Phrynus marginemaculatus* in the Florida Keys archipelago. Ecological Society of America 102nd Annual Meeting. Oral Presentation. Portland, Oregon.
- 2017 **Winkler DE**, Chapin KJ, Huxman TE. Introduction of Sahara mustard (*Brassica tournefortii*) in North America: Evolutionary and environmental mechanisms promoting invasive species success. The XIX International Botanical Congress. Oral Presentation. Shenzhen, China.

- 2017 **Winkler DE**, Amagai Y, Kaneko M, Huxman TE, Kudo G. Bamboo invasion above and below a treeline ecotone: seasonal soil moisture dry-down rates and high stress tolerance promote invasion success of *Sasa kurilensis*. The XIX International Botanical Congress. Oral Presentation. Shenzhen, China.
- 2017 **Winkler DE**, Filippone C, Hubbard JA, Swann D. Seasonal water use of Saguaro cacti (*Carnegiea gigantea*) and their nurses. George Wright Society Biennial Conference. Oral Presentation. Norfolk, Virginia.
- 2017 **Winkler DE**, Chapin KJ, Huxman TE. The unusual population structure of a pervasive invasive: Sahara mustard in National Parks. George Wright Society Biennial Conference. Oral Presentation. Norfolk, Virginia.
- 2017 Brooks E, **Winkler DE**. Drought at Extremes: Connecting Biological, Socio-Economic, and Cultural Extremity in a Water Dependent Ecology. Oral presentation as part of panel titled: Water Traditions and New Directions for Drought: Risk and Disaster in Southern California Water. Society for Applied Anthropology Annual Meeting. Santa Fe, New Mexico.
- 2016 **Winkler DE**, Chapin KJ, Garmon D, Huxman TE. Using population genomics to uncover the rapid colonization of Sahara mustard (*Brassica tournefortii*) in the United States. California Invasive Plant Council Symposium. Oral Presentation. Tenaya Lodge at Yosemite, California (**1st place Student Paper Competition**)
- 2016 Sforza RFH, Augé M, Marini F, Bon MC, **Winkler DE**, M Cristofaro, L Smith. Biocontrol of Sahara mustard: An updated on exploration in the native range. California Invasive Plant Council Symposium. Oral Presentation. Tenaya Lodge at Yosemite, California.
- 2016 **Winkler DE**, Chapin KJ, Garmon D, Huxman TE. Genomic analyses of the rapid colonization of Sahara mustard in the US. Ecological Society of America 101st Annual Meeting. Oral Presentation. Fort Lauderdale, Florida.
- 2016 **Winkler DE**, Brooks E, Huxman TE, Olson VA. Extremes of Borrego: biological and social life in a changing desert ecosystem. Ecological Society of America 101st Annual Meeting. Poster Presentation. Fort Lauderdale, Florida.
- 2016 **Winkler DE**, Chapin KJ, Garmon D, Huxman TE. Population genomics and the rapid colonization of Sahara mustard (*Brassica tournefortii*) in the United States. Botany 2016. Oral Presentation. Savannah, Georgia.
- 2015 Jabis M, **Winkler DE**, Kueppers LM. Warming contracts flowering phenology in an alpine ecosystem. American Geophysical Union Fall Meeting. Poster Presentation. San Francisco, California.
- 2015 **Winkler DE**, Goulden ML, Kudo G, Kueppers LM, Huxman TE. The complex future of parks: effects of climate change on invasive species in protected ecosystems. George Wright Society Biennial Conference. Oral Presentation. Oakland, California.
- 2015 Swann, D, Aldred J, Bendana S, Bijak A, Devantier J, Filippone C, Haserodt M, Lancaster H, Luna Golya G, Schook D, **Winkler DE**. Park Break Study of vital resources in Saguaro National Park. George Wright Society Biennial Conference. Oral Presentation. Oakland, California.
- 2015 **Winkler DE**. What goes up must come down: morphophysiological variation in an invasive Japanese dwarf bamboo. Ecology and Evolutionary Biology Graduate Student Symposium. Oral Presentation. University of California, Irvine.

- 2014 **Winkler DE**, Huxman TE, Kudo G. Morphological and physiological compensation promote climate-induced invasions above and below treeline. American Geophysical Union Fall Meeting. Poster Presentation. San Francisco, California.
- 2014 **Winkler DE**, Kudo G. Invasive species biology in a changing climate: insights from deserts to alpine tundra. Japan Society for the Promotion of Science Summer Symposia. Poster Presentation. Sokendai, Japan.
- 2013 **Winkler DE**, Kueppers LM. Productivity responses of an alpine plant community to climate changes: A multi-scale approach. Ecological Society of America 98th Annual Meeting. Oral Presentation. Minneapolis, Minnesota.
- 2012 **Winkler DE**, Kueppers LM. Plant Cover Responses to Simulated Climate Change in Alpine Plant Species. Ecological Society of America 97th Annual Meeting. Poster Presentation. Portland, Oregon.
- 2008 **Winkler DE**, Smith R. Seasonal reproductive potential and iteroparity of the burying beetle (Coleoptera: Silphidae). Summer Research Symposium. Rocky Mountain Biological Laboratory. Oral Presentation. Gothic, Colorado.

Grants & Awards

- 2017 Physiological Ecology Section Travel Award, Ecological Society of America, \$500
- 2017 Graduate Fellowship Award, Ayala School of Biological Sciences, UC Irvine, \$1000
- 2017 American Alpine Club Research Grant, \$500
- 2017 Forrest Shreve Student Research Fund, Ecological Society of America, \$1200
- 2017 International Botanical Congress Award, NSF & Botanical Society of America, \$2400
- 2017 George Melendez Wright Student Travel Scholarship, George Wright Society, \$1175
- 2017 Graduate Assistance in Areas of National Need Research Grant, \$2075
- 2016 1st Place Student Paper Competition, California Invasive Plant Council, \$250
- 2016 Associated Graduate Students Travel Scholarship, UC Irvine, \$400
- 2016 PLOS Ecology Reporting Fellowship, \$500
- 2016 Tucson Cactus and Succulent Society Research Grant, \$2000
- 2016 Explorers Club Exploration Fund – Mamont Scholar, \$1400
- 2016 Real Brown Travel Award, Ecological Society of America, \$300
- 2016 NCAR Graduate Workshop on Environmental Data Analytics Award
- 2016 Graduate Assistance in Areas of National Need Research Grant, \$2146
- 2016 Associated Graduate Students Travel Scholarship, UC Irvine, \$400
- 2016 Mathias Symposium Award, UC Natural Reserve System \$600
- 2016 Western National Parks Association Research Grant, \$7476
- 2015 ARCS Award Nominee, UC Irvine School of Biological Sciences
- 2015 COTE Cluster of Excellence Summer School Award, U. of Bordeaux, France \$3,000
- 2015 California Drought Scholarship, Water UC Irvine & AGU Chapman Conference, \$325
- 2015 George Melendez Wright Student Travel Scholarship, George Wright Society, \$1150
- 2015 Associated Graduate Students Travel Scholarship, UC Irvine, \$400
- 2015 George Wright Society Park Break Fellowship, \$3000
- 2014 Howie Wier Memorial Conservation Grant, Anza-Borrego Foundation, \$1500
- 2014 School of Biological Science Travel Grant, UC Irvine, \$500
- 2014 Hardman Native Plant Award, California Native Plant Society, \$550
- 2014 Joshua Tree National Park Robert Lee Graduate Student Research Grant, \$3752
- 2014 Anza Borrego Foundation Student Travel Scholarship, \$190

- 2014 Water UCI/ Jet Propulsion Laboratory Research Fellowship, \$5000
- 2014 Mayhew Graduate Research Award, Boyd Deep Canyon Desert Res. Center, \$1166
- 2014 George Wright Society Park Break Fellowship, \$3000
- 2014 Annetta Carter Memorial Fund, California Botanical Society, \$1000
- 2014 UC MEXUS Student and Postdoctoral Research Small Grant, \$1500
- 2014 Mildred E. Mathias Graduate Student Research Grant, \$2860
- 2012 Sigma Xi Associate Membership
- 2011 Star Award, US National Park Service, \$750
- 2011 Science Program for Excellence in Science – AAAS
- 2009 AmeriCorps Education Award, \$1000
- 2008 University Honors Scholar, New York University
- 2004–08 New York University, Dean’s List
- 2004 New York University, Residence Hall Leadership Award

Academic Fellowships

- 2017 Ayala School of Biology Sciences Graduate Fellowship Award, UC Irvine, \$1000
- 2017 Public Impact Distinguished Fellowship, UC Irvine, \$12,000
- 2017 Steele/Burnand Anza-Borrego Desert Research Center Fellowship, \$22,139
- 2016 Newkirk Graduate Student Fellowship, Newkirk Center for Science & Society, \$6889
- 2016 GAANN – Graduate Assistance in Areas of National Need Fellowship, \$32,476
- 2016 GAANN – Graduate Assistance in Areas of National Need Fellowship, \$13,600
- 2015 UC Irvine Data Science Initiative Graduate Fellowship, UC Irvine, \$6039
- 2015 Young Leaders in Climate Change Fellowship, National Park Service, \$15,000
- 2014 School of Biological Sciences Fellowship, UC Irvine, \$11,524
- 2014 East Asia and Pacific Summer Institute Fellowship, NSF, \$11,748
- 2014 Steele/Burnand Anza-Borrego Desert Research Center Fellowship, \$22,139
- 2013 School of Biological Sciences Fellowship, UC Irvine, \$3198
- 2012 Graduate Student Researcher Fellowship, UC Merced, \$9978
- 2012 Graduate Division Fellowship, UC Merced, \$3885
- 2012 Environmental Systems Graduate Bobcat Fellowship, UC Merced, \$3750
- 2008 Rocky Mountain Biological Laboratory Fellowship, \$5000
- 2004–8 New York Lottery Leaders of Tomorrow Scholarship, \$4000
- 2004–8 New York University Academic Scholarship, \$60,000

Teaching experience

- 2015 Guest Lecturer, Global Sustainability, UC Irvine (US13B, 4 credits). *Global climate change, the carbon budget, and carbon sequestration.*
- 2013 Teaching Assistant, Physiological Plant Ecology, UC Irvine (E127, 4 credits).
- 2013 Guest Lecturer, Physiological Plant Ecology, UC Irvine (E127, 4 credits). *Plant allocation and growth strategies*
- 2012 Teaching Assistant, Life: Concepts and Issues, UCLA (LS15, 4 credits)
- 2012 Guest Lecturer, Ecosystems of California, UC Merced (ESS050, 4 credits). *Urban ecosystem structure and the heat-island effect*
- 2012 Teaching Assistant, Ecosystems of California, UC Merced (ESS050, 4 credits)
- 2011 Teaching Assistant, Biological Anthropology, UC Merced (ANTH005, 4 credits)

Student Mentoring

UC Irvine undergraduate research (Bio Sci 199 credits):

- 2014–16 Michelle Yu-Chan Lin, 10 credits + Summer Undergraduate Research Fellowship Program Awardee + Excellence in Research Honors + Robert Ernst Prize for Excellence in Research in Plant Biology
- 2014–16 Melanie Kao, 12 credits + Excellence in Research Honors
- 2015–16 May Li, 10 credits
- 2014–15 Jun Dong, 6 credits
- 2016 Anh Dang, 3 credits
- 2016 Angela Choi, 3 credits
- 2016 Aarohi Desai, 5 credits
- 2016 Herman Kooner, 3 credits
- 2016 Khanh Phan, 2 credits

UC Irvine undergraduate and local high school research volunteers

- 2016 Herman Kooner
- 2016 Jeffrey Vozzo
- 2015 Keon Rabbani
- 2014 Alyssa Collins
- 2014 Phuongthao Nguyen

Conference mentoring

- 2016 Vincent Oliveras, Ecological Society of America meeting, Fort Lauderdale, Florida
- 2016 Liz Sampson, Botany meeting, Savannah, Georgia

Service & Outreach

Editorial positions

- 2016– Reviewing Editor, *Applications in Plant Sciences* (2016 – present: 11 manuscripts reviewed)

Peer-reviewer

- 2015 Arctic, Antarctic, and Alpine Research (2015: 1 manuscript)
- 2017 Biological Invasions (2017: 1 manuscript)
- 2015 Ecosphere (2016: 2 manuscripts, 2015: 1 manuscript)
- 2012 Plant and Soil (2013: 1 manuscript, 2012: 1 manuscript)

Conference abstracts reviewer

- 2017 Ecological Society of America Student Section Travel Awards
- 2016 North American Congress for Conservation Biology

Conference session chair

- 2017 George Wright Society Conference on Parks and Protected Areas (OCC 64: Understanding Aquatic & Riparian Invasive Species and Outbreak Events)
- 2016 Ecological Society of America meeting (COS 46: Invasion: Dynamics, Population Processes)

2015 George Wright Society Conference on Parks and Protected Areas (OCC 208: Responding to Invasive Plant Species).

Outreach

2016 Guest Instagrammer for American Geophysical Union (June 2016: [link](#))

2016– Master Plant Science Team mentor and liaison for the PlantingScience elementary school online program

Service

2015–16 Graduate Admissions Committee, UC Irvine Ecology & Evolutionary Biology

2015 U.S. National Park Service Young Leader in Climate Change

Media and Press

2017 Ecological Society of America [link](#)

2017 Phys.org [link](#)

2017 Science Newsline [link](#)

2017 Environmental News Network [link](#)

Training, certifications & study abroad

2016 3rd Annual Graduate Workshop on Environ. Data Analytics, NCAR, Boulder, CO

2016 Safe Zone Training, UC Irvine LGBT Resource Center

2015 Next Gen. Sequence Data Analysis, UC Irvine Data Science Initiative short course

2015 Introduction to Linux, UC Irvine Data Science Initiative short course

2015 COTE Summer School: Biodiversity and Global Change, U. of Bordeaux, France

2015 CPR & AED certified, National Park Service, Tucson, Arizona

2014 Summer Abroad, Hokkaido University, Sapporo, Japan

2013 Wilderness First Aid, Remote Medical, Institute of Arctic and Alpine Research

2011 Wilderness First Responder, Wilderness Medical Associates, University of Arizona

2011 High Occupancy Vehicle Training, University of Arizona

2009 Wildland Firefighter Red Card Certification (Type 2), Bureau of Indian Affairs

2009 Training, All Terrain Vehicle Safety Institute, Basic Safety Training

2005 Semester Abroad, New York University in Florence, Italy

2005 Semester Abroad, New York University in Paris, France

ABSTRACT OF THE DISSERTATION

Effects of climate change on protected and invasive plant species

By

Daniel Edward Winkler

Doctor of Philosophy in Ecology and Evolutionary Biology

University of California, Irvine, 2017

Professor Travis E. Huxman, Chair

Climate change will affect populations differentially through space and time. These impacts will also vary depending on species' life history strategies and the nativity of the species in question. Long-lived perennial plant species may be resilient to short-term or even within season drought stress and may only respond to long-term climate change given their generation times. At the same time, annual plant populations may respond rapidly to change given their short generation times and relatively direct selection pressures. However, which climate drivers and how they will impact populations through space and time remain poorly understood. Thus, this dissertation aims to fill in these gaps by examining: (i) the influence of drought on populations of the long-lived, protected saguaro cactus (Chapter 1), (ii) the ability of a dwarf bamboo to alter its growth strategies in response to changes in soil hydrology, thereby enabling its invasion into new habitats (Chapter 2), (iii) the genetic structure of the rapidly invading Sahara mustard in the southwestern US (Chapter 3), and (iv) Sahara mustard's capacity to adapt to variable water limitation across its invaded range (Chapter 4). Overall, this dissertation examines the influence of climate change on plant species at multiple scales and biological and conservation categories. In doing so, this dissertation tackles questions in evolutionary and population biology and

provides advice for land managers working to conserve protected species and control invasive species.

INTRODUCTION

Both natural and human systems will experience substantial impacts from future climate change and the coupled spread of invasive plant species as populations of native species respond. This may be especially true in arid and semi-arid systems that are highly dependent on water resources for biodiversity and ecosystem functioning. Native and invasive species may respond differentially to altered environments depending on their life history strategies as perennial or annual species. This dissertation uses multiple species to determine population responses to climate change using demographic analyses in the field and a common garden, and genetic analyses to examine the population structure of an invasive species occupying multiple environments in the southwest US.

Saguaro National Park and the surrounding Sonoran Desert are currently experiencing multiple climate change factors including altered precipitation regimes and increased temperatures (Weiss and Overpeck 2005, Saunders et al. 2009, Seager and Vecchi 2010). Native plant communities are also changing, seeing declines in native diversity, range shifts, loss of species, increases in non-native plants (e.g., Munson et al. 2012), and altered nutrient and water cycles (Cable et al. 2008). The impacts of climate change on the iconic Saguaro cactus, *Carnegiea gigantea*, remain unknown (Springer et al. 2015). Saguaro cacti are well-known for being part of a nurse-protégé relationship with desert trees and shrubs that provide them with protection from extreme heat during summer and cold during winter (Turner et al. 1966). However, this relationship is not fully understood (Vandermeer 1980), which suggests that research aimed at better understanding nurse-protégé relationships and how they will be impacted by human-induced climate changes should be assigned a high priority. Altered

precipitation regimes, increased temperatures, and altered nutrient and water availability due to changes in associated vegetation may have direct effects on saguaro regeneration and establishment (Drezner and Balling 2002, Drezner 2005), and may explain the reduced recruitment of saguaro cacti observed over in Saguaro National Park during the past two decades (Swann pers. comm.). It is also probable that climate change will have indirect effects on saguaro cacti by altering seasonal dynamics of water use by nurse species or other associated species saguaro depend on (McCluney et al. 2012).

Recognition of the necessity of understanding the influence of environmental drivers on saguaro distributions is nothing new. Yet, even a century after VM Spaulding published his seminal work on distribution and movement of desert plants (1909), we remain largely unaware of the mechanisms by which saguaro cacti thrive. Spaulding asserts that “[t]he role played by the roots in the distribution of [saguaro] is probably a most important one, but its precise value remains to be determined.” Long-term studies have shown saguaro establishment to be generally episodic and strongly influenced by precipitation and temperature. Water limitation through lower-than-average seasonal rainfall and elevated temperatures increasing evaporative loss can reduce survivorship of recent germinates. Thus, multi-year, extended drought could cause populations to decline as older saguaros die without replacement.

Previous studies have related establishment to temporal variation in rainfall, but most studies have been on non-randomized plots in ideal habitat and thus might not have captured the full variability within the local area. Chapter 1 addresses how saguaro establishment varied in space and which habitat features may buffer responses to drought on 36, 4 ha plots located randomly across an elevation gradient, including substantial replication in landscape position (bajada, foothills, and slopes) in the two disjunct districts of Saguaro National Park in southern

Arizona, USA. Recent, severe drought coincided with drastic declines in saguaro establishment across this ca. 25,000 ha area. Establishment patterns derived from the park-wide dataset was strongly correlated with drought, but the park's two districts and diversity of plots demonstrated substantially different population outcomes. Saguaro establishment was best explained by the interaction of drought and habitat type; establishment in bajada and foothill plots dropped to near-zero under the most severe periods of water limitation but remained higher in slope plots during the same time span. Combined with saguaro density estimates, these data suggest that the most suitable habitat type for saguaro establishment shifted to higher elevations during the time span of the recent drought. These results place into context the extent to which historical patterns of demography provide insight into future population dynamics in a changing climate and reveal the importance of understanding dynamics across the distribution of possible local habitat types with response to variation in weather.

The interaction of invasive species and climatic change will impact ecosystem structure and function, including a reduction in biodiversity (Ryan et al. 2008). Alpine ecosystems will experience some of the highest levels of warming globally and show signs of change before most ecosystems (Cannone et al. 2007; McCain and Colwell 2011; but see Rangwala and Miller 2012). Regional climate models predict that mid-latitude alpine environments will experience less snow and more rain – precipitation changes that will dynamically impact several climate variables important for maintaining alpine biodiversity (e.g., timing of snowmelt; Billings and Bliss 1959; IPCC 2007). These changes can create ideal windows of opportunity for invasions to occur (Kudo et al. 2011). In the Taisetsu Mountains, Japan, plant communities are already changing as a result of increased temperatures and species invasions (Kudo et al. 2011; Kudo and

Suzuki 2003). However, it remains unclear how changes in the timing of snowmelt and resulting soil moisture will contribute to changes in community structure (Backlund 2009).

Climate change may create temporal shifts that favor invasive species and facilitate range expansions (Colautti and Barrett 2013; Novak 2007). Alternatively, future conditions may promote alpine plant growth and invasives could be outcompeted. Understanding invasive plant phenotypic plasticity is key to predicting the future of biodiversity in alpine systems worldwide. Functional trait-based predictive models have been employed to understand community-wide dynamics (Huxman et al. 2013; Sutherland et al. 2013; Wright et al. 2004), including biodiversity loss, species invasions, and responses to climate change (Suding et al. 2005; 2008). Biomass allocation (a key functional trait) has had particular success in predicting how species invade new habitat and respond to environmental variation (James et al. 2006; Williams and Black 1994). Despite these advances, it is unclear how generally these models can be applied.

Species of the dwarf bamboo genus *Sasa* are commonly found throughout northeastern Asia (Makita 1992; Numata 1970). *Sasa* have extensive rhizome networks and a high density of culms, allowing them to outcompete natives and dominate large areas. *S. kurilensis* is the most common dwarf bamboo species in the snowy regions of Hokkaido. Dense patches of *S. kurilensis* can produce aboveground biomass of up to 3 kg/m² (reviewed in Kudo et al. 2011), creating shade effects intense enough to prevent forest regeneration (Hiura et al. 1996; Nakashizuka 1988; Takahashi 1997). At the treeline, *S. kurilensis* grow only in areas sheltered by alpine dwarf pines. Recent changes in winter precipitation and the timing of snowmelt have allowed *S. kurilensis* to expand beyond the treeline and invade alpine communities (Kudo et al. 2011). This has serious implications for alpine plant species that are adapted to high light and irradiation levels typical of alpine environments where a canopy layer is nearly nonexistent (Körner 2003).

This chapter includes using remote sensing imagery to quantify *S. kurilensis* expansion patterns across its range, measuring growth and stress tolerances of *S. kurilensis* above and below treeline, and evaluating components of growth to reveal how shifts in light and water limitations influence the ontogeny of height, branching, and leaf production. Results show that *S. kurilensis* more than doubled its abundance across its range, but more than tripled its abundance near and above treeline. Soil dry-down rates were a key driver of invasion above and below treeline, where growth rates decreased with more rapid rates of soil moisture dry-down. Results also found *S. kurilensis* responds to competition and climate stress by increasing allocation to belowground structures at high elevations. Further, it invests more carbon in fewer—yet taller and heavier—aboveground structures in low-light, low elevation environments. It appears this species' success is driven by considerable morphological and physiological flexibility, coupled with changes in water balance associated with snowmelt that in each habitat results in sites increasingly hospitable to bamboo. Overall, this study links resource allocation strategies and physiological responses to climate change and provides a mechanistic explanation of invasion success.

Human-induced climate change is promoting invasive species success globally (Dukes and Mooney 1999, Smith et al. 2000, Wilson et al. 2009) and, in some cases, causing an apparent feedback loop in which invasives accelerate climate change (Vitousek et al. 1996, Steers and Allen 2011). Human activities are further facilitating invasive success by acting as vectors of invasive dispersal via road, train, air, and marine traffic, and human-built corridors including canals, drainages, and roadway ditches. Now is an opportune time to learn how to control the spread of Sahara mustard (*Brassica tournefortii*). At the same time, this system provides a unique opportunity to test theoretical questions in invasion biology that hotly debated (Diham et

al. 2005, MacDougall and Turkington 2005, Valéry et al. 2013, Blondel et al. 2014, Simberloff and Vitule 2014). To this end, we can investigate how Sahara mustard has invaded, how climate change is affecting it, elucidating information on how it can be stopped.

Native plant communities across the desert Southwest are suffering because of the exceptional competitive ability of Sahara mustard (Esque & Schwalbe 2002, Marushia et al. 2010, Schneider & Allen 2012). Additionally, National Parks and surrounding lands are already experiencing direct impacts of climate change, which might make them increasingly susceptible to further invasion (Monahan and Fisichelli 2014). Other invasive plants in North America, such as *Bromus spp.*, have had widespread negative effects over the last 150 years (Bradley et al. 2009). We can anticipate Sahara mustard to spread with similar impact. If *Bromus* had been controlled early after it invaded in the 1800's, western North America landscapes would now be profoundly different. An essential goal toward controlling invasions is understanding where non-native introductions occur, what invasion routes they follow, and what climatological and biological mechanisms can promote or inhibit their success (Gurevitch & Padilla 2004, Ascunce et al. 2011, Fitzpatrick et al. 2012, Colautti & Barrett 2013).

These chapters include using genome-wide single nucleotide polymorphisms to identify the population structure and spatial geography of Sahara mustard using 760 individuals from 52 sites across its invaded range. Herbaria records are also used to model species expansion rate since its presumed introduction in the 1920s. Overall, Sahara mustard experienced atypical expansion patterns with a relative constant rate of expansion since its introduction. Results show that Sahara mustard exists as three genetically distinct populations in the United States without clear geographic pattern. High genetic divergence in one small population identified at only one site is likely a new introduction still in an initial phase of establishment prior to invasive spread.

However, overall low diversity within and between the other populations suggest that this primarily selfing species has undergone an isolate-breaking event that may explain its recent rapid expansion. This study is the first to explore the genetic composition of Sahara mustard in its invaded range and our results warrant the examination of the species in its native range. Management efforts aimed at control should prioritize targeting the isolated, novel population identified. Leveraging this current phylogeographic study to better understand the species in its historic range would identify native source populations that may lead to effective control and eradication measures in the arid and semi-arid United States.

Many studies argue that phenotypic plasticity plays an important role in invasions by allowing individuals to express advantageous phenotypes in a larger range of novel environments (Richards et al. 2006). Yet differences among invasive populations may exhibit unique selective pressures and limitations (Valladares et al. 2007), including differences in abiotic conditions such as drought, day length, seasonality, and biotic conditions such as herbivore pressures (Barrett et al. 2008, Prentis et al. 2008). As a result, these differences may determine range limits of populations (Sexton et al. 2009). Abiotic variation across the invaded range may explain observed variation in Sahara mustard phenology, plant size, and fecundity (pers. obs.). If plasticity enables an individual to persist in a novel environment, selection can then lead to local adaptation followed by genetic assimilation, or the loss of plasticity (Parker et al. 2003, Ghalambor et al. 2007, Crispo 2008). Overall, plant traits related to form, function, and defense are useful in examining local adaptation in relatively distinct environments (Linhart and Grant 1996, Dudley 2004, Richards et al. 2006, Gratani 2014). However, whether traits are genetically controlled and the extent to which they vary across the invaded range of Sahara mustard remains unknown.

Chapter 4 asked whether populations showed significant differentiation in functional traits across the invaded range, and whether that variation related to spatial and climatic variables associated with population locations. Two generations of plants were grown in a common garden sourced from 10 distinct locations across the species invaded range. Functional traits related to phenological, morphological, and physiological performance were tested for significant variation within and among populations. Using hierarchical partitioning analyses, this study evaluated the relative importance of spatial and climatic variables in explaining trait variation across populations. Analyses revealed that nine traits varied significantly among populations, specifically those related to phenology and early growth strategies, such as the timing of germination and flowering, as well as relative allocation to reproduction and seed mass. Variation in these traits observed in the common garden was related most strongly to variation in winter precipitation patterns at the source populations, though variation in temperature and latitude also showed significant contributions. These results are consistent with local adaptation, and identifies key functional traits that may explain the successful colonization of an invasive across a broad geographic and climatic range. Given that Sahara mustard has been in the US for less than a century, is capable of producing tens of thousands of seeds per plant, and exhibits divergence across 10 distinct environmental sites, the species will require substantial future research if land managers are to succeed in controlling the species.

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CHAPTER 1

The interaction of drought and habitat explain space-time patterns of establishment in saguaro (*Carnegiea gigantea*)

Abstract

The long-lived columnar saguaro cactus (*Carnegiea gigantea*) is among the most studied plants in the world. Long-term studies have shown saguaro establishment to be generally episodic and strongly influenced by precipitation and temperature. Water limitation through lower-than-average seasonal rainfall and elevated temperatures increasing evaporative loss can reduce survivorship of recent germinates. Thus, multi-year, extended drought could cause populations to decline as older saguaros die without replacement. Previous studies have related establishment to temporal variation in rainfall, but most studies have been on non-randomized plots in ideal habitat and thus might not have captured the full variability within the local area. We studied how saguaro establishment varied in space, and which habitat features may buffer responses to drought on 36, 4 ha plots located randomly across an elevation gradient, including substantial replication in landscape position (bajada, foothills, and slopes) in the two disjunct districts of Saguaro National Park in southern Arizona, USA. Recent, severe drought coincided with drastic declines in saguaro establishment across this ca. 25,000 ha area. Establishment patterns derived from the park-wide dataset was strongly correlated with drought, but the park's two districts and diversity of plots demonstrated substantially different population outcomes. Saguaro establishment was best explained by the interaction of drought and habitat type; establishment in bajada and foothill plots dropped to near-zero under the most severe periods of water limitation but remained higher in slope plots during the same time span. Combined with saguaro density

estimates, these data suggest that the most suitable habitat type for saguaro establishment shifted to higher elevations during the time span of the recent drought. These results place into context the extent to which historical patterns of demography provide insight into future population dynamics in a changing climate and reveal the importance of understanding dynamics across the distribution of possible local habitat types with response to variation in weather.

Introduction

Understanding how long-lived plant species may respond to projected climate change depends on how plants responded to climate in the past (Doak and Morris 1999, Davis and Shaw 2001, Butterfield et al. 2010, Hampe and Jump 2011) as well as interactions with other species, climatic variability, land use change, and other physical factors (Wright et al. 2014, Morris et al. 2008, Lawler et al. 2014, Kroiss and HilleRisLambers 2015). As a keystone species in the Sonoran Desert (Drezner 2014a) and symbol of the U.S. Southwest, the saguaro cactus (*Carnegiea gigantea* [Englm.] Britt. and Rose) is one of the most well-studied plants in the world. Saguaros principally grow during the summer monsoon season, and precipitation during this time is thought to be the primary limiting factor for establishment, growth, survival, and reproduction in the western, drier edge of their range (Steenbergh and Lowe 1977). However, temperature likely plays an important role in saguaro population dynamics at both the warm and cool edges of its range (Shreve 1911, Nobel 1980, 1982). For example, saguaro distribution in the eastern and northern extent of the range are limited by conditions of > 24 consecutive hrs of subfreezing temperatures (Hastings 1963, Steenbergh and Lowe 1976). Little is known about the combined effects of moisture and temperature limitation in driving saguaro dynamics across the

varied landscapes in which they are present. Nonetheless, more than 100 years of research on this plant has demonstrated how climatic features, especially precipitation variability, have interacted with land use factors such as cattle grazing and wood-cutting to influence large-scale patterns in saguaro establishment and survival (Spalding 1909, Niering et al. 1963, Steenbergh and Lowe 1977, 1983, Rogers 1985, Parker 1993, Pierson et al. 2013).

Saguaro height can be used to estimate age (Drezner and Turner 2014), given knowledge of rainfall in the area (Hastings and Alcorn 1961, Niklas and Buchman 1994, Drezner 2003, 2008), and has proven to be a helpful tool in estimating the year of establishment of individuals in a population (Steenbergh and Lowe 1977, 1983). Using this age-height relationship, many studies have shown saguaro establishment to be episodic, with periods of high germinant success transitioning to a maturing plant, often followed by long periods with very little evidence of new plants entering populations (Pierson et al. 1998, Drezner and Balling 2002, Orum et al. 2016). Drezner (2006b) and Pierson et al. (2013) used age-height data throughout the northern range of the saguaro to develop inferences in saguaro response to large-scale climatic factors using areas where saguaros were well established. Regeneration tracked strong ENSO (El Niño Southern Oscillation) events at large scales in these areas (Drezner 2006b). Although there were regional differences, establishment was generally favorable in the period between 1780 and 1860, declined in the early 1900s, and then many populations increased after 1960. Since the early 1990s, establishment appears to have again slowed dramatically throughout its range (Pierson et al. 2013, Orum et al. 2016, Conner et al. 2017). However, these studies could present a constrained view of saguaro demographics as they all sampled on non-randomly located plots in ideal habitats where saguaros are already well-established. A truly random design may better

explain saguaro establishment patterns by incorporating areas of varying saguaro densities and habitats that are historically less than ideal.

Most studies attribute drought to be the main environmental barrier resulting in episodic establishment in saguaro, which is primarily a function of lower precipitation but also results from how higher air temperatures influence landscape water balance. Drezner and Balling Jr. (2008) showed strong correlations of saguaro establishment and a number of global environmental patterns, including the levels of volcanic dust in the atmosphere, which can reduce summer temperatures to make water balance more favorable. The range-wide decline in saguaro recruitment observed since the mid-1990s coincides with extended periods of major drought in western North America (Seager and Veechi 2010, Woodhouse et al. 2010). Further, these events appear to be exacerbated by human-induced climate change resulting in negative impacts on nearly all ecological systems (Woodhouse et al. 2010, Hansen et al. 2014). Indeed, 2000 to 2009 was the hottest decade on record within the saguaro's range in southeastern Arizona (NOAA 2010). Furthermore, climate models predict increasing temperatures for the Southwest (Lenart et al. 2007, Munson et al. 2012) and an increasing frequency of drought (Seager et al. 2007). Additional drought effects include imperiled water and agricultural resources (MacDonald 2010, Seager and Veechi 2010), shifts in community composition (Huxman et al. 2013), species ranges contracting or shifting (Williams et al. 2010), diversity declining (Munson et al. 2013), and ecosystem processes reaching tipping points (Overpeck and Udall 2010, Anderegg et al. 2013).

Within the larger general picture of saguaro establishment, trends related to climate are shaped by local differences that are usually associated with land use change. For example, at Tumamoc Hill in Tucson, where saguaros have been studied for over a century, Pierson and

Turner (1998) found that establishment was very high in the mid-1800s, very low during 1870–1910, and high again during the middle of the 20th century. In contrast, in the Cactus Forest area of Saguaro National Park, less than 20 miles from Tumamoc Hill, saguaro establishment was high in the 1800s and declined throughout the first half of the 20th century before surging from the 1960s through the early 1990s (Pierson et al. 2013, Orum et al. 2016, Conver et al. 2017). There is strong evidence that these differences can be attributed to different land uses, particularly cattle grazing, which continued at Saguaro National Park long after it ended at Tumamoc Hill, and other human factors such as poaching and wood-cutting that removed protective nurse trees (Orum et al. 2016, Conver et al. 2017). In addition, local variability in population dynamics (Pierson et al. 2013) and observational evidence suggests that patterns of saguaro establishment may be complex and depend largely on the interaction with climate, land use, and other landscape features (Turner 1990). At Saguaro National Park, observers have long noticed that the population structure of saguaros on steep rocky slopes appears to be more even-aged than nearby populations in flatter areas with fine soils (Wilder and Wilder 1939, Steenbergh and Lowe 1983), and that these areas did not experience the same past declines despite somewhat similar climate and grazing histories. However, we are not aware of any studies that have examined such space-time differences in detail.

Our goal was to examine how saguaro responses to drought may vary locally across the landscape in an effort to better predict future responses to climate change. We used height data from saguaros on 36 large (4 ha) randomly-located plots to estimate year of establishment for each individual. These data were then evaluated in the context of the relative contribution of precipitation and temperature, aspect, slope, elevation, and soil type to determine long-term patterns of establishment for this long-lived desert plant. We examine saguaro establishment

across a range of physiographic locations within Saguaro National Park in relationship to drought using the Palmer Severity Drought Index, which combines both precipitation and temperature data in a single measure. We were particularly interested if establishment was buffered by potential hydrologic refugia in some areas, and if the plant's ideal habitat may shift in response to increased drought. We hypothesized that saguaro recruitment was negatively influenced by drought in the Southwest US and recruitment patterns would differ across districts and habitats within Saguaro National Park. Finally, we predicted that rocky habitats would mitigate some of the negative effects on recruitment during periods of drought by providing high resource patches due to increased infiltration and water retention rates, with broader implications of how saguaros may respond to future climate change throughout its range.

Methods

Study site

Saguaro National Park is located near Tucson, Arizona, and is divided into two districts. The Rincon Mountain District (RMD) encompasses a range of habitats across a 1817 m elevation gradient (816–2633 m asl) in the Rincon Mountains on the east side of the Tucson Basin. The Tucson Mountain District (TMD) borders the city of Tucson to the west and includes an elevation range of 730 m (670–1429 m asl). Saguaro presence is limited above 1550 m in the Rincon Mountains. Despite being separated by less than 40 km, the two districts differ topographically, hydrologically, edaphically, and thus have distinct plant communities. Less than 20% of the RMD contains Sonoran desert thornscrub and saguaros, but nearly 90% of the TMD

is composed of thornscrub and saguaros (Shelton 1985, McAuliffe 1996). Human impacts have also altered plant communities; woodcutting removed vital nurse plants (Turner et al. 1966) in the RMD and the district was actively grazed until the late 1950s (Conver et al. 2017), but the TMD remained free from intensive direct human impact due to a lack of perennial water sources (McAuliffe 1996). The majority of the TMD is lower in elevation than the RMD, which results in generally drier and warmer landscape conditions (e.g., Harlow et al., 2004) than the RMD (Steenbergh and Lowe 1969, 1983).

Sampling design & measurements

In 1990, 45 4 ha plots were established as part of a long-term monitoring project by the National Park Service (Duriscoe and Graban 1992). Plots were randomly distributed in the entire TMD, where saguaros are common throughout, and stratified randomly in the RMD within saguaro habitat < 1340 m asl as delineated by vegetation maps (Duriscoe and Graban 1991). A subset of the original plots ($n = 36$; TMD $n = 15$, RMD $n = 21$) were surveyed during the fall, winter, and spring of 2010–2011 (results are referred to as 2010 for simplicity) by National Park Service staff and citizen scientists (Appendix S1: Figs. S1–2). Crews of 3–5 individuals moved systematically through plots and measured the height of every saguaro to the nearest 1 cm using a folding ruler. Saguaros taller than 4 m were measured with a clinometer by two independent observers to the nearest 0.1 m. Each area was searched twice with separate crews to locate as many small saguaros as possible.

Because of the inter-relationships of geomorphic features and plant communities in deserts (e.g., McAuliffe, 1994), we captured variability in site characteristics by classifying plots

into three distinct physiographic locations (i.e., bajada, foothill, and slope) for each district of the park according to the variation of slope, elevation, and soil type (Table 1.1; Steenbergh and Lowe 1983). Bajada is a fluvial piedmont, usually composed of gravelly alluvium but may have large boulders embedded in the soil. Bajada plots were located on flat to gently sloping areas (< 10% slope) that were formed by the lateral coalescence of several alluvial fans, typically at the lowest elevations (Appendix S1: Fig. S1.1–1.3). Foothill plots were located in the transition zone between the bajadas and true eroding slopes, which typically were at higher elevations with steeper angles of incidence (10–25%). Here, soils formed from the rock pediments and erosion surfaces on older basin fill. Slope plots were typically positioned at higher elevations than the foothills, which included steeper inclines (> 25% slope). Slopes are the starting point of the erosional process and where the coarsest, rockiest soils were found (Table 1.1).

We obtained summer (June–August) Palmer Drought Severity Index (PDSI) data reconstructed from tree-rings (Cook et al. 1999; grid point 105) for the years 1950–2003 for southeastern Arizona. PDSI data quantify the water balance between precipitation and evapotranspiration and have been used in previous saguaro studies to explain saguaro regeneration within relatively small sites (Pierson and Turner 1998, Drezner and Balling 2002). The index values range from –6 to +6, where positive and negative values indicate wet and dry conditions. The annual summer PDSI values were smoothed with a 20-year low-pass filter in order to emphasize decadal variation (Cook et al. 1999). Drought effects are often cumulative and since we were interested in the effects of prolonged drought, this filter removed the high-frequency fluctuations, or periods of multi-year dry conditions punctuated with the occasional wet year, from the time series. The 20-year window has been used in previous studies of drought variability in the western United States (Hidalgo 2004); additionally, the saguaro cactus

transitions from the globose juvenile growth form to the larger club form at approximately 10–15 years of age (Steenbergh and Lowe 1977). Prolonged summer aridity results in the desiccation of globose juveniles and limits establishment of seedlings (Steenbergh and Lowe 1976; 1977) and the transition would not be possible during times of extreme, prolonged drought.

Statistical analyses

We estimated the ages of 11,961 individual saguaros across the districts of Saguaro National Park. We used height as a proxy to estimate age of each individual according to the age-height relationships for Saguaro National Park in Steenbergh and Lowe (1983). Each district of the park has its own age-height relationship resulting from the environmental variation between districts. Since our interest was in recent drought impacts on establishment, we analyzed establishment since 1950. Detecting young saguaros in the field is difficult because seedlings are 2–3 mm tall one year after germination and often obscured by the canopy of nurse plants. Despite this, a long-term monitoring study determined that approximately 40% of saguaros are detectable at 10 years old (0.052–0.064 m tall, Orum et al. 2016). The smallest saguaro detected during our field survey was 1.3 cm tall and was estimated to be 6 years old. However, only two saguaros that established after 2003 were detected during our 2010 surveys and were excluded from analyses because they occurred in different districts and established during different years. Truncating the data this way allowed for increased confidence in our tested establishment patterns as detectability at this age range is substantially higher (Orum et al. 2016).

We conducted initial exploratory analyses on the relationship between saguaro establishment and PDSI both across and between districts of the park. We used linear regression

to test for correlations between saguaro establishment and PDSI in each of the two park districts. We subsequently used analysis of covariance (ANCOVA) to test for differences between districts. Next, we evaluated how landscape characteristics explained park-wide differences in response to PDSI across years. We used multi-model comparisons of generalized linear models with Poisson distributions to determine the combination of factors that best predict saguaro establishment. We included PDSI, landscape type, and their interaction to determine the combination of factors that best predicted saguaro establishment (Aho et al. 2014, Barber and Ogle 2014, Burnham and Anderson 2014). We included estimates of pre-1950 establishment totals calculated using the full, un-truncated dataset as a covariate to control for potential density-dependent establishment patterns. We then tested for the predictive ability of each independent variable by comparing our full model with simpler variants and the change in Akaike Information Criterion corrected for small sample sizes for each model ($\Delta AICc$; Johnson and Omland 2004, Aho et al. 2014).

We continued with a second set of model comparisons by adding soil type and aspect as additional explanatory variables to the best-fit model, and district as a covariate to control for preexisting variation between districts. Elevation and slope were autocorrelated with habitat (Appendix S1: Fig. S1.3), and thus were not included in our models. We used $\Delta AICc$ to compute Akaike weights (w_i) as a measure of the relative likelihood that a given model is the best of all models. We calculated marginal r^2 to estimate the predictive power of explanatory variables for the best model (*sensu* Nakagawa and Schielzeth 2012). We then visualized the interaction of PDSI and habitat from the best fit model to examine the variability in habitat type that drives saguaro establishment in response to drought. Last, we tested for differences in establishment between habitats through time and since the 1997–98 El Niño event to explore potential shifts in

ideal habitat during periods of drought. We did this by plotting accumulation curves for each plot, calculating mean establishment by habitat during the recent drought, and used a pairwise *t*-test with *P* values adjusted with the Holm method to test for differences between habitats during the recent drought. All statistical analyses were carried out in R 3.3.2 (R Core Team 2014), and models were evaluated using the *piecewiseSEM* and *effects* packages (Lefcheck 2015, Fox 2003).

Results

Saguaro establishment was strongly and positively correlated with PDSI across and within districts of Saguaro National Park (Fig. 1.1). A period of relatively low establishment (< 2 individuals established per hectare) in the 1950s coincided with severe drought that was followed by a period of more favorable water balance in the 1970–80s that saw per-hectare establishment rates nearly quadruple across the park. However, the most recent severe drought, beginning with PDSI decreasing to negative values after 1993, coincided with a drastic decline in saguaro establishment rates that had seen near-zero recruitment of individuals in any habitat type. Establishment rates were highest in the TMD nearly every year; rates were often two or three times higher than those observed in the RMD (Fig. 1.1b). However, the RMD showed higher rates during years when drought was most severe with periods of water limitation both in the 1950's and from 1997 on (Fig. 1.1b). Although the RMD establishment rates continued to decline in the most recent years of the time series, the district exhibited rates that were double those seen in the TMD during the short period from 1996–1998. These data suggest that new

recruitment in both park districts at the peak of new establishment exceeded 590 individuals, while in the most recent years, the entire ca. 25,000 ha protected area saw fewer than five new individuals per year. As a result, estimates of saguaro establishment in protected areas declined by ca. 130% over the last 25 yrs (Fig. 1.1).

Overall, there was a positive relationship between establishment and PDSI in both districts of the park (Fig. 1.1b); establishment rates increased as drought severity was ameliorated. Establishment in the TMD demonstrated a tighter relationship with PDSI ($r^2 = 0.81$, $P < 0.001$) than in the RMD ($r^2 = 0.70$, $P < 0.001$). However, the RMD exhibited slightly steeper declines in saguaro establishment with increasing drought ($b = 47.55$) than the TMD did ($b = 43.17$; $F_{104,105} = 26.13$, $P < 0.001$). This differential sensitivity underlies the per hectare variation in performance associated with establishment in the different parks during extreme drought.

Habitat types differed in elevation, slope, and soil type between the two districts (Table 1.1). Bajada plots in the TMD were located 681.77–964.84 m asl, foothill plots 744.46–1056.55 m, and slope plots were located between 792.63–1273.44 m. Slopes in the district's plots ranged from 0–18° and represented 6 soil types defined by geologic age and level of erosion (Table 1.1; Appendix S1: Fig. S1.1–1.2). While there were overlaps in distribution, plots in the RMD were generally located at higher elevations than the TMD (Appendix S1: Fig. S1.1–1.2). Bajada plots were located between 818.47–918.97 m asl, foothill plots 896.58–1057.52 m, and slope plots were found between 1024.86–1214.75 m. Plots in the RMD also saw steeper slopes ranging from 0–30° and represented 6 soil types in the district. Only two soil types were found in both districts plots (Table 1.1).

Saguaro establishment was best predicted by the interaction of PDSI and habitat type with pre-1950 establishment estimates as a covariate (marginal $r^2 = 0.35$; Table 1.2). Models

without PDSI (marginal $r^2 = 0.09$ – 0.14) or its interaction with habitat type (marginal $r^2 = 0.25$ – 0.33) were worse at predicting saguaro establishment. Pre-1950 estimates of saguaro establishment improved all models; suggesting that saguaro establishment is partially density-dependent (Tables 1.2–1.3). Additionally, accumulation curves suggest saguaro populations have increased since 1950 but that there is substantial variation between habitats and plots (Appendix S1: Fig. S1.4). Compared to the first models, subsequent models were improved by including soil type (marginal $r^2 = 0.38$) and aspect (marginal $r^2 = 0.35$) as predictor variables, and district (marginal $r^2 = 0.35$) as a covariate to control for preexisting variation across the districts of the park (full model marginal $r^2 = 0.38$; Table 1.3). However, model performance was similar with and without district when soil was included in the model (Table 1.3), indicating that soil type classification of plots accounts for potential differences in establishment between the park's districts.

The best model identified bajada and foothill plots as responding somewhat similarly to PDSI regardless of severity (Fig. 1.2), but that foothill plots always outperformed bajada plots regardless of drought severity. Foothill plots outperformed all habitat types during periods with the least amount of drought (Fig. 1.3). However, the predicted number of saguaros to establish in bajada or foothill plots dropped to near-zero under the most severe drought but remained higher in slope plots (Fig. 1.2–1.3). Saguaro establishment in slope plots during a period of severe drought in the 1950s was more than double what was estimated in foothill or bajada plots (Fig. 1.3) but this varied from year to year with interannual climate variability. However, all plot types experienced substantial declines in establishment during the recent drought beginning in the late 1990s ($F_{2,31} = 2.96$, $P < 0.001$) with slope plots outperforming the other plot types (slope vs.

bajada $P < 0.001$, slope vs. foothill $P = 0.001$); suggesting that the most suitable habitat type for establishing saguaros shifted during periods of drought, especially during the recent drought.

Discussion

Determining how plant populations across a complex landscape may change with respect to future climates is a grand challenge. This is especially true for long-lived organisms, where patterns of establishment may be episodic or changing in non-obvious patterns that have implications for long-term population viability. Therefore, we sampled a number of large plots to document changes in demography for saguaro, across a range of habitat conditions that captured the variability in landscape features on which saguaro populations occur. Additionally, we used a randomized design that, in addition to capturing landscape variability across robust elevational gradients, accounted for size differences in saguaro populations and local water limitation. However, temperature also plays an important role in how populations responded to water limitation (Turner et al. 1966, Nobel 1980, 1982). Thus, we used the PDSI that tracked both precipitation and temperature as it may be a more robust indicator of the true factors that are driving saguaro establishment than precipitation alone. Overall, our study suggested that saguaro responses to drought were largely contingent upon regional and local variability in habitat characteristics and that recent establishment in Saguaro National Park has closely tracked patterns of regional drought.

Over a 60+ year period of saguaro establishment, we found a negative relationship between establishment and drought severity in both districts of Saguaro National Park. Although

the strong relationship between PDSI and saguaro establishment at Saguaro National Park is a novel finding, many previous studies, beginning with Shreve (1910), have indicated that saguaro establishment is often cyclic and correlated with long-term precipitation patterns (Despain 1974, Pierson and Turner 1998, Drezner and Balling 2002, Drezner 2004, Pierson et al. 2013) and is even affected by global events such as volcanic activity (Drezner and Balling 2008). As in our study, saguaro establishment elsewhere in eastern Arizona increased in the 1960s and 1970s when precipitation was high (Pierson and Turner 1998, Drezner 2006a, 2006b, Conner et al. 2017), and declined during the past two decades as precipitation declined (Pierson et al 2013, Orum et al. 2016, Conner et al. 2017). However, these previous studies were based largely on observations made in ideal saguaro habitat where plots encompassed existing populations that were essentially healthily established and where little to no variation in slope defined the landscape. Our randomized study design obtains substantial improvements in predictive power when explaining saguaro establishment in relation to drought. District-level correlations ($r^2 = 0.70\text{--}81$) of establishment with drought are orders of magnitude greater than those reported, if at all, in previous studies (Steenbergh and Lowe 1969, Brum 1973, Pierson and Turner 1998, Pierson et al. 2013, Drezner 2004, Drezner and Balling 2013).

Although the long-term relationship between drought and saguaro establishment appears to be fairly robust, local variability has complicated past studies of the relationship between climate and saguaro establishment across the saguaro's geographic range. For example, four of the long-term monitoring sites in Pierson et al. (2013) are within 40 km of each other, yet have experienced dramatically different periods of establishment in the 20th century. While most of these differences appeared to be attributable to different land use histories, particularly the cessation of cattle grazing, the relatively small plots also varied greatly in habitat, ranging from

flat areas with few rocks to relatively steep, rocky slopes. Our study provides a potential explanation for these differences, as noted but untested by Pierson et al. (2013): saguaro population dynamics vary substantially across the landscape, even at relatively small scales. Saguaro National Park contains a remarkable array of saguaro habitat types. The TMD is defined by desert habitats from flat bajadas to steep, rocky mountain slopes occurring in all four cardinal directions whereas the RMD is defined by large elevational and environmental gradients, with saguaros occurring primarily on west- and south-facing aspects. Among the two districts, we found steeper declines in establishment in response to drought than within the warmer, drier, TMD than within the cooler, wetter RMD. Overall, establishment in the TMD appears to be more consistent over time, but fails to maintain recruitment patterns during periods of severe drought. In the RMD, the steeper and rockier habitats at higher elevations may buffer the larger population from the negative impacts of rainfall variability given the few saguaros that successfully established there during the recent period of drought beginning in the late 1990s, whereas in the TMD few to no establishment events were documented. These results suggest that perhaps there is sub-surface or contributory overland flow from upslope areas to supplement water balance on the RMD plots given that there is substantial area above the upper elevational limit of saguaros in RMD.

Within districts, our results suggest that saguaro establishment is best explained by the interaction of drought and habitat type. Saguaros are locally influenced by microclimate effects created by nurse species including trees, shrubs, and even rocks that provide multiple benefits for a plant as it establishes (Shreve 1910, Steenberg and Lowe 1983, McAuliffe 1984, Goldberg and Turner 1986, Bowers 1994, Bowers and Pierson 2001, Drezner 2014a, 2015). In general, it appears that saguaro responses to drought are at least partially controlled by habitat differences

and that steep, rocky slopes appear to buffer saguaros from the negative impacts of drought. Nurse trees may be more common on these slopes, but it also seems likely that precipitation runoff that infiltrates rock crevices and confined soils may evaporate more slowly than in open areas, creating hydrologic refugia that promote higher survival of individuals in these microclimates. This is consistent with previous work showing that habitat suitability varied across elevational gradients in southwestern Arizona (Shreve 1922, Parker 1988) as is further supported by research in different systems also defined by water limitation (Kelly and Goulden 2008, Graff and Aguiar 2016, McLaughlin et al. 2017). These results suggest that saguaro populations may already be responding to climate change as individual sites shift to less-than-ideal habitat and hydrologic refugia are revealed (McLaughlin et al. 2017). Interestingly, this shift occurred in an upslope fashion similar to other changes in vegetation seen throughout the southwestern U.S. (Kelly and Goulden 2008).

In general, our study corroborates similar patterns observed in the historic plots at Tumamoc Hill and other sites (Pierson and Turner 1998, Pierson et al. 2013), and indicates that saguaro establishment is largely dependent on available moisture and is linked to ENSO patterns in which mild and wet winters allow for populations growth (Drezner and Balling 2002). However, our results highlight the difficulty in making predictions for how an entire species or community will respond to drought and other climate change factors (Hansen et al. 2014), and suggest that a fine-scale approach is required to understand saguaro populations. If local patterns in habitat suitability shift in response to drought, then it seems possible that the long-term result could be varying age structures within current saguaro habitats that could, over time, translate to the larger landscape scale.

Today, the Sonoran Desert is being impacted by climate change, which makes it increasingly difficult to disentangle the episodic recruitment nature of the saguaro cactus from its natural range of variability (Butterfield et al. 2010, Pierson et al. 2013). Although saguaro population demographics have been studied for over 100 years (Spalding 1909, Shreve 1910, Rodriguez-Buritica 2013, this study) our full understanding of the species in relation to its environment is inherently limited by past site selection and the difficulties of studying a species that may live for 200 years (Drezner 2014b). Long-lived species are traditionally expected to be more resilient to short term environmental variability (Morris et al. 2008) but extreme changes may result in more pronounced impacts on saguaros (Despain 1974, Steenbergh and Lowe 1983, Pierson and Turner 1998, Drezner and Balling 2002, Springer et al. 2015). Within Saguaro National Park, the end result could be dramatically different populations in each district, with potential loss of saguaros from the drier areas of the TMD. Our random design enabled us to capture variability in physiographic features (e.g., slope, elevation, aspect, soil type) and, as a result, the differential responses of saguaro populations to drought between and within districts. In doing so, our study is the first to provide a more holistic understanding of saguaro establishment patterns in relation to their environment.

Shreve (1910) wrote that, “It is bootless at this time to attempt to explain the apparent decadence of the Giant Cactus. A fuller knowledge of its germination and behavior of its seedlings, together with a more complete knowledge of the periodicity of certain climatic elements within its range, will be sure to throw light on the fall in its rate of establishment.” A similar declaration was made by Despain (1974), “Perhaps it is too soon to give an answer and after more than 50 years we are in relatively the same position described by Shreve in 1910.” In addressing the impacts of drought on establishment at multiple scales (e.g., within region and

between habitats), our study provides a different perspective on saguaro population dynamics and changes in suitable habitat.

We should be clear that declines in establishment and their correlation with drought do not imply causation. Saguaro National Park has undergone numerous changes in management and climate since the park was established. These changes are common throughout the National Parks and other protected lands (Saunders et al. 2009, Hansen et al. 2014). Future studies will need to address long-term management practices and, if possible, how they have influenced saguaro populations across habitats. Further, it is imperative to fully understand the biology of the saguaro if we are to attempt any further explanation of establishment patterns. This includes some large unknowns about life history strategies including how saguaros access water, the full role that nurse species play in saguaro establishment, and the extent to which human uses of desert waters influence the keystone saguaro and its associated species as they respond to increasing occurrences of drought in the southwest. We may remain in a similar predicament as Shreve (1910) until these challenges are addressed, in which we have a long way to go before we fully understand this iconic species and its relation to its environment as it continues to change.

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Table 1.1. Habitat characteristics for each district.

District	Habitat	<i>n</i> plots	Elevation (m)	Slope (°)	Soils
Rincon Mountain District	Bajada	11	818.47–918.97	0–3	Pantano-Granolite Complex, Palos Verdes-Jaynes complex, Cellar-Lehmans Complex, Cellar-Rock Outcrop Complex, Chimenea very gravelly fine sandy loam
	Foothill	6	896.58–1057.52	5–17	Cellar-Lehmans Complex, Cellar-Rock Outcrop Complex, Lampshire-Romero-Rock outcrop Complex
	Slope	4	1024.86–1214.75	14–30	Cellar-Lehmans Complex, Cellar-Rock Outcrop Complex, Lampshire-Romero-Rock outcrop Complex
Tucson Mountain District	Bajada	7	681.77–964.84	0–2	Pantano-Granolite Complex, Hayhook Sandy Loam, Pinaleno very cobbly sandy loam, Anklam-Cellar-Rock Outcrop Complex
	Foothill	6	744.46–1056.55	2–11	Pantano-Granolite Complex, Pinaleno very cobbly sandy loam, Chimenea very gravelly fine sandy loam, Anklam-Cellar-Rock Outcrop Complex, Chimenea-Cellar-Rock Outcrop Complex
	Slope	2	792.63–1273.44	13–18	Chimenea-Cellar-Rock Outcrop Complex, Anklam-Cellar-Rock Outcrop Complex

Table 1.2. Results of generalized linear models predicting saguaro establishment rates with the Palmer Drought Severity Index (PDSI), habitat type, and their interaction. Estimates of pre-1950s establishment are included as a covariate to control for potential density-dependent establishment. AICc are Akaike Information Criterion values corrected for small sample sizes, w_i are Akaike weights, which indicate the probability of each model being the best fit relative to others shown. The first model is the best-fit model.

model	AICc	Δ AICc	k	w_i
PDSI * habitat + pre-1950	16180.24	0	6	1.00
PDSI + habitat + pre-1950	16490.21	309.97	4	< 0.001
PDSI * habitat	17534.78	1354.54	5	< 0.001
PDSI + habitat	17844.75	1664.51	3	< 0.001
PDSI + pre-1950	16815.33	635.09	2	< 0.001
habitat + pre-1950	19842.92	3662.68	3	< 0.001
PDSI	19072.08	2891.84	1	< 0.001
habitat	21197.46	5017.22	2	< 0.001
pre-1950	20168.04	3987.80	1	< 0.001
intercept only	22422.80	6242.56	0	< 0.001

Table 1.3. Results of generalized linear models predicting saguaro establishment rates with the Palmer Drought Severity Index (PDSI), habitat type, their interaction, pre-1950 establishment estimates, district, aspect, and soil type. AICc are Akaike Information Criterion values corrected for small sample sizes, w_i are Akaike weights, which indicate the probability of each model being the best fit relative to others shown. The first model is the best-fit model.

model	AICc	Δ AIC	k	w_i
PDSI * habitat + pre-1950 + aspect + soil	15703.03	0	14	0.70
PDSI * habitat + pre-1950 + district + aspect + soil	15705.02	1.99	8	0.26
PDSI * habitat + pre-1950 + district + aspect	16074.08	371.05	13	< 0.001
PDSI * habitat + pre-1950 + district + soil	15711.04	8.01	13	0.01
PDSI * habitat + pre-1950 + district	16180.83	477.80	7	< 0.001
PDSI * habitat + pre-1950 + aspect	16078.66	375.63	7	< 0.001
PDSI * habitat + pre-1950 + soil	15709.18	6.15	12	0.03
PDSI * habitat + pre-1950	16180.24	477.21	6	< 0.001
intercept only	22422.80	6721.77	0	< 0.001

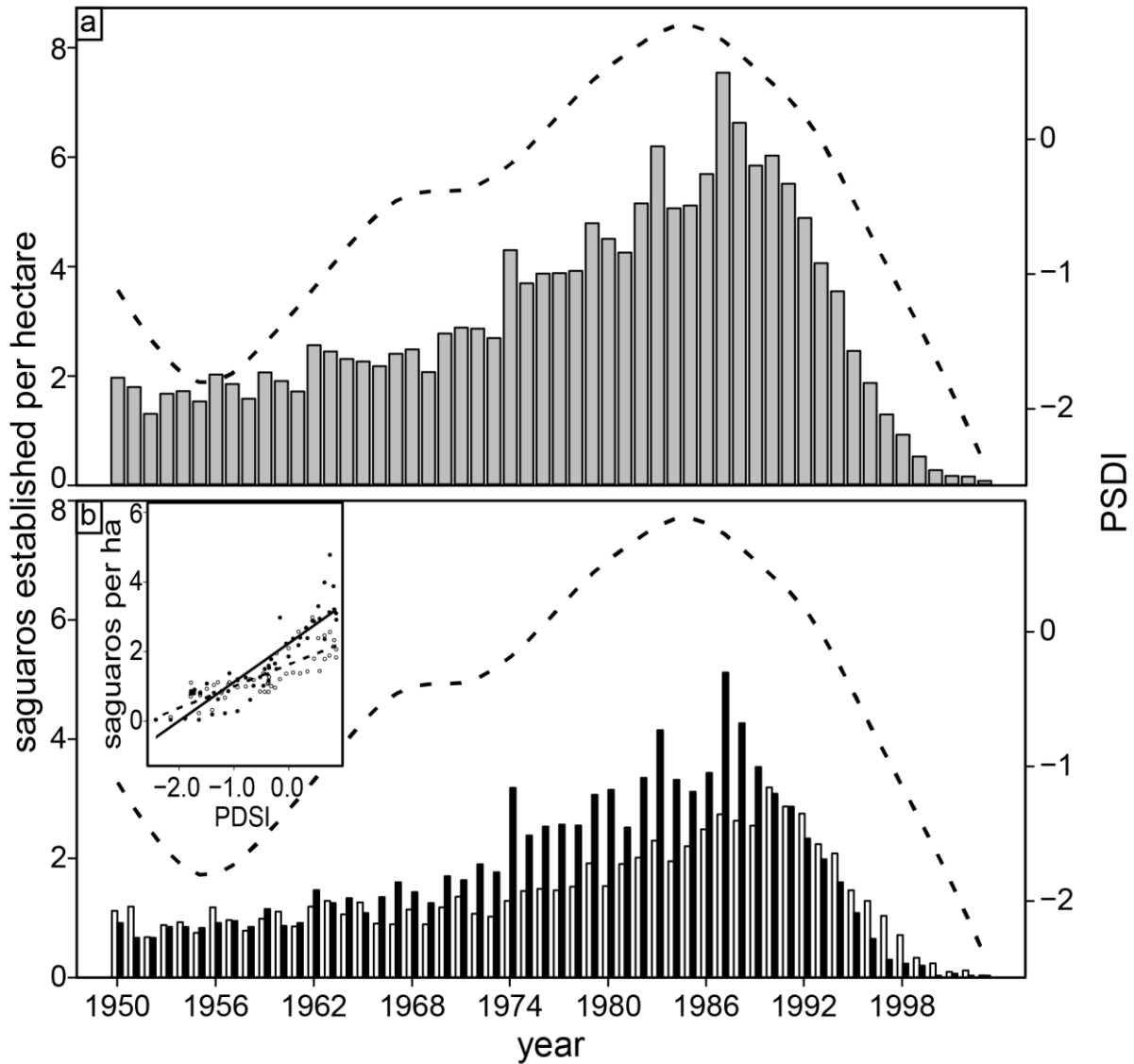


Fig. 1.1. (a) Total number of saguaros established per hectare at Saguardo National Park from 1950–2003 in relation to 20-year smoothed Palmer Drought Severity Index (PDSI). (b) Number of saguaros established per hectare in the Tucson Mountain District (TMD; filled bars) and the Rincon Mountain District (RMD; hollow bars) in relation to PDSI. (inset) Linear regressions are shown for each district of Saguardo National Park (filled circles = TMD, $r^2 = 0.81$, $P < 0.001$; hollow circles = RMD, $r^2 = 0.70$, $P < 0.001$). ANCOVA results show slopes of the regressions are significantly different ($F_{104,105} = 26.13$, $P < 0.001$).

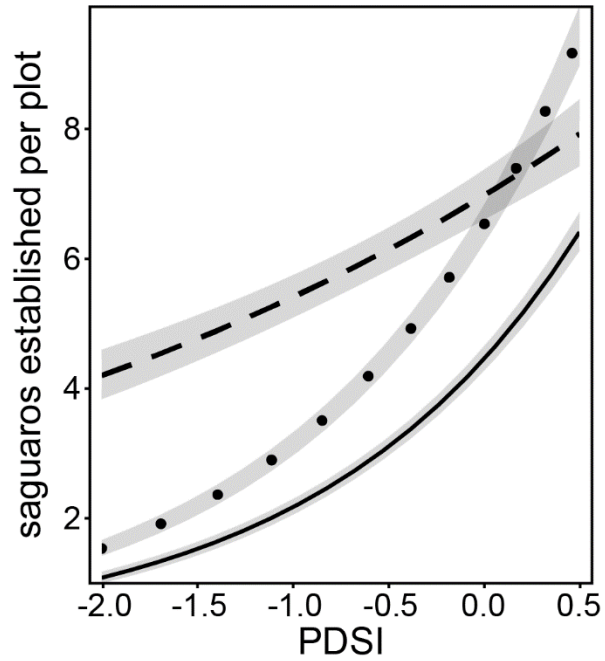


Fig. 1.2. Interaction plot of the best fit Poisson generalized linear model with the 20-year smoothed Palmer Drought Severity Index (PDSI) predicting the number of saguaros established in bajada plots (solid line), foothill plots (dotted line), and slope plots (dashed line). Shaded regions indicate 95% confidence intervals.

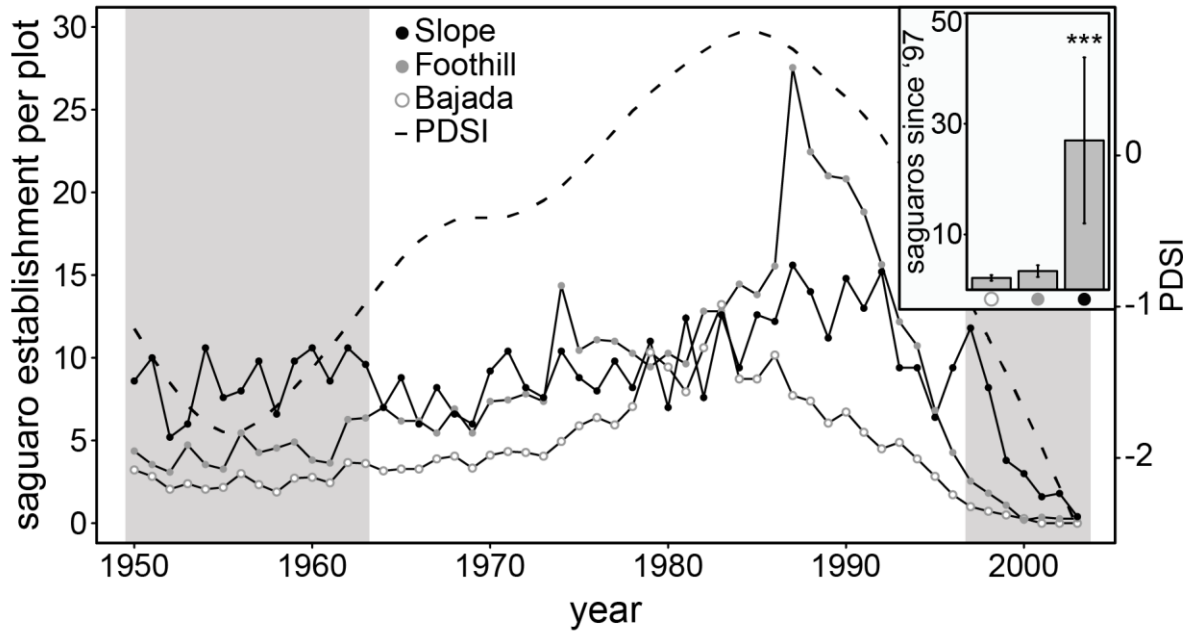


Fig. 1.3. Saguaro establishment by habitat (black circles = slope, gray circles = foothill, open circles = bajada) averaged each year with the 20-year smoothed Palmer Drought Severity Index (dashed line). Gray boxes show periods of severe drought (PDSI < -1) during 1950–62 and 1998–2003. (inset) Means \pm SEM of saguaro establishment following the 1997–98 El Niño are shown by habitat ($F_{2,31} = 2.96$, *** = $P < 0.001$).

Appendix S1

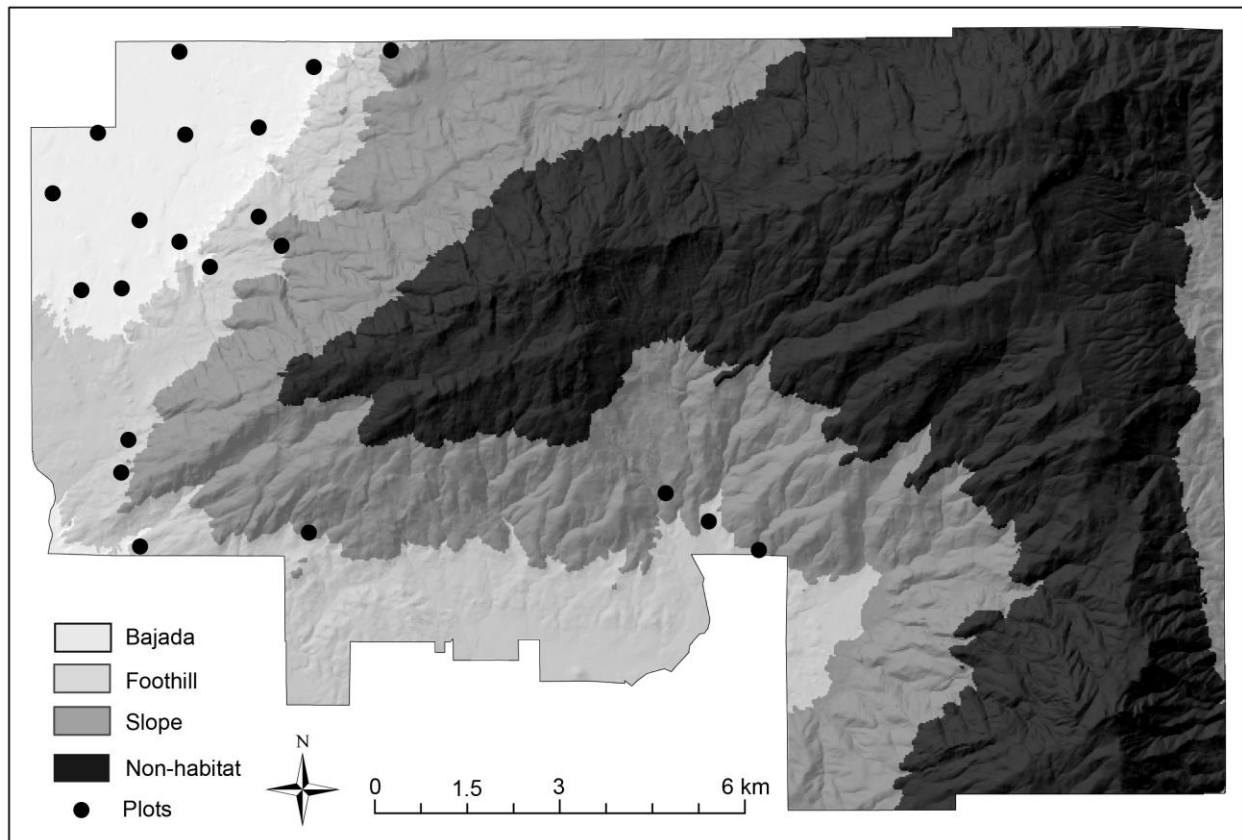


Fig. S1.1. Shaded relief of Saguaro National Park's Rincon Mountain District with plot locations (black circles) and each habitat highlighted (bajada = light gray, foothill = medium gray, dark gray = slope).

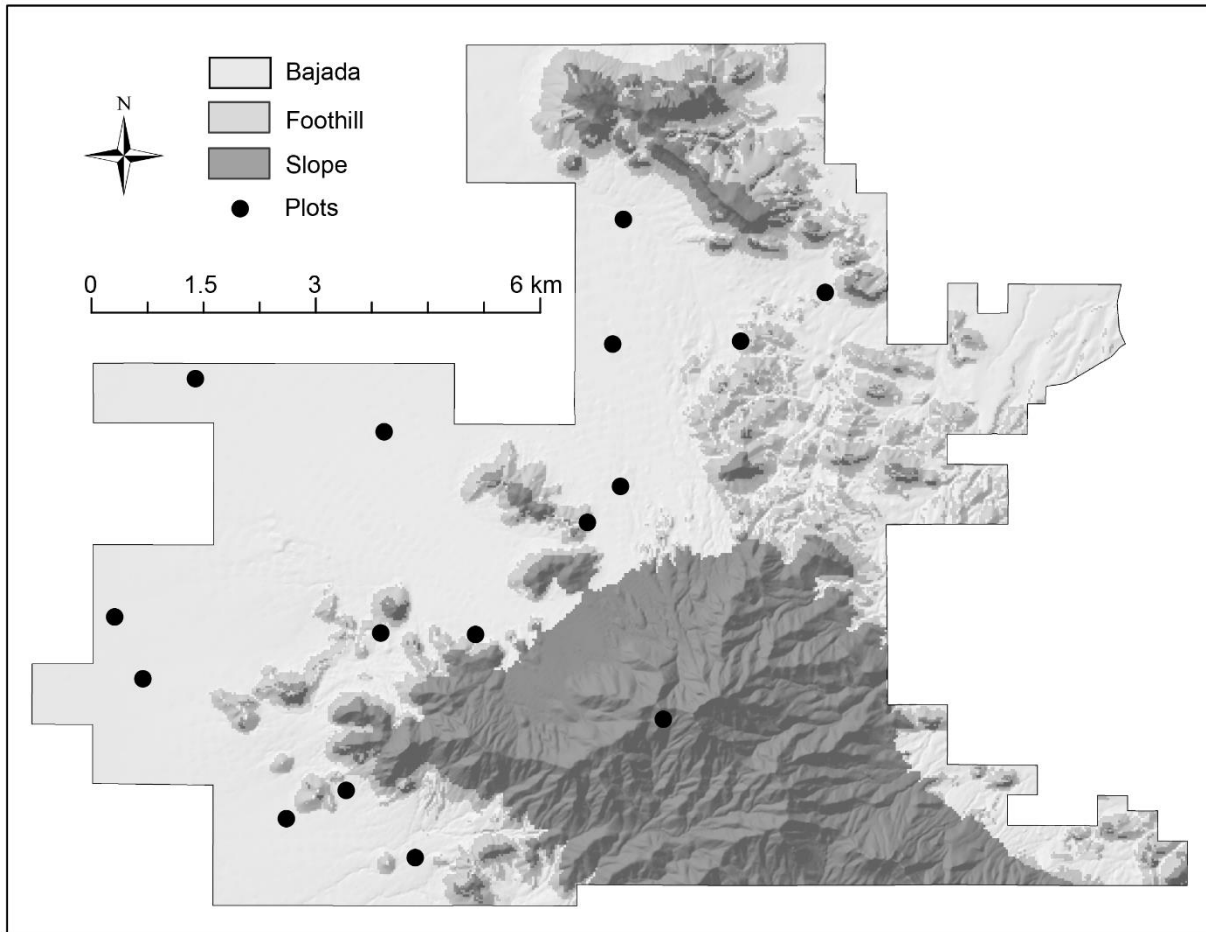


Fig. S1.2. Shaded relief of Saguardo National Park's Tucson Mountain District with plot locations (black circles) and each habitat highlighted (bajada = light gray, foothill = medium gray, dark gray = slope).

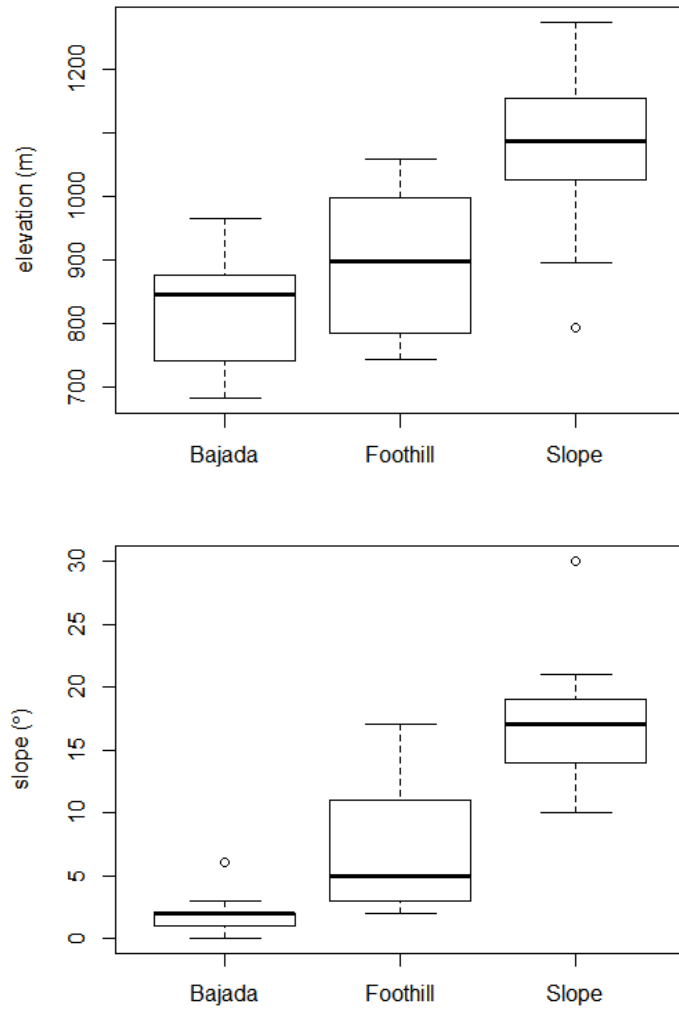


Fig. S1.3. Elevation (meters) and slope (°) ranges for plots in each habitat type across Saguaro National Park.

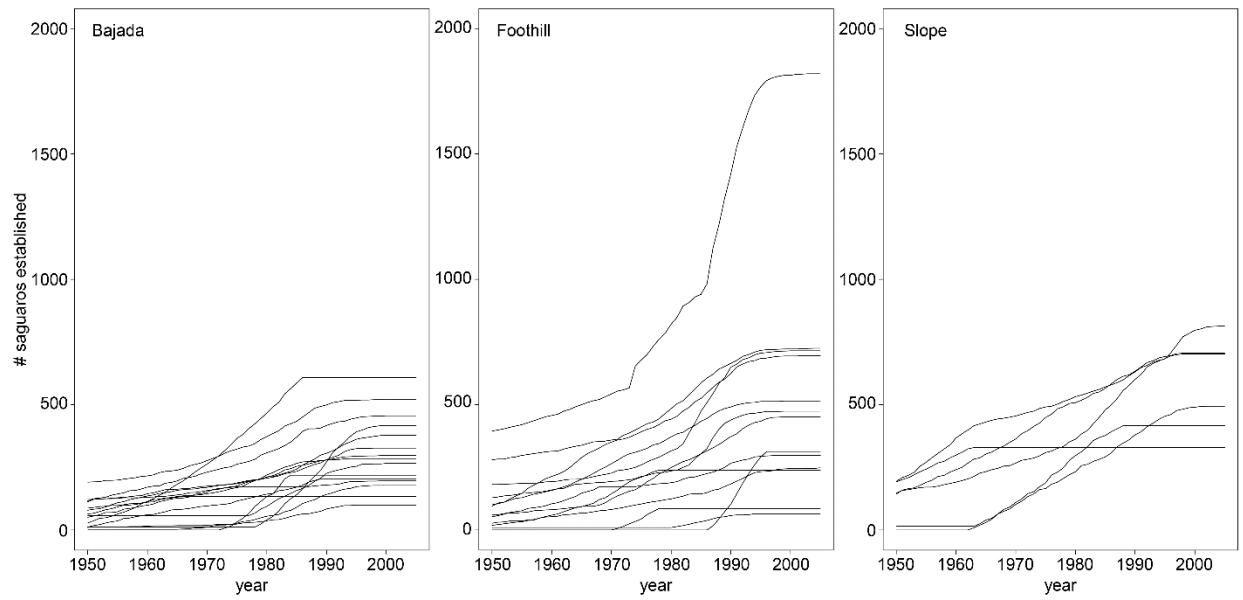


Fig. S1.4. Accumulation curves of saguaro establishment in individual plots through time by habitat (bajada = left panel, foothill = middle panel, slope = right panel).

CHAPTER 2

Seasonal dry-down rates and high stress tolerance promote bamboo invasion above and below treeline

Winkler DE, Y Amagai, TE Huxman, M Kaneko, G Kudo. 2016. Seasonal dry-down rates and high stress tolerance promote bamboo invasion above and below treeline. *Plant Ecology* 217(10): 1219–1234. doi: 10.1007/s11258-016-0649-y

Abstract

How species invasions impact ecosystem structure and function at important ecotones or boundaries is unknown, but may provide insight into the impacts of climate change and the mechanisms underlying community change. The dwarf bamboo, *Sasa kurilensis*, may be a good system to understand these issues, as the species impacts ecosystem features as it encroaches beyond treeline into alpine systems. We used remote sensing imagery to quantify *S. kurilensis* expansion patterns across its range, measured growth and stress tolerances of *S. kurilensis* above and below treeline, and evaluated components of growth to reveal how shifts in light and water limitations influence the ontogeny of height, branching, and leaf production. We show that *S. kurilensis* more than doubled its abundance across its range, but more than tripled its abundance near and above treeline. Soil dry-down rates were a key driver of invasion above and below treeline, where growth rates decreased with more rapid rates of soil moisture dry-down. We found *S. kurilensis* responds to competition and climate stress by increasing allocation to belowground structures at high elevations. Further, it invests more carbon in fewer—yet taller and heavier—aboveground structures in low-light, low elevation environments. It appears this species' success is driven by considerable morphological and physiological flexibility, coupled with changes in water balance associated with snowmelt that in each habitat results in sites

increasingly hospitable to bamboo. Overall, this study links resource allocation strategies and physiological responses to climate change and provides a mechanistic explanation of invasion success.

Introduction

Mass biological invasions are occurring globally in response to human-induced climate change (Ricciardi 2007). Aside from non-native introductions (e.g., Bradley et al. 2012), shifting environmental drivers at ecosystem boundaries are promoting species movement into novel habitats (Allen and Breshears 1998; Harsch et al. 2009; Chen et al. 2011), thereby altering ecosystem structure and functioning (Eldridge et al. 2011). While breaking down dispersal barriers is an important driver to invasive species presence (Wilson et al. 2009), elucidating the biological mechanisms underlying invasive species success in adjacent novel habitats remains a challenge (Sutherland et al. 2013; Colautti et al. 2004). Climate change may promote select species to expand their ranges into adjacent settings by favoring species with flexible phenotypes (Richards et al. 2006; Colautti and Barrett 2013) over those that are either slow to respond or have fixed traits (Etterson and Shaw 2001; Jump and Peñuelas 2005). Uncovering the phenotypes and environmental pressures that promote movement across system boundaries would enable much-needed predictions of future changes in biodiversity, biogeography, and biogeochemistry (Whitney and Gabler 2008).

Treeline ecotones, areas of gradual transition from forest to treeless areas, are eminently suited to address these challenges given their sensitivity to climate change and the variation in

abiotic filters occurring over short distances (e.g., timing of snowmelt, seasonal soil dry-down, microclimate; Taylor and Seastedt 1994; Körner 1998; Germino et al. 2002; Körner 2012). Similarly, forecasted species range shifts in treeline systems will likely result in increasingly homogenous communities with negative impacts on nutrient cycling (Scheffer 2001; Dullinger et al. 2004; Cannone et al. 2007). Furthermore, treeline ecotones typically contain steeper gradients of biological diversity compared to adjoining systems (Cadenasso et al. 2003). As a result, treelines are more difficult to characterize due to varying functional types and species compositions. Understanding these interactions has proved difficult because biological and environmental timescales are often challenging to characterize using most available datasets, which are either too short-term, do not account for ontogenetic effects, or evaluate only morphological or physiological processes (Holtmeier and Broll 2005; Yang and Rudolf 2010).

The invasive dwarf bamboo *Sasa kurilensis* is presumed to be highly sensitive to climate change (Tsuyama et al. 2008) and has been identified as an ideal ecological indicator species to monitor climate change impacts as species boundaries have been predicted and, in some cases, observed to have shifted upward in elevation and northward towards the poles (Higa et al. 2013). *S. kurilensis* range shifts likely exemplify the phenotypic variation and environmental drivers that determine successful invasion of species into adjacent systems and, thus, can provide critical insight into the ways system boundaries will respond to future change.

Species of the dwarf bamboo genus *Sasa* are common throughout northeastern Asia (Makita 1992; Numata 1970). *Sasa kurilensis* is the most common dwarf bamboo species in the snowy regions of Hokkaido where it often forms monotypic patches in the understory of montane forests. *S. kurilensis* is an evergreen shrub typically 2 m tall (Oshima 1961a). Dense patches of *S. kurilensis* can produce aboveground biomass of up to 3 kg m⁻² (Oshima 1961b). At

treeline, *S. kurilensis* grow only in areas sheltered by dwarf pines or under subalpine forest canopies. *S. kurilensis* and related species have extensive rhizome networks and produce a high density of culms, allowing them to outcompete natives and dominate large areas. Thus, *S. kurilensis* invasions have serious implications for subalpine and alpine plant communities adapted to high light and irradiation levels.

Sasa kurilensis has expanded beyond the treeline and begun to invade alpine communities, leading to the loss of over 1/3 of native species diversity in some areas (Kudo et al. 2011). Its invasion is reportedly due to its competitive ability coupled with recent changes in winter precipitation / snowmelt timing (Franklin et al. 1979; Ohsawa et al. 1998; Herben 2004; Kudo et al. 2011). Similar changes in precipitation and snowmelt timing are occurring below treeline in subalpine meadows (Dullinger et al. 2004; Sloat et al. 2015). Thus, it is likely *S. kurilensis* will experience or has already experienced additional windows of opportunity to invade similar habitats at lower elevations below treeline. Whether this is the case remains unresolved. Furthermore, it remains unknown how environmental changes are directly driving invasion and influencing the species' form and function.

In this study, we aimed to identify ecological and organismal components that facilitate *S. kurilensis* invasions and success. We asked the following questions: (1) What are the expansion patterns of *S. kurilensis* into meadows and mires above and below treeline? (2) What are the morphological and physiological traits that facilitate *S. kurilensis* invasion into novel habitats? (3) How do these traits align with environmental drivers in each habitat? We used satellite imagery to examine *S. kurilensis* expansion patterns over the past 35 years. In the field, we compared growth and allocation patterns between individuals of different ages in the historical (i.e., montane forests) and invaded (i.e., a subalpine mire and alpine meadow)

communities of *S. kurilensis*. We also tested for variation in water and nutrient stress in each of the populations to examine whether there is a fundamental trade-off between stress tolerance and growth capacity that may explain invasion strategies in different environments. Lastly, we examined environmental variation within and among sites and related it to corresponding biological patterns to infer how climate change is promoting the rapid expansion of this species above and below treeline.

Methods

Study site

Our study sites are located along a 800 m elevation gradient (900–1700 m asl) situated in central Hokkaido, northern Japan along the western slope of Mount Asahi (2290 m asl; 43° 16' N, 142° 28' E) in the Taisetsu Mountains in Daisetsuzan National Park (Fig. 2.1). Summits within the park reach 1900–2100 m asl with treeline present between 1400–1600 m asl depending on aspect and exposure. The Taisetsu Mountains are characterized by low air temperatures (mean annual air temperature at 1635 m asl: -2.0°C) and high amounts of precipitation (mean annual precipitation for 1951 to 1980: 1158 mm; Ishikawa 2002). Snowmelt typically occurs in May on upper slopes, and July at the bottom of snowfields, which receives a monthly average of 223 mm precipitation (Kudo et al. 2011). Much of the parent materials on western slopes of Mount Asahidake are characterized by Asahidake lava consisting of olivine-augite-hypersthene andesites (reviewed in Zhou and Tachibana 2004). Meadow soils contain thick peat layers (up to

58 cm deep) formed by sand and ash from volcanic eruptions over the last 1600 years with some peat layers dating back as far as 7,500 years bp (Takahashi 1990).

Our study includes three sites: an alpine snow meadow, a subalpine mire, and a montane forest. Our alpine site is a snow meadow plant community at 1600 m asl. Snow meadow communities are typical of leeward slopes where wind-blown snow accumulates and melts relatively late in the summer (typically July; Kudo and Ito 1992). This results in a short growing season with species adapted to wet alpine soils dominated by herbaceous species including *Anemone narcissiflora*, *Trollius riederianus*, *Saussurea riederi*, and *Pranunculus acris* (Kudo et al. 2011). The site is adjacent to treeline communities composed of *Pinus pumila*, *S. kurilensis*, and occasionally stunted subalpine species *Picea glehnii* and *Betula ermanii* (Okitsu and Ito 1984). Our subalpine site is a mire, or wetland, plant community at 1310 m asl and surrounded by subalpine forest. Intact peat soils are dominated by herbaceous species including *Sphagnum* spp., *Rhychospora yasudana*, *Scirpus caespitosus*, *Carex omiana*, and *Hosta atropurpurea* among others (Zhou and Tachibana 2004). The site is surrounded by subalpine forest dominated by *P. glehnii* with associated species *B. ermanii*, *Abies sachalinensis*, and *S. kurilensis*. Last, our forest site is a typical montane forest dominated by *B. ermanii* at 997 m asl. The understory is dominated almost entirely by *S. kurilensis* and is close to the lower altitudinal limits of *S. kurilensis* in the Taisetsu Mountains (Toyooka et al. 1983).

Remote sensing

To place our knowledge of plant growth in context, we evaluated historical changes in the distribution of *S. kurilensis* across a 2.2×2.5 km² (i.e., 550 hectares) plot of the western slope of

Mount Asahi. The area analyzed included our alpine and subalpine sites, and the lower portions of subalpine forest at 1300–1700 m asl. Distributions were compared between 1977 and 2012 using aerial imagery provided by the Geospatial Information Authority of Japan (1977 imagery) and Photec Co. Ltd. (Sapporo, Japan; 2012 imagery). Imagery for both time points was georeferenced with aerial photographs, had a Ground Sampling Distance of 30 cm, and was acquired in September after peak biomass when most species are senescing, which enabled us to readily distinguish species by color tone (Key et al. 2001).

Imagery was digitized and transformed into orthogonal projections. Digital surface model (DSM) data were generated on a 50 cm² cell grid using ArcMap 10.1 (ESRI, Inc., Redlands, CA, USA). DSMs were separated into 100 m elevation bins to estimate expansion patterns by elevation. The total area of each elevation bin was equal across time points. Patches of *S. kurilensis* were extracted as polygons using non-hierarchical clustering with an ISODATA clustering algorithm that grouped pixels based on reflectance levels of each wavelength (e.g., Townsend and Walsh 2001; Amagai et al. 2015). Area of bamboo abundance in each elevation category was calculated for the 1977 and 2012 images. Additionally, we evaluated *S. kurilensis* expansion beyond our study sites and into backcountry areas with subsets of DSM data.

Monitoring growth

To understand patterns of growth, 10 current year culms and 10 previous year culms (1+ year(s) old) of *S. kurilensis* were tracked weekly in the alpine, subalpine, and forest sites throughout the summer growing season until approximate peak productivity was reached at the end of August.

We recorded height (cm), and counts for all leaves and branches of each tagged individual weekly for 7 weeks.

Biomass measurements

To translate the aboveground growth into patterns of whole plant activity, we established 12 harvest plots to measure variation in above- and belowground biomass production between forest ($n = 7$) and alpine ($n = 5$) sites. We established a second alpine site within a similar snow meadow community located approximately 10 km from our primary alpine location where destructive harvests were performed. Aboveground portions of harvest plots measured 1.0×0.5 m² in the forest site and 0.5×0.5 m² in the alpine site. Belowground portions of harvest plots at all sites measured 0.3×0.3 m². Sample and plot sizes differed due to permitting restrictions and the difficulty of excavating and carrying large belowground samples.

We harvested the above- and belowground biomass of all individual culms in each of the harvest plots. We counted the total number of culms (shoots + branches) and leaves for aboveground samples. Belowground samples were sieved to remove soils using a no. 30-mesh sieve pan, washed in water baths, sieved again, and rewashed. We dried all samples for 48 hours at 60 °C. Additionally, we counted the number of rhizome buds (next year's culms) in each plot to estimate asexual reproductive investment.

Physiological measurements

Relative water content of leaves was measured at the end of the summer growing season as an indirect measurement of leaf turgor (Smart and Bingham 1974). In an effort to minimize impact on other measurements, leaves from 20 current year culms were sampled from each site for relative water content measurements after all other morphological and physiological measurements were completed. We sampled the healthiest, fully emerged leaf from each plant. Leaves were immediately placed in hermetically sealed vials and stored in a cool, dark container during transport to our lab. Leaf tissue was preserved for isotopic analysis and only leaf punches were used to estimate relative water content. We obtained fresh weights of leaf punches and then floated leaves in distilled water in a petri dish for 6 hours in a dark room to allow for rehydration. The assumed turgid weights were then obtained and leaves were subsequently dried overnight to obtain dry weights. Relative water content (RWC) was then calculated (Weatherley 1950; González and González-Vilar 2001),

$$RWC = \frac{w_f - w_d}{w_t - w_d}$$

where w_f , w_d , and w_t are fresh weight, dry weight, and turgid weight.

Leaf ^{13}C and ^{15}N isotopes, and total carbon and nitrogen were analyzed at the University of California, Davis Stable Isotope Facility via an elemental analyzer interfaced to a mass spectrometer (PDZ Europa ANCA-GSL and PDZ Europa 20-20, Secron Ltd., UK). Carbon isotope ratios were converted to discrimination values (Δ , per mil ‰ – a time-integrated measure of water-use efficiency; Dawson et al. 2002) by the equation:

$$\Delta = \frac{\delta_a - \delta_p}{(1 + \delta_p) \times 0.0001}$$

where δ_a is the carbon isotope ratio of atmospheric CO₂ (assumed to be -8 ‰) and δ_p is the measured carbon isotope ratio of the leaf tissue (Farquhar et al. 1989). Lower values of Δ indicate higher intrinsic water-use efficiency values (Dawson et al. 2002; Gremer et al. 2013).

Nitrogen isotopes are expressed as $\delta^{15}\text{N}$ calculated by the equation,

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where R_{sample} is the measured isotope ratio (¹⁵N/¹⁴N) of the leaf tissue and R_{standard} is the ratio of atmospheric N₂ (Sharp 2007). Last, measurements of stomatal conductance (g_s ; mmol m⁻² s⁻¹) were taken at peak productivity on a randomly selected, recently matured leaf on each individual (SC-1 Leaf Porometer, Decagon Devices, USA).

Environmental measurements

Soil volumetric water content (%) was measured within 10 cm of each individual weekly using a HydroSense soil moisture probe (HydroSense CD620, Campbell Scientific, USA). These measurements were used to track the seasonal soil moisture dry-down experienced by each individual. Photosynthetically active radiation (PAR, in μmol of photons m⁻² s⁻¹) was also measured above and below each tagged individual via a LI-190R quantum sensor and LI-250A light meter (LI-COR Inc., USA).

Statistical analyses

Historical changes in *S. kurilensis* abundance were estimated as percent change between 1977 and 2012. Expansion patterns were calculated for each 100 m elevation bin and summed to obtain an overall area expansion for the 35-year period analyzed. Measurements were transformed to meet statistical assumptions when appropriate. Relative growth rates (RGR) were estimated for each individual as the slope of separate linear regressions between log-transformed height (hRGR), leaf count (lRGR), the number of branches (bRGR) and time (c.f., Gremer et al. 2013). Total relative growth rate (tRGR) was estimated for each individual by summing relative growth rates of height, leaves, and branches. Mean relative growth rate values were calculated separately for current and previous year shoots at each site to examine between-site and ontogenetic variation.

We used a linear regression to test for a trade-off between relative growth rate and water-use efficiency (WUE) across sites as a corollary between stress tolerance and growth capacity. We also tested the relationship between relative growth rate and relative water content (RWC, a useful measurement of cellular water deficit and water stress; González and González-Vilar 2001), and $\delta^{15}\text{N}$ and C:N to test for the potential influence of nutrient stress and substrate availability in relation to growth between study sites (Robinson et al. 2000). All regression *P*-values were adjusted via Bonferroni correction for multiple testing.

Biomass production measurements were scaled to 1 m² when necessary to allow for between site comparisons. We ran a Welch two sample t-test with degrees of freedom corrected for uneven sample sizes to test for differences between the two alpine sites using number of

leaves per individual culm to ensure the second alpine site was similar to the main alpine site. We then compared biomass measures between alpine and forest sites with Wilcoxon tests using Bonferroni correction for multiple tests. We calculated a relative seasonal soil moisture dry-down rate for each individual as the absolute value of the slopes of separate linear regressions between the volumetric water content of soil and time. Linear regression was used to relate dry-down rates to estimates of tRGR and WUE, RWC, $\delta^{15}\text{N}$, and C:N at peak productivity. Last, we analyzed site differences in dry-down rates and physiological measures (g_s , WUE, RWC, $\delta^{15}\text{N}$, and C:N) with one-way ANOVA and Tukey's HSD tests. All statistical analyses were conducted in R 3.3.1 (R Core Team 2016).

Results

Sasa kurilensis more than doubled in abundance across elevations since 1977 (Fig. 2.2, Table 2.1). The largest changes occurred near treeline in the 1400–1500 m elevation range where the species more than tripled in spatial footprint from 11.61 hectares to 34.55 hectares, within the monitored region. Overall, the treeline ecotone saw increases in *S. kurilensis* abundance both above and below treeline with a total change from 59.517 hectares in 1977 to 120.70 hectares in 2012. The smallest changes were observed at the upper and lower elevation limits of the species with a 1.89 hectare increase in abundance below 1300 m asl and a 0.14 increase above 1700 m asl where sparse vegetation occurs. Our measurements below treeline are likely underestimates given that it is not possible to fully measure bamboo cover under the forest canopy. However, we presume error is small since bamboo expansion rates are faster than changes in forested canopy

during the 35-year measurement period. *S. kurilensis* spread was observed not only along trail systems but also in backcountry areas inaccessible to tourists (Appendix S2: Fig. A2.1–A2.2).

Sasa kurilensis growth strategies shifted from the typical less dense and taller forest site morphology to the denser and dwarfed form in the invaded sites above and below treeline (Fig. 2.3). tRGR decreased with elevation and was greatest in current year culms compared to older culms. Plants became increasingly dwarfed with elevation as hRGR decreased in both previous year culms and current year culms. Leafing rates (lRGR) were equivalent across all current year culms but lRGR were lower in older culms in the alpine relative to subalpine and forest sites (Fig. 2.3). Current year culms in the forest never produced branches and older culms in the forest site had the lowest branching rates compared to subalpine and alpine sites. Branching rates within subalpine and alpine sites were nearly identical for current and older culms, but decreased slightly between sites (Fig. 2.3).

Allocation to above- and belowground structures differed between forest and alpine sites. The number of leaves and culms produced per m² ground area increased with elevation. However, total aboveground biomass and its components decreased with elevation (Table 2.2). Asexual reproduction (i.e., the number of rhizome buds) was more than 3 times greater in the alpine site (462.96 ± 118.69 buds m⁻²) than the forest site (136.51 ± 28.14 buds m⁻²) but no differences were detected for overall rhizome dry weight and total belowground biomass between forest and alpine sites. However, root biomass that did not include rhizomes increased three-fold with elevation, suggesting an increase in belowground stressors (Table 2.2). As a result of the changes in total biomass and relative component allocations, AGB:BGB did not differ between forest and alpine sites. There was no difference in leaf counts between alpine sites.

Seasonal dry-down rates were highest in the subalpine site (1.06 ± 0.04 % vwc wk⁻¹; $\bar{x} \pm$ SEM) where mire soils dried twice as fast as alpine soils (0.65 ± 0.04 % vwc wk⁻¹), and more than three times as fast as forest soils (0.29 ± 0.03 % vwc wk⁻¹; Table 2.3). Light intensity in the forest site was nearly 1/4 of that observed in the subalpine and alpine sites where canopy cover above the tallest bamboos was non-existent (Table 2.3). However, current year shoots experienced significant shading from older shoots as maximum heights were reached at peak canopy expansion (and presumably peak standing biomass) within the developing bamboo canopy in the alpine meadow. Due to the subalpine mire's proximity to the neighboring forest on all sides, light intensity equaled alpine light conditions but only during peak daylight hours (Table 2.3). Shading from surrounding trees occurred early and late in the day. Last, g_s increased with elevation such that leaves from alpine settings had four times the g_s values as the forest site (Table 2.3). There was no detectable difference in g_s values in the remaining site comparisons. All sites differed in dry-down rates, but physiological measures were matched for subalpine and alpine sites and differed from the forest site (Table 2.3). However, RWC values were similar among forest and subalpine sites and differed from the alpine site.

Water-use efficiency (determined through Δ of leaves) decreased as tRGR increased across all sites and is indicative of how functional strategies reflect a fundamental tradeoff associated with performance in novel habitats ($r^2 = 0.34$, $P < 0.001$; Fig. 2.4). A similar negative correlation was identified between tRGR and leaf-level measurements of relative water content ($r^2 = 0.32$, $P < 0.001$), and carbon:nitrogen ($r^2 = 0.20$, $P < 0.001$). These findings are consistent with the nature of a functional relationship between RGR and WUE above; leaf water content and C:N decreased as tRGR decreased. Additionally, tRGR was positively related to $\delta^{15}\text{N}$ across all sites ($r^2 = 0.36$, $P < 0.001$), but this pattern was independent of changes in growth rate with

elevation. Total relative growth rates were negatively related to seasonal soil moisture dry-down rates across sites ($r^2 = 0.18$, $P < 0.001$) and so were $\delta^{15}\text{N}$ values of leaves ($r^2 = 0.54$, $P < 0.001$; Fig. 2.5). Water-use efficiency increased with increasing seasonal dry-down rates ($r^2 = 0.40$, $P < 0.001$) and so did leaf-level carbon:nitrogen ($r^2 = 0.35$, $P < 0.001$).

Discussion

Our study's results suggests that the rapid invasion of the dwarf bamboo *Sasa kurilensis* into alpine and subalpine meadows and mires is partly driven by an accelerated seasonal soil moisture dry-down and biomass production patterns relating to that process. A shift in resource allocation and growth strategy was evident in *S. kurilensis*' invaded ranges across the treeline ecotone, consistent with a trade-off between physiological stress tolerance and growth capacity aligned with changes in site water balance. Furthermore, it is likely the considerable flexibility in strategies associated with this species' morphological and physiological responses to variation in weather across the elevation range promoted its establishment and success, and will likely enable its future spread.

As plants expanded their range into higher elevations, individuals increased asexual reproduction, were increasingly dwarfed, grew in denser patches, and allocated resources towards increased branching patterns and more numerous photosynthetic structures. However, these individuals also grew slower, and increased investment in root systems relative to shoots. Additionally, *S. kurilensis* showed signs of increased stress with increasing stomatal conductance in its higher elevation, invaded ranges. *S. kurilensis* in its invaded subalpine and alpine sites also

exhibited distinct responses between sites: subalpine current year and previous year culms were taller than alpine counterparts were and previous year bamboo in the subalpine mire produced leaves 1.5 times faster than alpine plants of the same age. These differences likely relate to soil dry-down rates that were fastest in the subalpine mire.

Boundary studies like ours provide experimental evidence of contemporary and anticipated climate change impacts, along with offering inference to impacts on other global systems and their margins (Scott et al. 2006; Eldridge et al. 2011; Cable et al. 2012). Continued changes in species composition, dominance, and invasion of *S. kurilensis* into alpine and subalpine areas will likely alter landscape function by increasing snow accumulation given its shrubby perennial form, thereby altering snowmelt patterns and hydrological function (Hudson and Henry 2009; Drexler et al. 2013). Its canopy height and density will also continue to reduce diversity by outcompeting alpine and subalpine species for light, water, and nutrient resources (Kudo et al. 2011). *S. kurilensis* exhibits the key traits found in many of the world's invasive wetland species: it forms dense monotypic stands taller than competing plant species, primarily disperses via asexual reproduction, and produces abundant, high quality litter (Larkin et al. 2011). This trait syndrome and its response to varying soil moisture patterns may be, in part, responsible for *S. kurilensis* rapid invasion above and below treeline. Our results support this by examining site-specific differences in growth and functional traits responding to variable moisture patterns, a direct mechanism that identifies the fine-scale environmental controls of plant success (Walker et al. 2015).

Our study provides experimental evidence for the role of moisture in determining *S. kurilensis* success. Many montane systems experience a summer soil dry-down as snowpack melts, followed by warm summers with relatively low precipitation (Billings and Bliss 1959;

Taylor and Seastedt 1994; Grant et al. 2004). It is possible climate change is exacerbating a post-snowmelt dry-down period, impacting treeline communities (Barnett et al. 2005; Moyes et al. 2013; Winkler et al. 2016). This would act to promote *S. kurilensis* invasion observed near treeline. We calculated seasonal soil moisture dry-down rates as an indirect measure of site-specific and within-site variation in snowpack cover that may serve as a more concrete environmental measurement determining *S. kurilensis* success in terms of growth and asexual spread. It is likely systems changing towards a more hospitable environment depend on suitability for a given species (Sorte et al. 2013) and *S. kurilensis* is responding to shifts towards a favorable environment at the invasion sites we examined (Fujita 2009).

Changes in hydrological conditions within our subalpine mire site are likely promoting *S. kurilensis* invasion, which has serious consequences not only for native species impacted but also for nutrient cycling. As a long-lived, perennial, with rapid growth rates, *S. kurilensis* has greater net CO₂ exchange rates compared to typical mire species (223.8 vs. 5.8 g C m⁻² of ground area y⁻¹) and coupled CH₄ and N₂O emissions, resulting in a system that has a higher global warming potential compared to a typical mire ecosystem (1009 ± 73 vs 408 ± 129 g CO₂ m⁻² y⁻¹; Nagata et al. 2004). Additionally, the role of mires and similar wetland peat soils in carbon sequestration is of global concern given their role in global carbon cycling (Gorham 1991; Belyea and Malmer 2004). Our study is the first to examine the potential for *S. kurilensis* to disrupt cycling and is essential in forecasting impacts of climate change.

Soil moisture has previously been shown to affect the foraging strategies of a bamboo species in temperate China, increasing allocation to belowground roots and rhizomes as soils dry (Qing et al. 2004). Our study examines similar measures along a moisture gradient at multiple sites but also includes increased physiological stress responses with elevation that correspond to

changes in growth rates and carbon allocation. In fact, belowground allocation to roots and rhizome buds (i.e., next year's culms) was higher in our wetter sites. Although counterintuitive, it is likely the observed increase in aboveground density of culms that followed the elevation gradient induced belowground changes as a mechanism to prevent competition among related individuals. This also likely explains the observed increase in allocation to branching patterns and photosynthetic surfaces at higher elevations where soil moisture was greater but also decreased at faster rates than in the forest site. We believe the increased stress brought on by increased exposure and competition for resources with related individuals is alleviated by the species flexible morphology and selective allocation to above and belowground structures as it expands its range.

Growth strategies of *S. kurilensis* varied not only with elevation and associated environmental drivers, but also as a result of ontogeny. Current year culms grew exceedingly faster than older culms in terms of height, the rate at which leaves were produced, and in terms of total relative growth rates (Figure 3). Current year forest culms responded to relatively poor light conditions by rapidly growing tall to reach the canopy of neighboring related culms. This is in contrast to current year culms in the subalpine and alpine and that were essentially free from any light limitation. Current year culms so efficiently responded to light limitation in the forest that no single branch was produced during the first year of growth and, instead, branching did not occur until individuals matured to their second year (Figure 3). Again, this is in stark contrast to current year culms in the subalpine and alpine that branched relatively quick during their first year of growth and mostly maintained branching rates during later years. This is likely explained by competition between culms and explains the dramatic increase in the number of rhizome buds

observed at higher elevations, compensating for increased turnover rates of culms with elevation due to stress.

Trade-offs in species performance are commonly explored in ecology to explain resource limitations, allocation strategies, species coexistence, and biodiversity at multiple spatial scales, among others (Kneitel and Chase 2004; Fynn et al. 2005; Angert et al. 2007). Here, we tested for a trade-off between stress tolerance and growth capacity that may explain *S. kurilensis* invasion success. We observed a trade-off between water-use efficiency and total relative growth rate across the variation in form and function we observed in *S. kurilensis*. Those individuals in the invaded subalpine mire and alpine meadow were more similar to each other than to the forest individuals in terms of water-use efficiency, potentially illustrating the influence of an open canopy and high solar radiation in driving the observed stress response. This goes against the expected trend of isotopic discrimination decreasing with elevation (Körner et al. 1991) and, instead, may be further indicative of additional limitations beyond light operating in each site. A similar trade-off was identified between *S. kurilensis* growth rates and leaf relative water content. This trade-off was different in that relative water content only strongly varied as a mean comparison between sites and in areas of the alpine where soil dry-down rates were highest, leading to the highest observed levels of cellular water deficit.

Although we did not test nitrogen availability in soils, leaf $\delta^{15}\text{N}$ and C:N also did not follow the expected trend of decreasing discrimination and increasing nitrogen content with elevation (Körner et al. 1991). This likely reflects nutrient availability and plant requirements in the different sites. In addition, *S. kurilensis* has been shown to have a higher nitrogen-use efficiency than its forest competitor *Betula ermanii* (Tripathi et al. 2006) and, as a result, appears to have evolved a relatively conservative nitrogen-use strategy that may further enhance its

ability to deal with variable nutrient conditions in its invaded ranges. Hence, we observed increased allocation to foraging roots at the higher elevation, invaded range while rhizome mass did not change with elevation. Rhizomes likely enable *S. kurilensis* to conserve nitrogen resources via translocation, storage, and remobilization during periods of stress or with ontogeny. Our results are consistent with previous studies examining nutrient stress responses belowground (e.g., Rengel and Marschner 2005) and further illustrate site-specific variation in the fine-scale environmental drivers determining success.

The ability of *S. kurilensis* to invade treeline communities is likely driven by the species flexible morphology and physiological stress tolerances driven by changes in seasonal soil moisture dry-down rates. *S. kurilensis* successfully altered its growth strategies in the two invaded communities by shifting its allocation of resources and driving its physiology towards a more conservative approach. We revealed that *S. kurilensis* responded to increasing levels of stress (i.e., high levels of irradiance and increased rates of soil moisture decline) by altering both its morphological and physiological allocation of resources. This study is the first to reveal strategies of invasion taking place in two distinct communities (i.e., above and below treeline). *S. kurilensis* has been identified numerous times as a species sensitive to the impacts of climate change given its limitations by snow conditions (Noguchi and Yoshida 2005; Tsuyama et al. 2008), and one that is ideal to monitor climate impacts as an indicator species of ecosystem decline (Higa et al. 2013). These modelling studies suggest that *S. kurilensis* will continue to thrive in Japan only in northwestern Hokkaido and in mountainous areas along the Sea of Japan. This indicates that *S. kurilensis* at our study sites will likely be extirpated. It is possible that the invasion our study characterizes is the species response to recent climate change; driving the species upward and into new habitats as sites become increasingly hospitable. However, it is not

yet apparent if negative effects are being experienced in the species lower elevation habitats including our forest site. Future research should examine lower elevation responses of the species, and further explore the role of water in determining invasion success.

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Table 2.1. Expansion of *Sasa kurilensis* in hectares and as a percentage of the total survey area (550 hectares) from 1977 to 2012 at six elevation categories. The elevation bands sampled in this analysis had consistent sizes.

Elevation	1977	2012	Δ distribution
< 1300 m	9.498	11.387	1.889
1300–1400 m	10.904	21.235	10.331
1400–1500 m	11.607	34.552	22.945
1500–1600 m	15.502	27.494	11.992
1600–1700 m	11.875	25.764	13.889
> 1700 m	0.131	0.269	0.138
Total	59.517	120.701	61.184
% of survey area	10.82%	21.95%	11.12%

Table 2.2. $\bar{x} \pm \text{SEM}$ for above- and belowground biomass production measurements (m^{-2}) in forest ($n = 7$) and alpine sites ($n = 5$).

Biomass component	Forest	Alpine	<i>W</i>	<i>P</i>
number of leaves	1271.71 \pm 207.20	2834.40 \pm 244.97	35	< 0.01
number of culms	36.58 \pm 4.49	149.60 \pm 18.79	35	< 0.01
leaves dry weight (g)	401.44 \pm 75.42	241.06 \pm 21.16	4	0.03
culms dry weight (g)	3317.91 \pm 657.01	1440.45 \pm 147.60	4	0.03
aboveground biomass (g)	3719.53 \pm 727.93	1681.52 \pm 148.73	4	< 0.05
number of rhizome buds	136.51 \pm 28.14	462.96 \pm 118.69	21	0.02
rhizomes dry weight (g)	1505.61 \pm 429.88	1051.34 \pm 134.03	10	1
roots dry weight (g)	213.42 \pm 43.80	706.58 \pm 69.15	21	< 0.05
belowground biomass (g)	1719.03 \pm 527.36	1757.93 \pm 203.00	12	0.83
AGB:BGB	3.67 \pm 1.06	1.06 \pm 0.12	2	0.07

Results of Wilcoxon tests are reported with significance at $\alpha = 0.05$ with Bonferroni corrections for multiple testing.

Table 2.3. $\bar{x} \pm \text{SEM}$ environmental and leaf-level characteristics by site, including, soil dry-down rates as a percent change in volumetric water content (vwc) each week, ranges of photosynthetically active radiation (PPFD) above and below bamboo canopies, along with mean g_s ($\text{mmol m}^{-2} \text{s}^{-1}$), water-use efficiency (WUE), relative water content (RWC), $\delta^{15}\text{N}$, and C:N.

Variable	Forest	Subalpine	Alpine	<i>F</i>	<i>P</i>
soil dry-down (% vwc wk ⁻¹)	0.29 ± 0.03 ^a	1.06 ± 0.04 ^b	0.65 ± 0.04 ^c	5.43	< 0.001
Range PPFD above	9.53–930.60	24.84–2370.00	16.78–2289.00	–	–
Range PPFD below	4.87–99.30	15.25–2268.00	2.72–1755.70	–	–
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	65.48 ± 11.10 ^{ac}	204.42 ± 19.05 ^{bc}	310.04 ± 19.32 ^b	3.69	0.03
WUE (Δ)	19.94 ± 0.21 ^a	17.38 ± 0.14 ^b	17.44 ± 0.17 ^b	25.82	< 0.001
RWC	0.92 ± 0.01 ^a	0.89 ± 0.01 ^a	0.83 ± 0.02 ^b	13.32	< 0.001
$\delta^{15}\text{N}$	-1.89 ± 0.25 ^a	-5.12 ± 0.35 ^b	-4.27 ± 0.19 ^b	37.21	< 0.001
C:N	15.34 ± 0.41 ^a	21.11 ± 0.64 ^b	19.25 ± 0.61 ^b	25.82	< 0.001

^{a,b,c}Indicates differences between sites according to one-way ANOVA and Tukey's HSD tests.

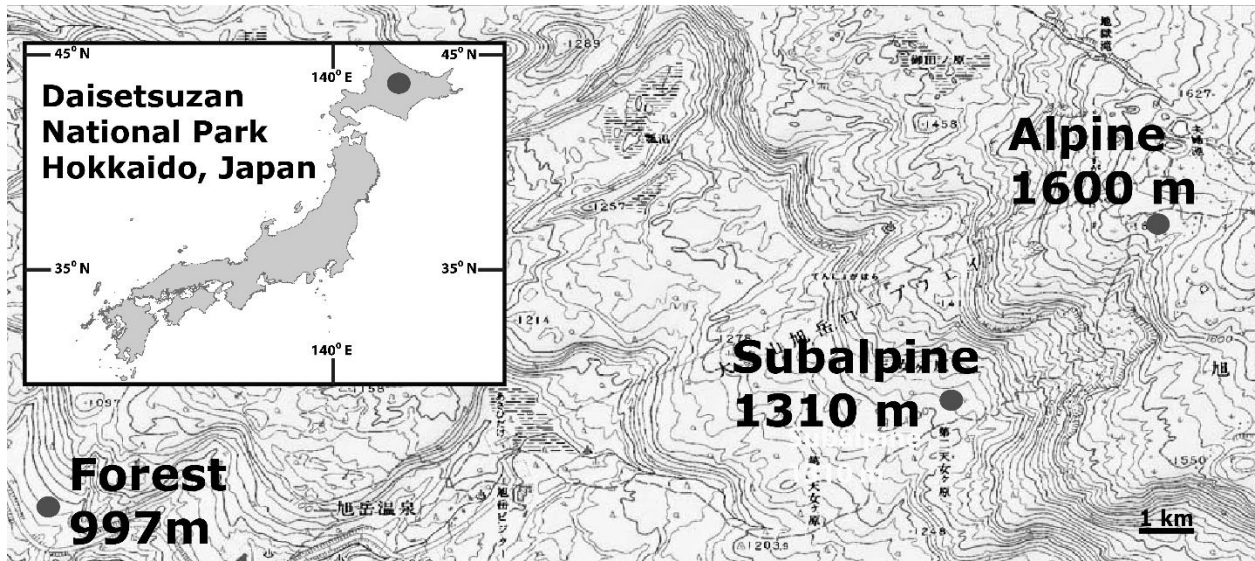


Fig. 2.1 Contour map of sites and sampling locations (circles). Inset: location of Daisetsuzan National Park (circle) in Hokkaido, Japan

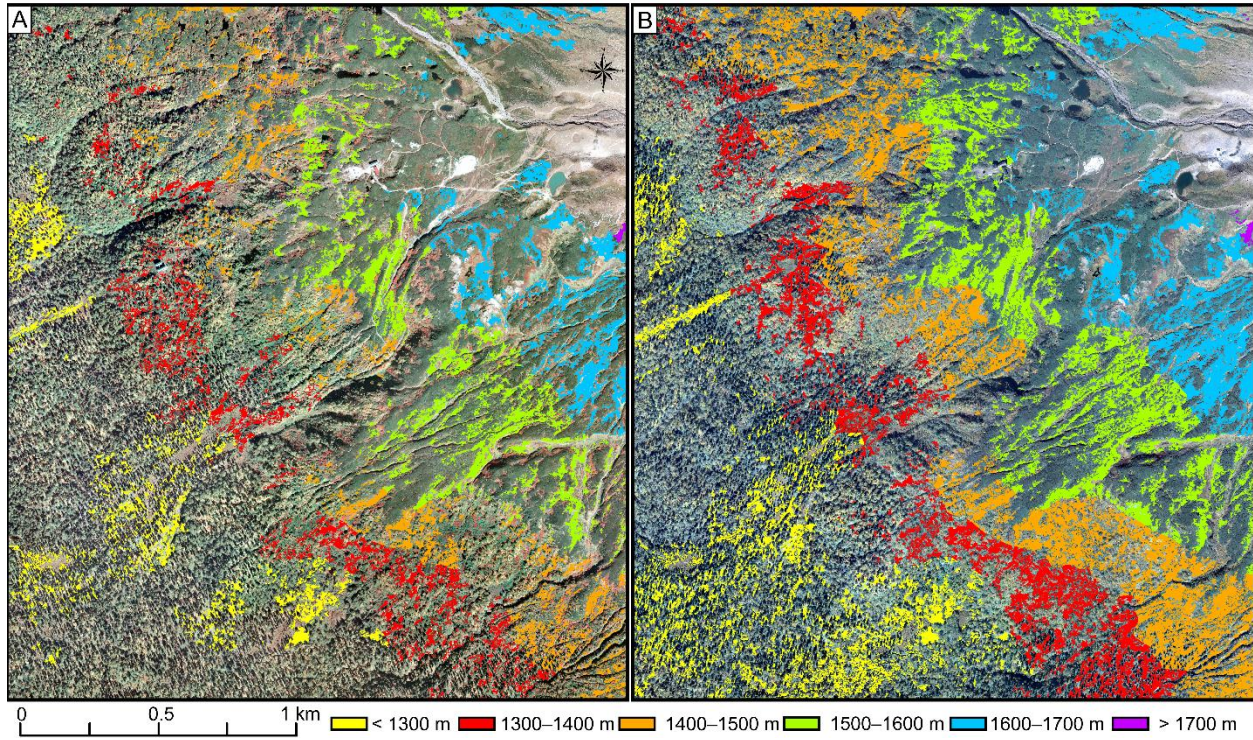


Fig. 2.2 Imagery from vegetation classification analysis using clustering of remote sensing spectral imagery showing changes in *Sasa kurilensis* distribution from 1977 (A) and 2012 (B). Identified plant patches (at a 30 cm pixel) are identified with a color, depending upon elevation

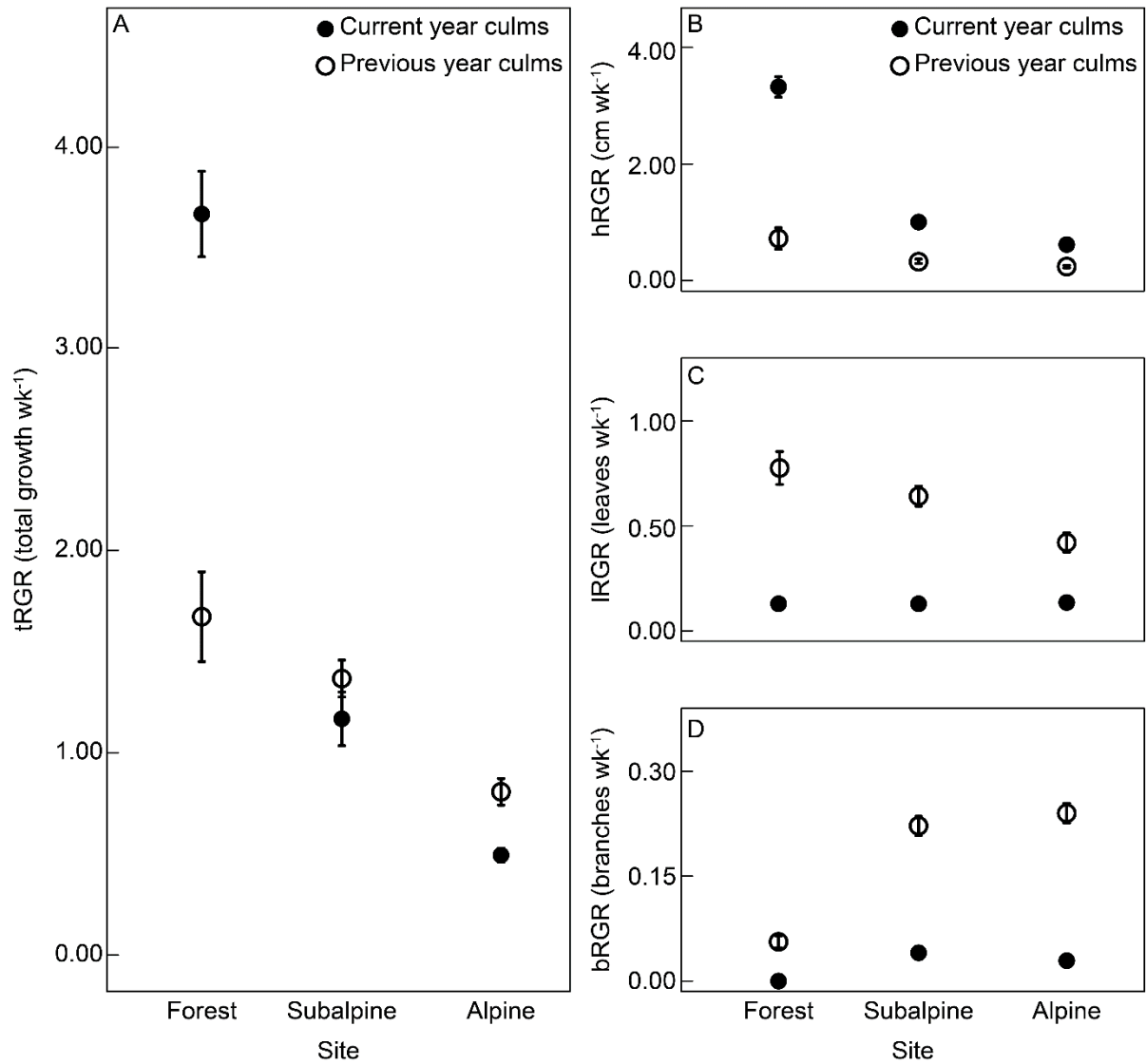


Fig. 2.3 Total relative growth rate (total growth wk^{-1} ; A) and individual component growth rates for height (hRGR, cm wk^{-1} ; B), leaves (leaves wk^{-1} ; C), and branches (branches wk^{-1} ; D). $\bar{x} \pm \text{SEM}$ are presented for current year (closed circles) and previous year culms (open circles) for each sites. Current year culms in the forest site did not produce branches during the duration of the experiment

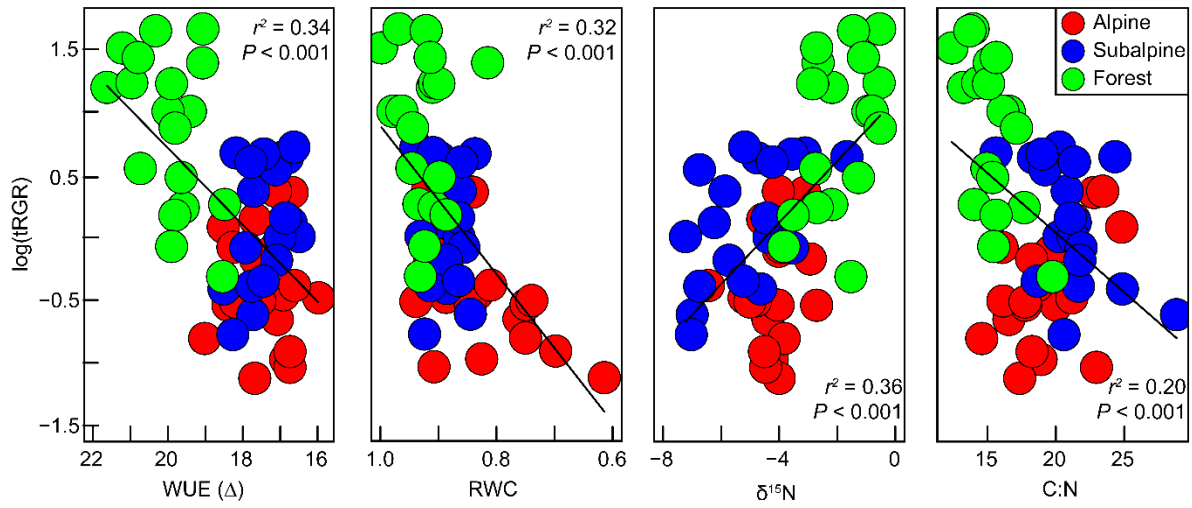


Fig. 2.4 Relationship of log-transformed Total RGR with water-use efficiency (WUE, Δ), relative water content (RWC), $\delta^{15}\text{N}$, and C:N across sites. Note: WUE and RWC values are plotted on a descending axis to illustrate an increasing WUE and cellular water deficit (RWC) as one moves left-to-right on the figure

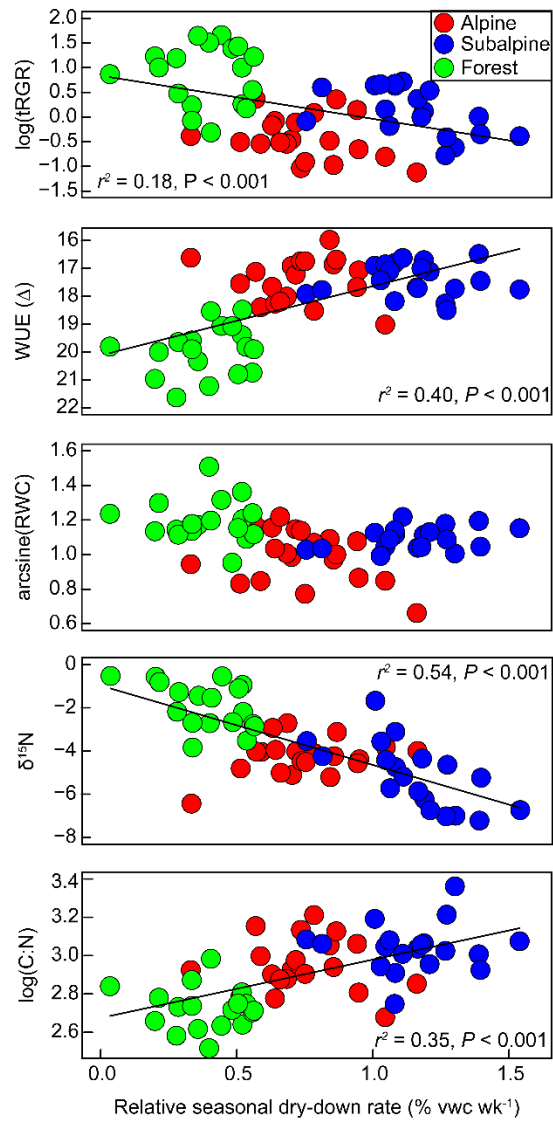


Fig. 2.5 The relationship between relative seasonal soil moisture dry-down rates and total relative growth rate, water-use efficiency, relative water content, $\delta^{15}\text{N}$, and C:N across sites

Appendix S2

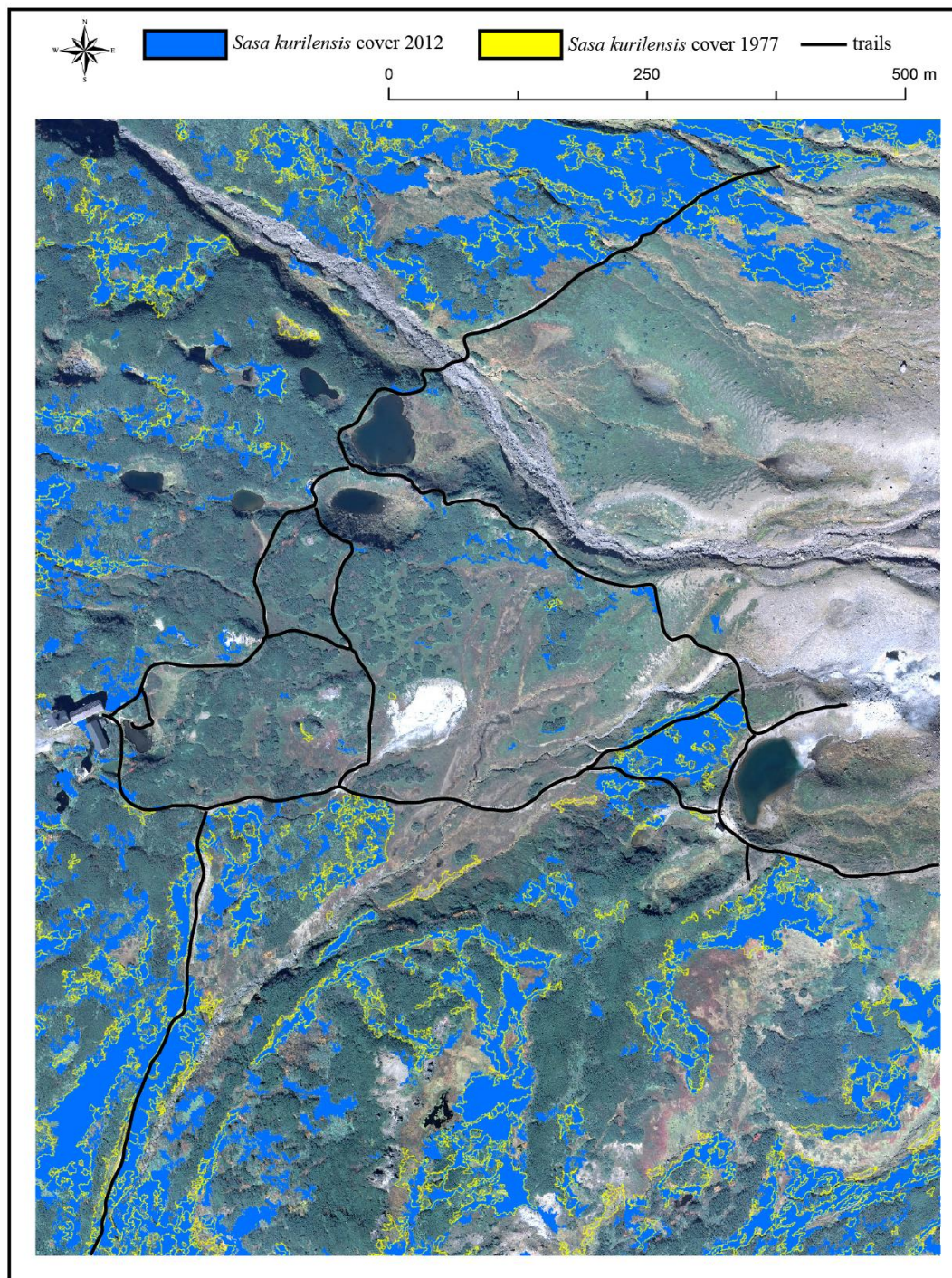


Fig. S2.1 Satellite imagery analyses highlighting changes in *Sasa kurilensis* distributions in our alpine meadow site in 1977 (yellow outlines) and in 2012 (blue patches). Imagery reveals *S. kurilensis* expansion is occurring independent of trail locations and is increasingly common in backcountry areas where visitor access is not permitted

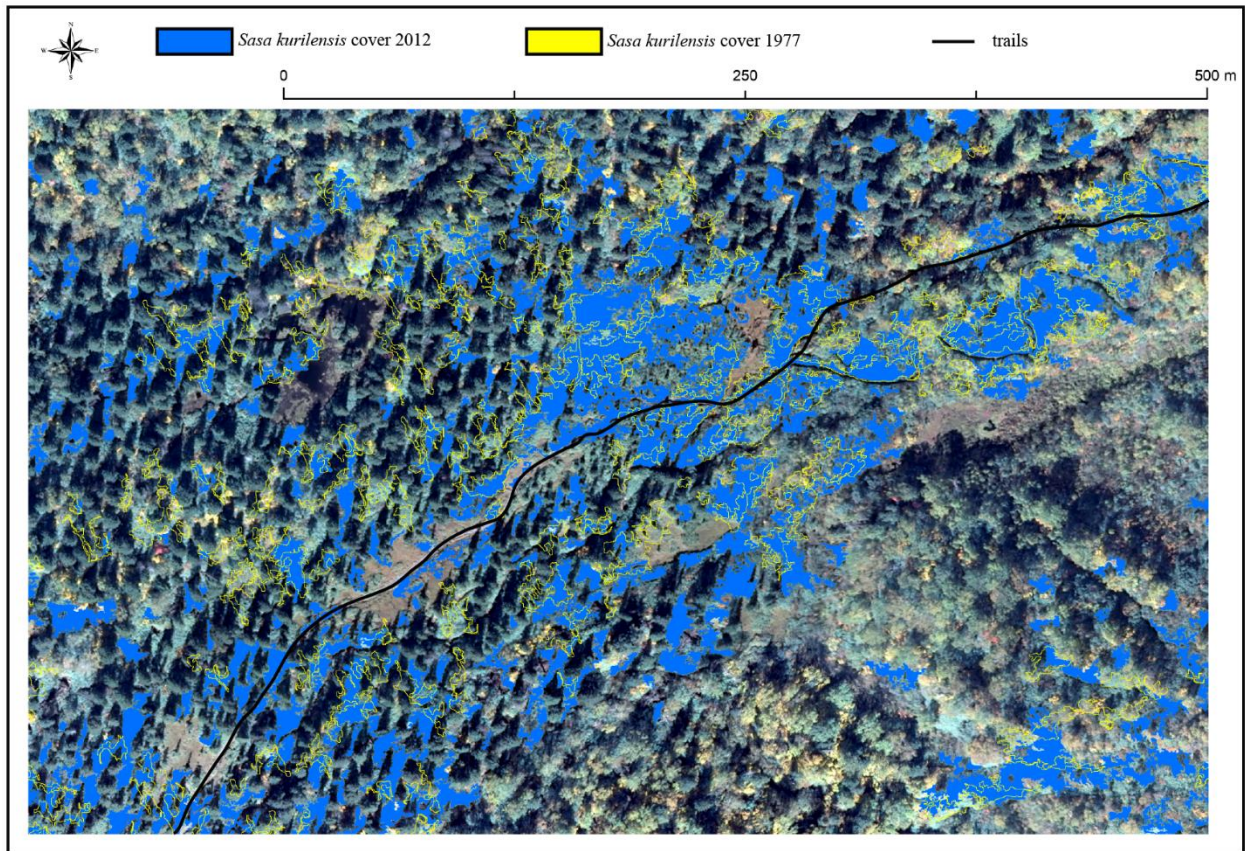


Fig. S2.2 Satellite imagery analyses highlighting changes in *Sasa kurilensis* distributions in our subalpine mire site in 1977 (yellow outlines) and in 2012 (blue patches). Imagery reveals expansion is occurring beyond our focal site in the center of the image

CHAPTER 3

The unusual population structure of the rapidly spreading invasive Sahara mustard (*Brassica tournefortii*) in the United States

Abstract

Human-induced climate change is promoting invasive species success globally and, in some cases, causing an apparent feedback loop in which these non-native species accelerate climate change. Human activities further facilitate invasive success by acting as vectors for dispersal via road, train, air, and marine traffic, and by producing efficient corridors for movement including canals, drainages, and roadway ditches. The invasive annual forb Sahara mustard (*Brassica tournefortii*) has rapidly invaded the southwestern United States and northern Mexico within this past century, displacing natives and altering these water-limited landscapes. We used genome-wide single nucleotide polymorphisms to identify the population structure and spatial geography of Sahara mustard using 760 individuals from 52 sites across its invaded range. We also used herbaria records to model species expansion rate since its presumed introduction in the 1920s. Overall, we saw that Sahara mustard experienced atypical expansion patterns with a relative constant rate of expansion since its introduction. We found that Sahara mustard exists as three genetically distinct populations in the United States without clear geographic pattern. High genetic divergence in one small population identified at only one site is likely a new introduction still in an initial phase of establishment prior to invasive spread. However, overall low diversity within and between the other populations suggest that this primarily selfing species has undergone an isolate-breaking event that may explain its recent rapid expansion. Our study is the

first to explore the genetic composition of Sahara mustard in its invaded range and our results warrant the examination of the species in its native range. Management efforts aimed at control should prioritize targeting the isolated, novel population we identified and identifying broad treatments, such as biological control, that may be developed over a longer time horizon. Leveraging this current phylogeographic study to better understand the species in its historic range would identify native source populations that may lead to effective control and eradication measures in the arid and semi-arid United States.

Introduction

Successful invasions often occur as dispersal barriers break down (Mooney and Cleland 2001; Wilson et al. 2007; van Kleunen et al. 2015). One potential barrier is inertia to adapt to new environments. The rate of population expansions for invasive species may depend on the time it takes to adapt to new environments. The evolutionary mechanisms enabling the success of invasives can be explained by elucidating the genetic variability and geography of a species (Lee 2002; Cristescu 2015; Colautti and Lau 2015). As such, understanding the population structure of invasive species can provide insight into the history of dispersal during the colonization and expansion process (Durka et al. 2005; Peccoud et al. 2008; Barker et al. 2016). Population genetic studies of invasive species have often revealed mixed strategies among cases, making it difficult to find a single suite of successful invasive characteristics (Sakai et al. 2001; Simberloff et al. 2013). Despite this, genetic research can explain how gene flow and selection events can lead to local adaptation (Dybdahl and Kane 2005; Lavergne and Molofsky 2007; Verhoeven

2011; Colautti and Barrett 2013), pinpoint ecological phenomenon such as bridgehead effects as a mechanism for invasion success (Eriksen et al. 2014; Barker et al. 2016), identify potential for impacts on interacting species (Magalhaes et al. 2011), and even be used to effectively manage potentially undesirable species (Simberloff 2003).

Although a variety of characteristics have been used to explain successful invasive species establishment and spread, reproductive strategies are often cited as a primary driver (Sakai et al. 2001; Richard et al. 2006; van Kleunen et al. 2015). Indeed, self-compatibility and other reproductive strategies (e.g., vegetative propagation, apomixis, etc.) are commonplace in invasive plant species (Baker 1955; Pappert et al. 2000; Colautti et al. 2005; Dlugosch and Parker 2008). These reproductive systems enable populations to expand from one or a few invaders and allow for rapid population growth regardless of the number of individuals introduced (Cheptou 2004; Dornier et al. 2008; Blackburn et al. 2015). One hypothesis predicts that invading species undergo an initial lag phase where populations remain small before a relatively sudden range expansion (Brock et al. 2015; Pannell 2015), and that the species' breeding system might determine the length of the lag phase or ameliorate it altogether (Crooks and Soule 1999; Parker 2004; Crooks 2005). In this case, it is possible that mixed breeding systems within a species (e.g., facultative autogamy, or being capable of, but not restricted to, self-fertilization) can speed up the spread of invasive species by reducing inbreeding and other negative consequences of small population sizes during colonization (Daehler 1998; Saltonstall 2003; Morgan et al. 2005; Arnaud-Haond et al. 2006; Ansell et al. 2008). However, the extent to which mixed breeding systems share similar characteristics across invasive species and the existence of associated lag phases in such systems remains largely unexplored, leaving explanations for rates of local adaptation and range expansion to speculation.

Invasions often arise from multiple introduction events and subsequent admixture can increase invasion success (Durka et al. 2005; Dlugosch and Parker 2008; Lombaert et al. 2010; Barker et al. 2016). Multiple introductions and subsequent admixture commonly enhance invasion success by increasing genetic diversity of invaded populations, thereby decreasing inbreeding depression and enabling adaptation (Parker et al. 2003; Prentis et al. 2008; Lawson Handley et al. 2011; Rius and Darling 2014; Dlugosch et al. 2015; Peischl and Excoffier 2015; Barker et al. 2016). However, there is mounting evidence that not all invasives experience reduced genetic diversity and, instead, can avoid the negative effects of bottlenecks with high propagule pressure in the form of larger migrant population sizes (Holle and Simberloff 2005; Roman and Darling 2007). Varied scenarios like these may explain why estimated lag phases have ranged from nearly no delay to over 300 years (Crooks and Soule 1999) and further reveal that evolutionary processes allowing for invasion success will be determined by standing genetic variation generated by either a single or multiple introductions (Kolbe et al. 2004).

Examining invasion routes along environmental gradients and diverse habitats can reveal adaptive strategies that would otherwise be difficult to detect in homogenous invaded habitats (Kolbe et al. 2004; Richards et al. 2012; Hamilton et al. 2015). That is, spatial patterns of invasion can provide insight on a species' potential for local adaptation to different environments across its invaded range (Leger et al. 2009). Assessing genetic variability across diverse ecological conditions might suggest potential for adaptation in response to selection (Reznick and Ghalambor 2001; Lee 2002; Genton et al. 2005; Facon et al. 2006; Colautti and Lau 2015) regardless of the level of variability in the species (Tsutsui et al. 2000; Facon et al. 2006; Dlugosch et al. 2015; Bock et al. 2015).

Sahara mustard (*Brassica tournefortii*; Family Brassicaceae) is an annual herb native to the Mediterranean basin and much of the Middle East through western India (Prain 1898; Thanos et al. 1991; Aldhebiani and Howladar 2013). Its genome is approximately 791 Mbp (Arumuganathan and Earle 1991). It is considered a weedy species in agriculture fields in parts of its native range (Ahmed et al. 2015; El-Saied et al. 2015) but also has traditional dietary uses and economic value in regions where it is cultivated (Guarrera and Savo 2016; Singh et al. 2015). Sahara mustard is an invasive throughout much of Australia (Chahuan et al. 2006), South Africa (McGeoch et al. 2009), Chile (Teillier et al. 2014), and western North America (Li et al. 2015). Sahara mustard germinates under a wide range of temperatures, light, and soil conditions and depths (Thanos et al. 1991; Jurado and Westoby 1992; Chahuan et al. 2006; Bangle et al. 2008). It is capable of self-fertilization (i.e., facultatively autogamous) and produces seeds rapidly (ca. 50 days from germination; Marushia et al. 2012) and in high quantities (Trader et al. 2006) that can remain viable at least 1 year after production (Chahuan et al. 2006; pers. obs.) and can likely undergo some level of dormancy similar to desert annuals with which it co-occurs (Adondakis and Venable 2004). Seeds contain a mucilaginous film that provides protection from desiccation and likely increases viability during dispersal via animals and water (Bangle et al. 2008). Individual plants can produce over 16,000 seeds annually that disperse via small mammals, flowing water, wind, and human transport (Trader et al. 2006; Bangle et al. 2008; Sánchez-Flores 2007; Berry et al. 2014).

The first documented occurrence of Sahara mustard in the United States comes from an herbarium sample collected in the Coachella Valley, California in 1927. It is thought to have been introduced as a contaminant of date palm crops (Sanders and Minnich 2000) and remained relatively unnoticed except in the Coachella and Imperial Valleys of California deserts where it

was observed to be locally established (Musil 1948, 1950; Robbins et al. 1951). Herbaria records tracked its spread to coastal California in the late 1950s and to Tucson, Arizona and Sonora, Mexico in the early 1970s. The species apparently saw a population boom beginning in the 1980s when it spread rapidly throughout the southwest (Sanders and Minnich 2000).

Sahara mustard is having ever-greater impacts on natural ecosystems across the southwestern United States (Barrows et al. 2009; VanTassel et al. 2014). Since its presumed introduction in the 1920s, this invader has become increasingly common in semi-arid regions including all counties in Southern California (Sanders and Minnich 2000) and throughout the Southwest US and Northwest Mexico. Although a few ecological studies have examined the species performance and impacts in a few invaded areas (Barrows et al. 2009; Marushia et al. 2010, 2012; VanTassel et al. 2014; Li et al. 2015), no research has been conducted to examine the genetic structure of this rapidly-spreading invasive.

In this study, we use next generation sequencing to generate genome-wide polymorphism data from across the invaded range of Sahara mustard. We also calculated allele frequencies within sites to better understand connectivity of sites based on the species' reproductive strategies. We use these data and historical herbaria records to answer three questions. First, what is the historical geographic expansion rate of Sahara mustard? Second, is there genetic variability across its range? Third, what are the rates of selfing in each Sahara mustard population? We expected regional grouping of populations, given that both barriers to dispersal and multiple introductions were likely. We also expected genetic diversity to be low given that Sahara mustard is facultatively autogamous.

Methods

Historical range expansion

We utilized herbaria records to examine the geographic spread of Sahara mustard in North America through time. We realize that these data often provide an incomplete picture that can be misleading because of uneven collection efforts and time-lags that do not accurately illustrate the invasion patterns (Williamson 2006). Nevertheless, herbaria records can provide insights into invasion patterns and can be used as a conservative underestimate of range expansion (Crawford and Hoagland 2009; Delisle et al. 2013). We obtained a total of 2834 records containing collection dates and spatial data from online databases including the Consortium of California Herbaria (<http://ucjeps.berkeley.edu/consortium/>), the Global Biodiversity Information Facility (<http://www.gbif.org>), and the Southwest Environmental Information Network (<http://swbiodiversity.org/seinet/index.php>). Additionally, we included observation data from our field collections, bringing our total number of localities to 2915.

Sampling

We sampled up to 30 individuals each from 70 locations (more than 2000 individuals total) spanning a ca. 10° latitudinal and ca. 15° longitudinal gradient across the species' invaded range in Spring 2015 (Fig. 3.1; Table 3.1). We selected the highest quality samples for genetic analysis, amounting to 760 individuals from 52 populations. Sites ranged from coastal Mediterranean to hot desert ecosystems with elevation ranging 0–1500 m asl (Table 3.1). Tissue for genetic

analysis were desiccated with silica gel for preservation and transported to UCI after fieldwork concluded in Spring 2015.

DNA Extraction and genotyping

We extracted gDNA from 760 individuals from 52 populations using QIAGEN DNeasy Plant Mini Kits (Qiagen, Valencia, CA, USA). We estimated DNA concentrations via fluorometry (Qubit 2.0 Fluorometer, Invitrogen, Life Technologies) and tested DNA quality for a subset of samples via 1% agarose gel electrophoresis. We generated single-nucleotide polymorphism (SNP) data via nextRAD (Nextera-tagmented, reductively-amplified DNA) sequencing (Russello et al. 2015). nextRAD uses short oligonucleotide primers to amplify arbitrary loci across samples. We integrated primers into the Nextera library prep protocol (Illumina, Inc), which also ligates short adapter sequences to the ends of the DNA fragments. We then amplified DNA fragments with one of the primers matching the adapter sequence. Initial reads indicated successful amplification and sequencing. We barcoded pooled samples before purification and size selection to 350–500 bp. We sequenced multiplexed segments on an Illumina HiSeq2000 platform (Genomics Core Facility, University of Oregon) producing 100 bp read lengths. In total, we obtained ca. 22 billion paired-end Illumina reads across the 760 individuals.

We processed raw sequence data using Trimmomatic software (Bolger et al. 2014) to remove adapter sequences and filter sequences less than 50 bp. We quality-filtered the sequences using the program `process_radtags` in STACKS (Catchen et al. 2011, Catchen et al. 2013). We retained sequences with at least 15–2500× coverage, and present in at least 10% of samples. We removed locus if more than two alleles were found in a sample in more than 5% of a sampling

locality to exclude paralogs (Hare 2001, Russello et al. 2015). We then mapped remaining sequences to a reference created using abundant reads across the combined set of samples using the program BBmap v.35.40 (<http://sourceforge.net/projects/bbmap>; *sensu* Russello et al. 2015). 15 individuals were removed from the final dataset due to >75% missing data. In total, 899 SNPs were identified across the 745 sequenced individuals. We took 200 reads randomly from each sample for comparison to known sequences and blasted them to the NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to test for contamination from species other than Sahara mustard. No plausible contamination was detected in the tested reads.

Analyses

We estimated the spread of Sahara mustard populations using herbaria record locality data by counting the number of spatial units that the species occupied across time (1927–2016). Spatial units were delimited by rounding geographic degrees to the nearest hundredth. Thus, records were classed into ca. 1 km² units across the invaded range. We created accumulation curves of the number of spatial units occupied by Sahara mustard per year, with the assumption that the species currently occupied all historic localities. This allowed us to examine rates of growth through time and identify a potential lag phase (Crooks 2005).

We first visualized the population structure of all sequenced individuals using a discriminant analysis of principal components (DAPC) to construct population differentiation relationships (Jombart et al. 2010). We tested this on both the full dataset and a subsetted dataset that removed one population that was substantially divergent from all other samples. These

analyses were performed using the adegenet package in R 3.3.2 (Jombart 2008; Jombart and Ahmed 2011; R Core Team 2014).

We estimated the number and location of genetic clusters using the spatial Bayesian clustering algorithm implemented in TESS 2.3.1 (Chen et al. 2007; Durand et al. 2009). TESS uses spatial locations of samples to construct a neighborhood network of individuals to measure spatial patterns of genetic relatedness. TESS makes stronger inference of population structure in selfing species (Guillot et al. 2009; Fogelqvist et al. 2010). We used the admixture model (CAR) set at the default spatial interaction parameter $\psi = 0.6$ with a burn-in length of 10,000, a run length of 50,000, and performed 10 iterations of $k = 2-10$. Deviance information criterion (DIC) were averaged and plotted for each k to select the optimum number of clusters following Chen et al. (2007). Mean membership scores per sampling site were plotted as admixture proportions following François (2016) in R 3.3.2 (R Core Team 2014). We visualized mean membership of sample sites using the LEA R package (Frichot and François 2014). Mean membership values from the ten TESS runs of the optimal k value were used to visual admixture at each site by overlaying results onto a map of the sampling range.

We examined the mean and standard error of the mean observed (H_o) and expected (H_{exp}) heterozygosities for each population as a measure of diversity. We also calculated inbreeding coefficients (F_{is}) for each site and used this to calculate selfing rates (S) and levels of outcrossing ($T = 1 - S$; Hedrick 2011). Last, we inferred the number of private alleles in each population to examine levels of isolation between groups. Diversity measures were calculated using the poppr and adegenet packages in R 3.3.2 (Kamvar et al. 2014; Paquette 2012; R Core Team 2014).

Results

Historical range expansion

Herbaria records suggest that Sahara mustard has undergone an atypical invasion history with no detectable lag phase and a relatively constant expansion pattern following its introduction (Fig. 3.2). Overall, Sahara mustard's range based on sampling sites currently stretches approximately 500,000 km² (Fig. 3.1). This, however, is likely an underestimate of its range edges given the observed expansion dynamics and the fact that sampling was carried out in 2015 for this current analysis. The most dramatic change occurred as Sahara mustard was identified as a management concern especially after 2000. Both latitudinal and longitudinal expansion rates appear to equal each other throughout much of the 20th century and only occasionally differ slightly since then. However, overall expansion patterns have slowed in recent years and might be reaching a stable population phase, are being bounded by environmental constraints, or are another example of collection effort bias (Fig. 3.2; Cousens and Mortimer 1995).

Population structure

In an effort to decipher to population structure, DAPC analyses revealed clear genetic structure across the invaded range of Sahara mustard (Fig. 3.3). This is mostly driven by one site (Nipomo, CA), which was exceptionally divergent relative to other sites. Repeating DAPC while excluding this population revealed additional structure; individuals from Palm Springs, CA, Coachella, CA, and Roosevelt, AZ separated from the remaining sites. Subsequent structure

assignments in TESS revealed that these sites represented three of four sites that had some significant probability of assignment to Cluster 2 (Fig. 3.4a). The fourth site is Parker, AZ which was not delineated in the DAPC but was in TESS analyses. The Nipomo, CA site was the only site assigned to Cluster 3; all others were assigned a 100% probability of belong to Cluster 1 (Fig. 3.4a). Overall, TESS analyses revealed that 3 genetically distinct populations exist in the United States based on the sites we sampled but that some individuals exhibited multiple assignment to Clusters 1 and 2. This was evidenced by replicates of each K value separating Cluster 2 and 3 sites from Cluster 1 across all runs of K . The mean log probability of the data increased with the successive addition of clusters to $K = 3$, after which it plateaued.

Mapping the mean TESS assignment probabilities revealed no clear spatial patterns across the invaded range (Fig. 3.4b). Cluster 1 was dominant throughout the entire invaded range and also occurred within sampling sites primarily assigned to the other Clusters. The Nipomo, CA site was the only with individuals assigned to Cluster 3 but that approximately 3% of individuals also exhibited multiple assignment with Cluster 1 (Fig. 3.4b). This was a similar pattern for the Cluster 2 sampling sites but with varying degrees of assignment probabilities. The Palm Springs, CA site had the highest assignment probability to Cluster 2, followed by Coachella, CA, Parker, AZ, and Roosevelt, AZ exhibiting declines in Cluster 2 assignments as geographic distance increased from Palm Springs.

Genetic diversity

All sites had very low levels of genetic diversity. Further, nearly all sites had slightly lower levels of heterozygosity than expected (Appendix S3: Table S3.1) but this was not true at the

population level (Table 2). Cluster 1 exhibited the highest levels of inbreeding ($F_{is} = 0.8425$) and selfing ($S = 0.9145$), and the lowest levels of outcrossing ($T = 0.0855$). Cluster 2 exhibited lower levels of fixation ($F_{is} = 0.7827$), selfing rates ($S = 0.8778$), and higher outcrossing rates ($T = 0.1222$) than Cluster 1. Cluster 3 exhibited similar levels of fixation ($F_{is} = 0.7865$), selfing rates ($S = 0.8805$), and outcrossing rates ($T = 0.1195$) to Cluster 2; suggesting Clusters 2 and 3 utilizing more of a mixed breeding system of self-fertilization and outcrossing. That being said, all three populations appear to overwhelmingly self-fertilize (Table 3.2). Cluster 3 contained 303 private alleles that were found nowhere else in the invaded range while Cluster 1 had only 40 private alleles and Cluster 2 had 1; suggesting levels of isolation between populations vary and that this is likely due to selfing rates and the geographic range of each population.

Discussion

Our study suggests Sahara mustard exists as three populations in the United States and that its historical range expansion and genetic diversity are likely a product of the species' proclivity to self-fertilize. The unusual spatial structure of Sahara mustard's populations are most likely the result of multiple introductions or evolutionary events, perhaps due to unique selection pressures at sites where population structure varies. Overall, however, low levels of genetic diversity are widespread regardless of location and selfing rates and limited outcrossing appear to be a main driver to Sahara mustard's invasion success. Our study further shows Sahara mustard successfully overcome typical invasion roadblocks (e.g., lag phase and inbreeding depression) by

avoiding them altogether. We argue that self-compatibility can act as a trait that ensures successful colonization and rapid population expansion.

The reproductive strategies invasives employ is a primary driver of invasive success (Baker 1955; Sakai et al. 2001; Richard et al. 2006). Self-fertilization reduces the role of biotic interaction (i.e., do not require pollinators or sexual partners) and can enable establishment and spread (Pannell 2015). Similarly, the type of reproduction can be a make-it-or-break-it strategy for non-native introductions responding to novel environments (Barrett et al. 2008). This is also true for germination traits that can set invaders up with a competitive edge over native species (Wolkovich and Cleland 2011) but that these traits and their relative importance likely vary as a non-native species goes through the phases of introduction, establishment, and spread (Bock et al. 2015; Pannell 2015). Sahara mustard exhibits all of these characteristics and our results further reveal the ability of a self-fertilizing plant to rapidly expand its invaded range within decades.

The first record of Sahara mustard in the United States dated back to 1927 and records were relatively infrequent and concentrated to the deserts of southern California until around the 1970–80s when it began appearing in neighboring states. Although the herbaria records we analyzed are a subset of the actual occurrences of Sahara mustard, they suggest that the species did not undergo a typical lag phase and, instead, was able to expand its range at a somewhat constant rate after its introduction, likely the result of the species breeding system. Taken together, our population structure analyses and estimated expansion patterns successfully revealed that reproductive strategies and putative multiple introductions enabled Sahara mustard to colonize the diverse range of habitats in which it currently occurs despite the common evolutionary roadblocks facing invasive species (Hargreaves and Eckert 2014).

Expansion load, or the accumulation of deleterious mutations during range expansion, can prevent species from colonizes new environments if local adaptation has not occurred (Peischl and Excoffier 2015; Gilbert et al. 2017). However, self-fertilization can overcome these potentially expansion-halting effects depending on the severity of the abiotic filters associated with establishment at a given site and inbreeding effects (Hargreaves and Eckert 2014). Additionally, severity of the abiotic environment can be re-categorized by how much of an environmental gradient an expanding population encounters (Hamilton et al. 2015; Gilbert et al. 2017). Our results reveal that self-fertilization can also overcome severe environmental gradients given the wide-range of the species in the United States. Further, if multiple introductions of Sahara mustard occurred, they are not evidenced in our genetic data, expect perhaps in Nipomo, CA. It is likely that a single introduction explains most of the range expansion and that adaptation is enabling the species to colonize a variety of ecosystems. Nonetheless, our herbaria analyses suggest that Sahara mustard has only recently undergone a rapid population growth and may have not yet reached its stabilizing point when expansion halts and potential barriers to success may be expressed.

Self-fertilization coupled with high propagule pressures can also reduce the potential negative impacts of inbreeding (Levin 2010; Hargreaves and Eckert 2014). A single individual Sahara mustard can produce over 16,000 seeds and disperse relatively large distances via animals, wind, water, and roadways (Trader et al. 2006; Bangle et al. 2008; Sánchez-Flores 2007; Berry et al. 2014). It is likely that even if a small number of Sahara mustard were initially introduced into the US, the species' huge reproductive investments would enable it to maintain adequate population sizes to overcome bottlenecks and establish itself at least locally. Our analyses of herbaria records corroborate this and suggest that a self-compatible species is capable

of avoiding the typical lag phase most invasives experience (van Kleunen et al. 2007). However, the particular adaptations or admixture that enabled the rapid expansion of Sahara mustard in the century since its introduction remains unknown.

Attempts have been made to project Sahara mustard presence and abundance under future climate scenarios (Curtis and Bradley 2015). Conservative models predict a considerable decline in suitable habitat while less conservative models predict continued expansion. However, these models were limited by herbaria records and survey data that are largely biased and, in some cases, not representative of the full extent of Sahara mustard presence and abundance in the United States (pers. obs.). Somewhat similar methods were employed in Li et al. (2015) with results indicating Sahara mustard matched its native range environment in its invaded range. This further suggests Sahara mustard is unlikely to have undergone local adaptation to the invaded environments. However, these conclusions assume the full suite of adapted genotypes from the native range were introduced in the invaded range. This is rarely the case and introduced species are often representative of a small regional population from the native range (Dlugosch and Parker 2008; Lombaert et al. 2010; Barker et al. 2015). Our study revealed that multiple introductions of Sahara mustard occurred in the United States and substantial admixture has yet to occur between all of these populations. This suggests that novel genotypes are capable of arising and would perhaps enable further colonization beyond the species current range if outcrossing were to occur.

We generally detected low levels of genetic diversity across the invaded range of Sahara mustard. Samples from the site at Nipomo, CA, however, show considerable divergence from the rest of the range. This was likely caused by a second introduction of Sahara mustard sometime after the initial Coachella Valley introduction. If this is the case, the population in Nipomo, CA

should receive high priority for eradication as it is in initial invasion stages pre-expansion. Further, the population has potential to hybridize with the other populations that, from experience with other invaders (e.g., Barker et al. 2016), would enable further range expansion. As is, the population appears to be relatively isolated but should still be treated with concern given that humans are likely facilitating the species dispersal (Trader et al. 2006; Sánchez-Flores 2007; Berry et al. 2014).

Sahara mustard has a similar invasion history in Australia where it was introduced in the early 1900s and is presumed to have dispersed via the transcontinental railroad (Kloot 1987). Since humans are likely facilitating the spread of Sahara mustard in the United States, it is unsurprising that the diversity is able to remain low across such a large range and that there are no clear geographic separations between populations. This is likely the result of a combination of self-fertilization coupled with human-mediated dispersal. Our results further reveal the ability of self-fertilization to maintain similar levels of genetic diversity across a range, regardless of expansion limitations and environmental gradients. Range edges are thought to have lower diversity during expansion but this is not always the case and often depends on geneflow and dispersal (Leys et al. 2014). Connectivity usually limits geneflow, but human-mediated dispersal can occur over hundreds of kilometers (Mona et al. 2014). Although we predicted populations would separate spatially, we also anticipated possible alternative spatial structuring given that Sahara mustard is presumed to disperse via human traffic—an artificial dispersal mechanism that might enable long-distance dispersal of unconnected haplotypes. Our results corroborate this and further reveal that low levels of genetic diversity can be maintained with little consequences in reproductive success if mating systems are combined with the forces of highway traffic.

In areas where Sahara mustard invasion rates are high, biodiversity is declining, native species are becoming displaced, and natural resources are being altered (Barrows et al. 2009, Schneider and Allen 2012; VanTassel et al. 2014). Additionally, conservation efforts are stalling and stakeholders are discouraged by the limited options for response. Other invasive plants in North America, such as *Bromus spp.*, have had widespread negative effects over the last 150 years (Bradley et al. 2009) and we can anticipate Sahara mustard to spread with similar impact. If *Bromus spp.* had been controlled early after it invaded in the 1800's, western North America landscapes would be profoundly different. Sahara mustard's rapid, annual growth form, ability to self-fertilize, and its dispersal modality via human traffic along roadways likely explain the species proliferation and continued spread into habitats ranging from California eastward to Texas, and northward to Nevada and Utah. An imperative follow-up to our study is the identification of source populations in Sahara mustard's native range. It is only then that a clearer invasion history can be understood. Additionally, identifying source populations as well as the abiotic and biotic mechanisms controlling native populations are the first step in designing effective control programs in a species invaded range (Müller-Schärer et al. 2004; Estoup and Guillemaud 2010).

Understanding the population structure of invasives is not only useful in solving evolutionary problems but is also a valuable tool for land managers in designing invasive control programs that effectively reduce the spread and future introduction of invasives (Mack et al. 2000). The effectiveness of controlling invasive populations already present in a given management area is often dependent on factors like genetic diversity and propagule pressure (Sakai et al. 2001, Roderick and Navajas 2003). Additionally, the ability of land managers to effectively control invasives often depends on what stage of invasion the species is in (Suarez

and Tsutsui 2008; Pannell 2015). Unfortunately, many invasives remain largely unnoticed until the species is undergoing rapid population growth and range expansion at which point management becomes more difficult and alternate strategies are required (Lodge et al. 2006). Population genetics combined with historical distribution records may shed light on the state of an invasive (Dlugosch et al. 2015), management potential (Lodge et al. 2006), and identify invasion hotspots (O'Donnell et al. 2012).

In summary, our study is the first to document the genetic patterns of Sahara mustard invasion in the United States and successfully reveal that the species exists as three genetically distinct populations with low levels of diversity likely the result of self-fertilization. Further, our results reveal that a lack of spatial structure is likely a combination of the species' mating systems and human-mediated dispersal modalities. However, the origins of these populations remains unclear, but we hypothesize at least two introductions. Future research is needed that will apply similar population genetic methods in the species' native range to identify sources and reconstruct the species' invasion history in the United States. Successful management efforts will likely be achieved if human-mediated spread is curtailed along roadways first, with special focus on newly introduced populations like that at Nipomo, CA, which have not yet expanded. Future introductions like Nipomo, CA should be expected, necessitating genetic testing as new localities are discovered. Additionally, future research focusing on phenotypic plasticity is needed to reveal the strategies that are enabling Sahara mustard to be successful in multiple environments in spite of its low levels of genetic diversity.

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Table 3.1. Sampling location and site characteristics.

Site	location	<i>n</i>	latitude	longitude	Elevation (m)	ecoregion
Irvine	South CA Coast	20	33.63791	-117.969	-13.33	California South Coast
Anza1	Anza Borrego	11	33.21406	-116.466	1026.09	California South Coast
Anza2	Anza Borrego	12	33.30025	-116.387	223.15	Sonoran Desert
Salton City	Imperial Valley	12	33.2687	-115.957	-27.75	Sonoran Desert
El Centro	Imperial Valley	17	32.82803	-115.5	-11.65	Sonoran Desert
Ocotillo	Imperial Valley	19	32.64709	-116.106	905.68	California South Coast
San Diego	South CA Coast	9	32.72335	-117.138	41.22	California South Coast
Coachella	Coachella Valley	12	33.77248	-116.304	24.64	Sonoran Desert
Glamis	West AZ	20	32.97718	-115.171	82.8	Sonoran Desert
Blythe	West AZ	20	33.56059	-114.657	70.54	Sonoran Desert
Parker	West AZ	19	33.99411	-114.216	195.51	Sonoran Desert
JOTR1	Joshua Tree	19	34.0488	-115.217	450.5	Sonoran Desert
JOTR2	Joshua Tree	19	34.13515	-116.137	771.34	Mojave Desert
MOJA1	Mojave	18	34.82923	-116.694	590.13	Mojave Desert
Ibis	West AZ	13	35.07073	-114.828	611.76	Mojave Desert
Leeds	Northern	7	37.22532	-113.406	985.23	Mojave Desert
MormonPk	Northern	19	36.83129	-114.373	850.17	Mojave Desert

LAKE1	Northern	20	36.01262	-114.731	466.12	Mojave Desert
Amargosa	Northern	18	36.56371	-116.123	871.79	Mojave Desert
Beatty	Northern	13	36.85232	-116.756	961.68	Mojave Desert
Las Vegas	Northern	11	36.0898	-115.233	726.88	Mojave Desert
MOJA2	Mojave	9	35.13412	-116.207	457.71	Mojave Desert
MOJA3	Mojave	10	35.26009	-116.068	290.92	Mojave Desert
MOJA4	Mojave	14	34.80259	-115.612	1193.11	Mojave Desert
Aguila	West AZ	7	33.94289	-113.161	667.28	Sonoran Desert
Phoenix1	Phoenix	18	33.80308	-112.24	501.69	Sonoran Desert
Phoenix2	Phoenix	10	33.39348	-111.662	429.83	Sonoran Desert
Tortilla Flat	Apache Highlands	9	33.52706	-111.388	549.03	Sonoran Desert
Roosevelt	Apache Highlands	19	33.64701	-111.112	824.45	Sonoran Desert
Fort Apache	Apache Highlands	19	33.79879	-110.507	1053.48	Apache Highlands
Las Cruces	New Mexico	12	32.18376	-106.678	1195.52	Chihuahuan Desert
Fort Hancock	Texas	11	31.29882	-105.832	1114.53	Chihuahuan Desert
El Paso	Texas	15	31.75388	-106.348	1182.78	Chihuahuan Desert
Dragoon	East AZ	16	32.08114	-110.051	1502.66	Apache Highlands
SAGU1	Tucson	11	32.17711	-110.739	962.64	Sonoran Desert
SAGU2	Tucson	18	32.32543	-111.122	717.74	Sonoran Desert

Malibu	Central Coast	12	34.0221	-118.809	50.84	California South Coast
Nipomo	Central Coast	12	35.04823	-120.512	128.22	California Central Coast
Chaney Ranch	Central Valley	14	36.6231	-120.609	188.78	Great Central Valley
Murray	Central Valley	11	36.07389	-120.103	189.74	California Central Coast
Bakersfield	Central Valley	14	35.29217	-118.752	272.18	Great Central Valley
Victorville	Mojave	16	34.50634	-117.597	1035.46	Mojave Desert
JOTR3	Joshua Tree	13	33.91534	-115.829	561.53	Mojave Desert
Palm Springs	Coachella	18	33.92079	-116.71	437.52	Sonoran Desert
Anza3	Anza Borrego	13	33.13015	-116.312	363.5	California South Coast
DEVA	Northern	14	35.90607	-116.641	-116.6	Mojave Desert
Dateland	South AZ	13	32.80141	-113.541	130.86	Sonoran Desert
Gila Bend	South AZ	19	32.93566	-112.68	244.3	Sonoran Desert
Rocky Pt	South AZ	15	32.60002	-112.871	346.92	Sonoran Desert
ORPI1	South AZ	17	32.02349	-112.802	522.6	Sonoran Desert
ORPI2	South AZ	9	32.00967	-112.709	697.56	Sonoran Desert
ORPI3	South AZ	12	32.13016	-112.768	556.48	Sonoran Desert

Table 3.2. Summary statistics for each population as defined by TESS results. n = number of individuals analysed, H_o (\pm SE) = the observed heterozygosity for SNPs, H_{exp} (\pm SE) = the expected heterozygosity for SNPs, F_{is} = index of fixation, S = selfing rate, T = outcrossing rate, and private = the number of private alleles. Standard errors are reported parenthetically.

population	n	H_o (\pm SE)	H_{exp} (\pm SE)	F_{is}	S	T	private
Cluster 1	668	0.0577 (\pm 0.0065)	0.0697 (\pm 0.0041)	0.8425	0.9145	0.0855	40
Cluster 2	68	0.0565 (\pm 0.0066)	0.0562 (\pm 0.0039)	0.7827	0.8778	0.1222	1
Cluster 3	12	0.0551 (\pm 0.0066)	0.0356 (\pm 0.0038)	0.7865	0.8805	0.1195	303

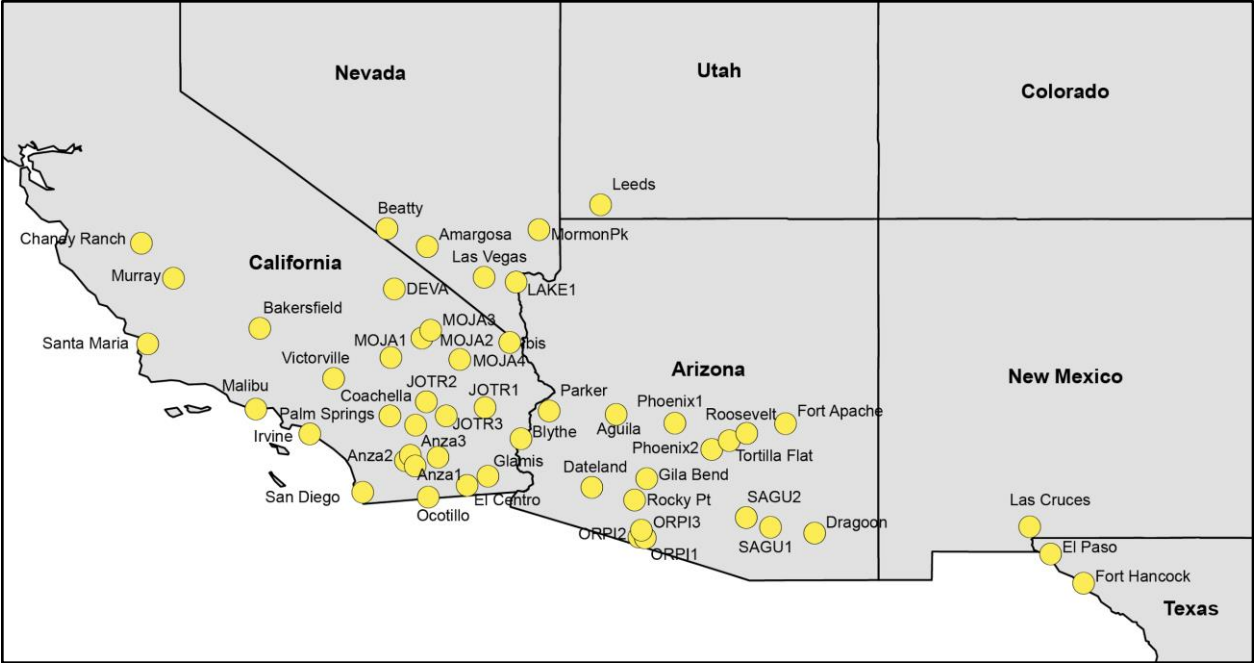


Fig. 3.1. Map of sampling sites in California, Arizona, New Mexico, Texas, Nevada, and Utah. Site codes correspond to those in Table 1.

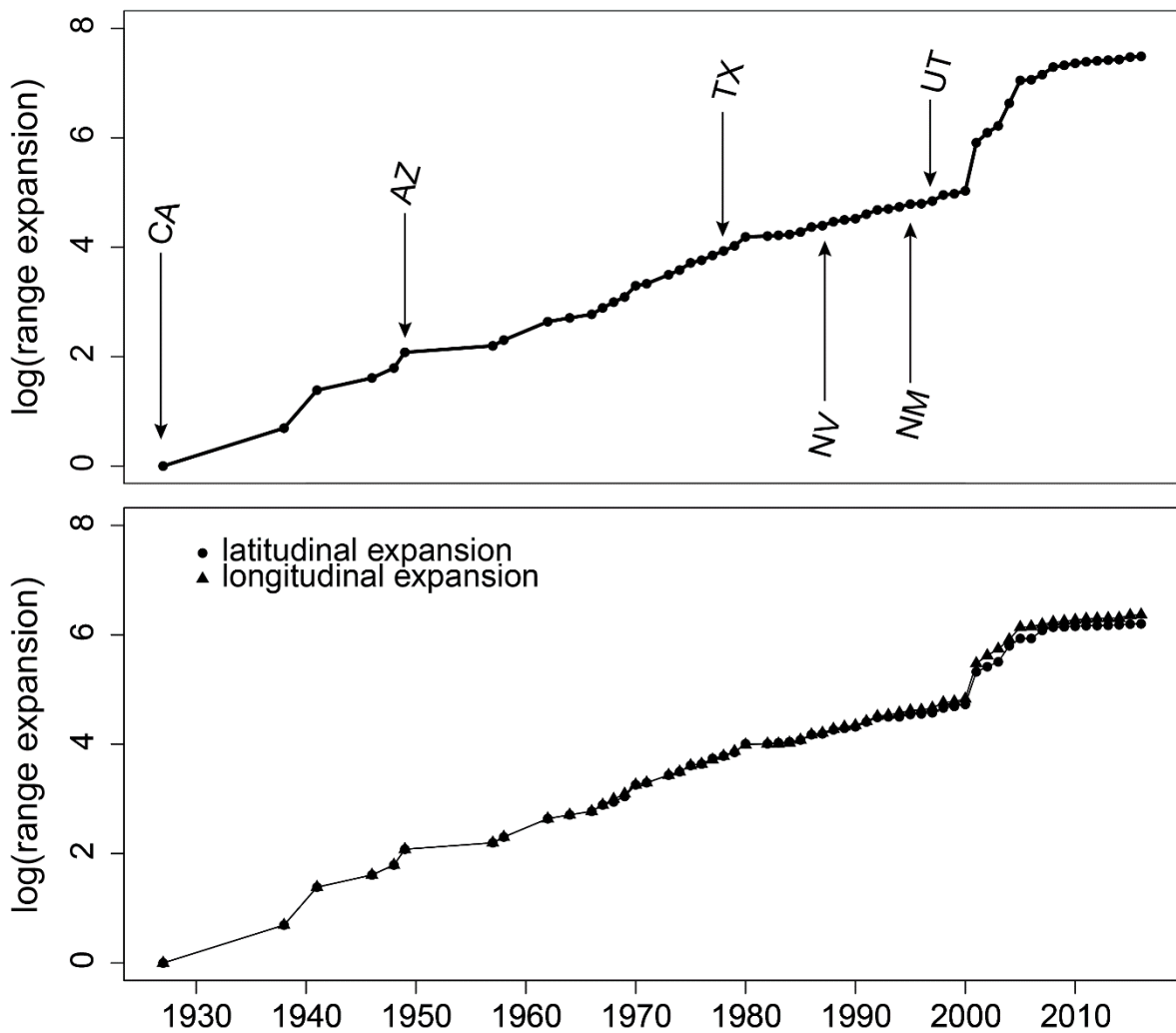


Fig. 3.2. Estimated range expansion using herbaria records through time (1927–2016). Top panel illustrates the accumulated unique ca. 1 km² observations across both latitudes and longitudes while the bottom panel illustrates latitudinal (circles) and longitudinal (triangles) expansion separately. Arrows indicate when the first occurrence of Sahara mustard (*Brassica tournefortii*) was reported in each state.

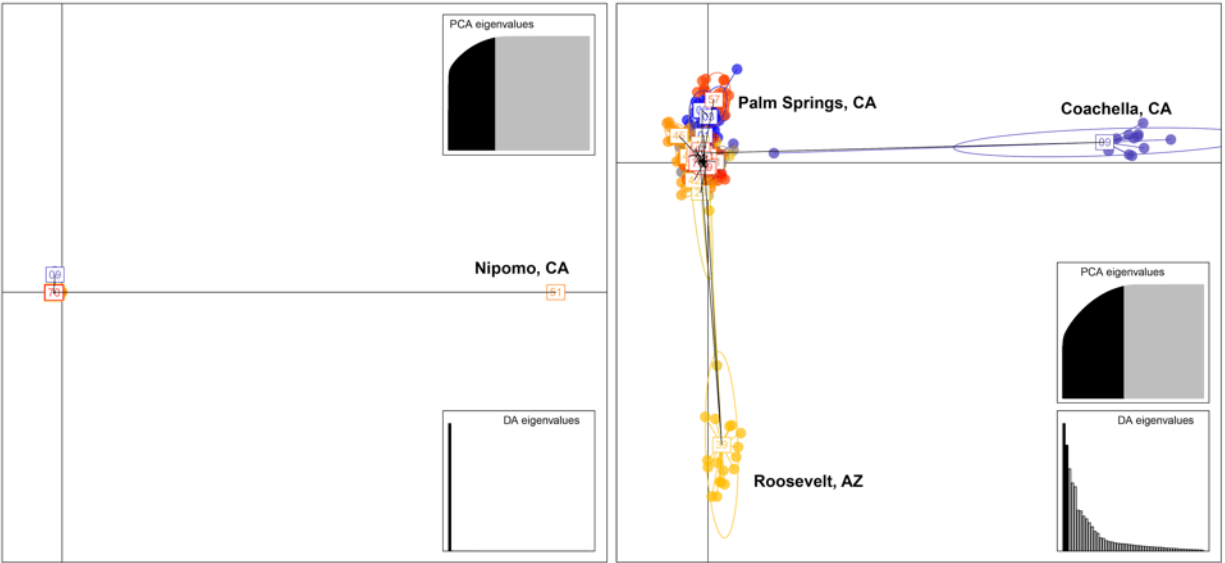


Fig. 3.3. Discriminant analysis of principal components (DAPC) based on 899 SNP loci for all sampling sites (left panel) and sampling sites without the Nipomo, CA site (right panel). Ovals in the right panel are 95% inertia ellipses. Individual genotypes are depicted with circles and are color-coded by sampling site.

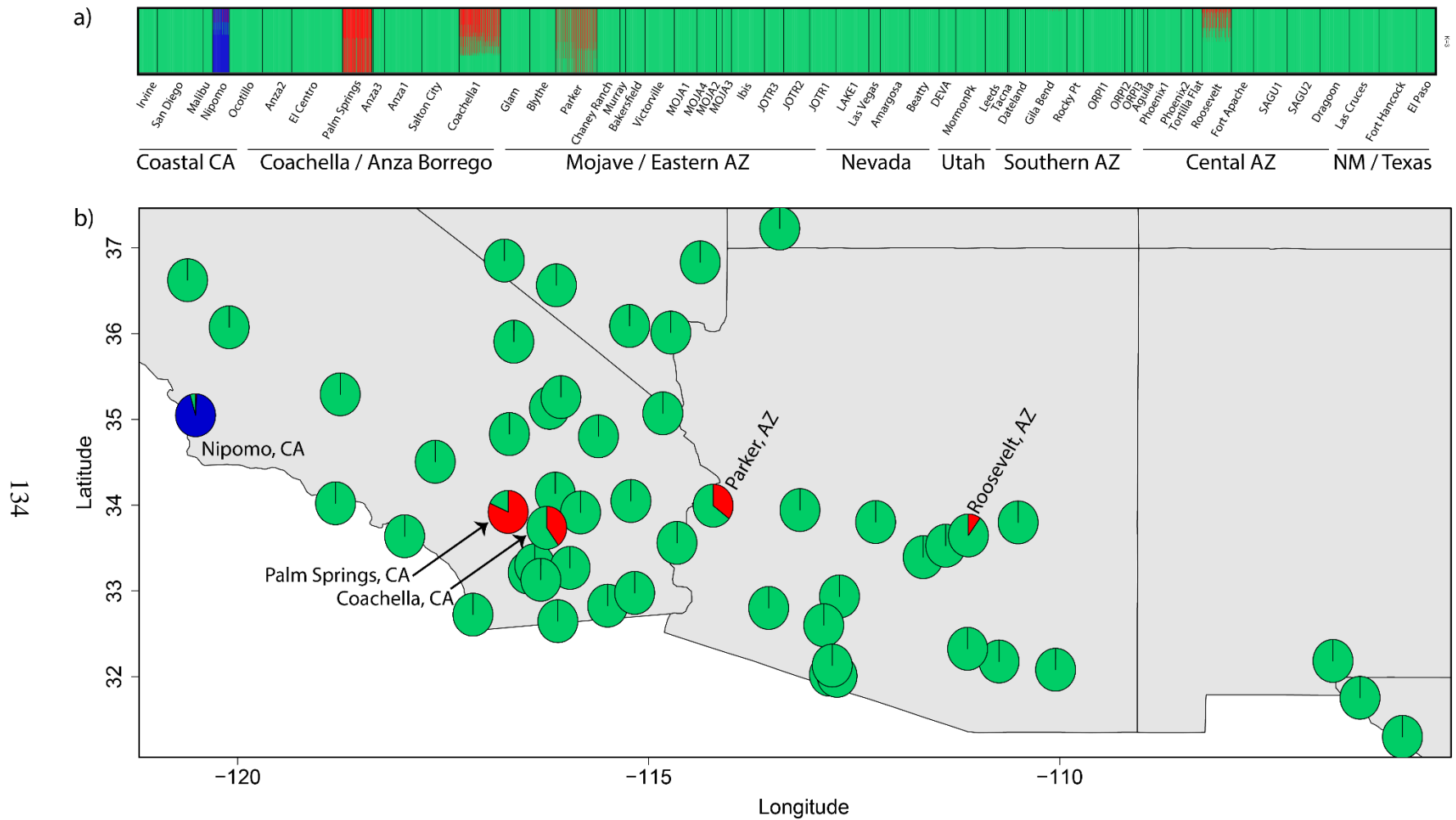


Fig. 3.4. (a) Individual assignments from TESS analyses based on 899 SNP loci of 745 individuals from across the invaded range of Sahara mustard (*Brassica tournefortii*). Barplots are averaged across ten runs of the highest likely number of clustered predicted to be $K = 3$. (b) Map illustrating average assignment probabilities to each cluster from TESS analyses (pie chart colors: cluster 1 = green, cluster 2 = red, cluster 3 = blue). Clusters 2 and 3 are named by sampling site.

Appendix S3

Table S3.1. Summary statistics for each sampling site. H_o (\pm SE) = the observed heterozygosity for SNPs, H_{exp} (\pm SE) = the expected heterozygosity for SNPs, F_{is} = index of fixation, S = selfing rate, T = outcrossing rate, and private = the number of private alleles. Standard errors are reported parenthetically.

location	H_o (\pm SE)	H_{exp} (\pm SE)	F_{is}	S	T	private
Irvine	0.055 (\pm 0.0063)	0.1301 (\pm 0.0053)	0.8363	0.9109	0.0891	1
Anza1	0.058 (\pm 0.0067)	0.0997 (\pm 0.0046)	0.8535	0.9210	0.0790	0
Anza2	0.0587 (\pm 0.0066)	0.1091 (\pm 0.0047)	0.8394	0.9127	0.0873	0
Salton City	0.0576 (\pm 0.0062)	0.1234 (\pm 0.0053)	0.8433	0.9150	0.0850	0
El Centro	0.0613 (\pm 0.0065)	0.1071 (\pm 0.0047)	0.8429	0.9148	0.0852	0
Ocotillo	0.0556 (\pm 0.0065)	0.0504 (\pm 0.0038)	0.8469	0.9171	0.0829	0
San Diego	0.0588 (\pm 0.0064)	0.1171 (\pm 0.0051)	0.8539	0.9212	0.0788	0
Coachella	0.0553 (\pm 0.0064)	0.0754 (\pm 0.0039)	0.7332	0.8461	0.1539	0
Glamis	0.0557 (\pm 0.0066)	0.0767 (\pm 0.0039)	0.8301	0.9072	0.0928	0
Blythe	0.0556 (\pm 0.0066)	0.0359 (\pm 0.0038)	0.8305	0.9074	0.0926	1
Parker	0.0547 (\pm 0.0065)	0.0356 (\pm 0.0038)	0.7854	0.8798	0.1202	0
JOTR1	0.0576 (\pm 0.0066)	0.0765 (\pm 0.004)	0.8342	0.9096	0.0904	0
JOTR2	0.0564 (\pm 0.0066)	0.0369 (\pm 0.0039)	0.8222	0.9024	0.0976	0
MOJA1	0.0584 (\pm 0.0068)	0.0373 (\pm 0.0038)	0.8434	0.9150	0.0850	1
Ibis	0.058 (\pm 0.0068)	0.0374 (\pm 0.0039)	0.8397	0.9129	0.0871	1

Leeds	0.0577 (± 0.0067)	0.0864 (± 0.0039)	0.8620	0.9259	0.0741	1
MormonPk	0.0588 (± 0.0067)	0.0775 (± 0.004)	0.8343	0.9096	0.0904	0
LAKE1	0.0547 (± 0.0064)	0.0891 (± 0.0039)	0.8354	0.9103	0.0897	0
Amargosa	0.0561 (± 0.0065)	0.0778 (± 0.004)	0.8419	0.9142	0.0858	1
Beatty	0.0586 (± 0.0066)	0.0926 (± 0.004)	0.8400	0.9130	0.0870	0
Las Vegas	0.0586 (± 0.0066)	0.0716 (± 0.004)	0.8544	0.9215	0.0785	2
MOJA2	0.0589 (± 0.0066)	0.0925 (± 0.0039)	0.8492	0.9185	0.0815	2
MOJA3	0.0566 (± 0.0064)	0.0833 (± 0.0039)	0.8443	0.9156	0.0844	1
MOJA4	0.0599 (± 0.0065)	0.0894 (± 0.004)	0.8345	0.9098	0.0902	0
Aguila	0.0543 (± 0.0063)	0.0884 (± 0.0038)	0.8609	0.9252	0.0748	0
Phoenix1	0.0582 (± 0.0066)	0.0902 (± 0.004)	0.8327	0.9087	0.0913	2
Phoenix2	0.0541 (± 0.0063)	0.0892 (± 0.0038)	0.8585	0.9239	0.0761	2
Tortilla Flat	0.0566 (± 0.0067)	0.0365 (± 0.0039)	0.8558	0.9223	0.0777	3
Roosevelt	0.057 (± 0.0067)	0.0369 (± 0.0038)	0.7933	0.8847	0.1153	0
Fort Apache	0.0546 (± 0.0065)	0.036 (± 0.0038)	0.8416	0.9140	0.0860	1
Las Cruces	0.0556 (± 0.0066)	0.0358 (± 0.0038)	0.8453	0.9161	0.0839	2
Fort Hancock	0.0557 (± 0.0066)	0.0363 (± 0.0038)	0.8414	0.9139	0.0861	2
El Paso	0.0555 (± 0.0066)	0.0355 (± 0.0038)	0.8405	0.9134	0.0866	2
Dragoon	0.0576 (± 0.0067)	0.0368 (± 0.0038)	0.8399	0.9130	0.0870	1

SAGU1	0.0548 (± 0.0066)	0.0356 (± 0.0038)	0.8493	0.9185	0.0815	2
SAGU2	0.0541 (± 0.0065)	0.035 (± 0.0038)	0.8383	0.9120	0.0880	0
Malibu	0.055 (± 0.0064)	0.0361 (± 0.0037)	0.8521	0.9201	0.0799	1
Nipomo	0.0551 (± 0.0066)	0.0356 (± 0.0038)	0.7865	0.8805	0.1195	303
Chaney Ranch	0.0553 (± 0.0065)	0.0362 (± 0.0038)	0.8462	0.9167	0.0833	0
Murray	0.0554 (± 0.0065)	0.0367 (± 0.0038)	0.8508	0.9194	0.0806	0
Bakersfield	0.0531 (± 0.0065)	0.0341 (± 0.0038)	0.8358	0.9106	0.0894	1
Victorville	0.0581 (± 0.0068)	0.0368 (± 0.0039)	0.8397	0.9129	0.0871	1
JOTR3	0.058 (± 0.0066)	0.0375 (± 0.0038)	0.8456	0.9164	0.0836	1
Palm Springs	0.0589 (± 0.0067)	0.0767 (± 0.004)	0.8189	0.9004	0.0996	1
Anza3	0.059 (± 0.0066)	0.0795 (± 0.0041)	0.8435	0.9151	0.0849	0
DEVA	0.0626 (± 0.0067)	0.0895 (± 0.0043)	0.8385	0.9122	0.0878	1
Dateland	0.059 (± 0.0066)	0.0761 (± 0.004)	0.8454	0.9162	0.0838	0
Gila Bend	0.0572 (± 0.0062)	0.1434 (± 0.0058)	0.8056	0.8923	0.1077	3
Rocky Pt	0.0608 (± 0.0063)	0.1117 (± 0.0048)	0.8379	0.9118	0.0882	3
ORPI1	0.0792 (± 0.0063)	0.1154 (± 0.005)	0.8451	0.9161	0.0839	0
ORPI2	0.06 (± 0.0066)	0.0474 (± 0.0038)	0.8499	0.9189	0.0811	1
ORPI3	0.0637 (± 0.0065)	0.0773 (± 0.0042)	0.8459	0.9165	0.0835	0

CHAPTER 4

Rapid alignment of functional trait variation with local conditions across the invaded range of Sahara mustard (*Brassica tournefortii*)

Abstract

The mechanisms by which invasive species can successfully perform in multiple contexts across broad environmental gradients are poorly understood. Local adaptation is often invoked to explain performance across extreme distances for widespread invasive species. However, the role of genetically controlled trait variation of growth strategies is rarely evaluated in the context of environmental variability. Here we evaluate the widespread, relatively new invasion of Sahara mustard (*Brassica tournefortii*) in the southwest US to understand the extent to which the species shows significant differentiation in functional traits across the invaded range, and whether that variation related to spatial and climatic variables associated with population locations. We used a common garden approach, growing two generations of plants in environmentally controlled conditions sourced from 10 distinct locations across the species' range. We measured traits underlying the functional variation in phenological, morphological, and physiological performance and tested for significant variation within and among populations. Using hierarchical partitioning analyses, we then evaluated the relative importance of spatial and climatic variables in explaining trait variation across populations. Our analyses revealed that nine key traits varied significantly among populations, specifically those related to phenology and early growth strategies, such as the timing of germination and flowering, as well as relative allocation of biomass to reproduction and seed mass. Variation in these traits observed in the

common garden was related most strongly to variation in winter precipitation patterns at the source populations, though variation in temperature and latitude also showed significant contributions. Our results are consistent with local adaptation, and identifies the importance in key functional traits relating to timing of growth and reproduction that may explain the successful colonization of an invasive across a broad geographic and climatic range.

Introduction

It is difficult to predict invasive species spread and to identify when and where introduced species will be successful (Kolar and Lodge 2001). Furthermore, the adaptive traits that characterize a successful invader do not hold for all species (Sakai et al. 2001). Invasive success and geographic spread have been explained by local adaptation for some successful invaders (Colautti and Barrett 2013), while phenotypic plasticity explains others (Sexton et al. 2002, Richards et al. 2006, Davidson et al. 2011, Castillo et al. 2014). That said, many iconic invasives occupy a wide breadth of environments (Colautti et al. 2009), suggesting that selection and subsequent adaptation likely occurs during the invasion process (Prentis et al. 2008). Which adaptive traits enable invasives to successfully spread and establish depends on the size of the area in question (Colautti et al. 2009; Erskine-Ogden et al. 2016) and likely represents a suite of traits that follow selection pressures across the species' range (Simberloff et al. 2013).

A common approach to predicting invasions relies on bioclimatic envelope models, which use environmental characteristics of a species' native range to project success in new areas, or a limited region of the invaded range to evaluate risk of spread in a larger area.

However, many successful invasive species occupy a range of environmental conditions in their native ranges, which may reflect several distinct populations (Lawson Handley et al. 2011; Dlugosch et al. 2015), each with its own selection pressures (Prentis et al. 2008). However, introductions into new ranges are often from only a small regional source population from the native range (Lombaert et al. 2010, Landley Dawson et al. 2011). Thus, the conditions across the entire native range may not represent those to which the invaded population is adapted. Further, one or a few introductions of a nonnative species is likely a subset of the genetic diversity of the native populations (Tsutsui et al. 2000). In this sense, successful invasions may only be possible when the invaded environment matches the conditions from the source population (Dietz and Edwards 2006) or subsequent adaptation occurs. Additionally, climate matching rarely predicts the full invasion range—adaptation to local environments within the invaded range often occurs after introduction (Peterson 2003). Thus, invasive species can expand their invaded range beyond their introduction point by three mechanisms: adaptive evolution, phenotypic plasticity, and multiple introductions from a diversity of native populations (Sakai et al. 2001, Blair and Wolfe 2004, Palacio-López and Gianoli 2011).

Trait-based ecology shows promise for identifying mechanisms driving invasive success and predicting future patterns (Leishman et al. 2007, Tecco et al. 2010, van Kleunen et al. 2010, Murphy et al. 2016) because functional trait variation represents the different phenological, morphological, and physiological characteristics influencing species responses to the environment. Species introduced to novel environments have been shown to rapidly evolve adaptive phenotypes, thereby allowing persistence and further spread (Weber and Schmid 1998, Reznick and Ghalambor 2001, Moran et al. 2004, Barrett et al. 2008, Dlugosch and Parker 2008, Prentis et al. 2008). Although phenotypic plasticity is still considered a key process in driving the

dynamics of invasions, rapid evolution has now been reviewed extensively as the evidence of its commonality builds (Mack et al. 2000, Mooney and Cleland 2001, Reznick et al. 2001, Sakai et al. 2001, Lee 2002, Roderick and Navajas 2003, Dlugosch and Parker 2008, Suarez and Tsutsui 2008, Colautti and Barrett 2013, among others). A number of functional traits have been related to invasive performance and colonization success, including rapid germination and flowering phenologies (Kimball et al. 2011, Colautti and Barrett 2013), increased allocation to photosynthetic and reproductive structures (Leishman et al. 2007, Moroney et al. 2013, Erskine-Ogden et al. 2016), and water-use efficiency matched to site-specific environments (Tecco et al. 2010, van Kleunen et al. 2010). Plasticity and rapid evolution of these critical traits in novel environments range is likely to facilitate expansion in invaded ranges.

Sahara mustard (*Brassica tournefortii*, family: Brassicaceae) is rapidly expanding in the southwestern U.S., and negatively impacting natural ecosystems across the region (Barrows et al. 2009; VanTassel et al. 2014). Since its presumed introduction in the 1920s, this invader has become increasingly common in arid and semi-arid regions throughout the Southwest US (Sanders and Minnich 2000), ranging from coastal mediterranean conditions in California to hot desert localities from Arizona to Texas. Further, Sahara mustard exhibits several biological features that likely hint at the capacity to rapidly evolve. It germinates under a wide range of temperatures, light, and soil conditions and depths (Thanos et al. 1991; Jurado and Westoby 1992; Chahuan et al. 2006; Bangle et al. 2008). It is capable of self-fertilization (i.e., facultatively autogamous) and produces seeds rapidly (ca. 50 days from germination; Marushia et al. 2012) and in high quantities (Trader et al. 2006) that can remain viable at least 1 year after production (Chahuan et al. 2006). It is also likely that its seeds undergo some level of dormancy similar to desert annuals with which it co-occurs (i.e., Adondakis and Venable 2004). Its ability

to self-fertilize and its large distribution increase its chances for local adaptation relative to other breeding systems (i.e., Sakai et al. 2001). This is partly because self-fertilization and inbreeding promotes genetic subdivision as populations experience isolation in multiple environments (Schmeske 1984). Further, individual plants can produce over 16,000 seeds annually that disperse via small mammals, flowing water, wind, and human transport (Trader et al. 2006; Bangle et al. 2008; Sánchez-Flores 2007; Berry et al. 2014).

Previous modelling work suggests that Sahara mustard might exist as a plastic, general purpose genotype with an invaded range that matches its native range in terms of environmental variability (Li et al. 2016), suggesting that its invasion in North America might reflect niche conservatism or the retention of ancestral traits and environmental distributions. However, Sahara mustard occupies a large native range (Marushia et al. 2012, Li et al. 2016) and likely exists as several genetically distinct populations that have been separated by thousands of kilometers for many generations (Parker et al. 2003, Lawson Handley et al. 2011). Further, it is most probable that Sahara mustard in the US is representative of only a small regional population from its native range (Lombaert et al. 2010, Arnesen et al. 2017). Although a few ecological studies have examined the species performance and impacts in a few invaded areas (Barrows et al. 2009; Marushia et al. 2010, 2012; VanTassel et al. 2014; Li et al. 2015), no research has been conducted to determine which adaptive traits may explain the success of this rapidly-spreading invasive. Similarly, it remains unknown whether the species exists as genetically-distinct populations across its range. Thus, Sahara mustard is an ideal system to explore some of the evolutionary questions related to invasions.

Our study aimed to identify key functional traits that may explain the success of this invasive species across the broad range of environments of its invaded range in the Southwest

US. We tested for population divergence in functional traits using multigenerational common garden experiments in a controlled environment setting, matching traits to local environment features associated with maternal lineage origination. In doing so, we distinguished between local environmental, maternal, and plant-level variation effects on observed phenotypes. Based on the growing mass of literature, we predicted that phenological traits would most closely match site-specific environmental cues and that precipitation would best predict trait variation in water-limited sites. We also expected genetically-determined investment in biomass to leaves would track variability across the species invaded range, suggesting increased competition for resources with native species. Last, we predicted water-use efficiency and leaf nitrogen investment would significantly differ, following aridity gradients across the range. Our presumption is that functional traits would explain the phenotypes of Sahara mustard across its range. Further, we also expected this rapidly growing annual to synchronize its reproductive efforts to precipitation variability during the winter growing season to ensure high fitness in subsequent generations.

Methods

Species natural history

Sahara mustard (*Brassica tournefortii*, family: Brassicaceae) is an annual native to the Mediterranean basin and much of the Middle East through to western India (Prain 1898; Thanos

et al. 1991; Aldhebiani and Howladar 2013). It is considered a weedy species in agriculture fields in parts of its native range (Ahmed et al. 2015; El-Saied et al. 2015) but also has traditional dietary uses and economic value in regions where it is cultivated (Guarrera and Savo 2016; Singh et al. 2015). Sahara mustard is an invasive throughout much of Australia (Chahuan et al. 2006), South Africa (McGeoch et al. 2009), Chile (Teillier et al. 2014), and western North America (Li et al. 2015). In North America, the first documented occurrence of Sahara mustard comes from an herbarium sample collected in the Coachella Valley, California in 1927. It is thought to have been introduced as a contaminant of date palm translocation into the hemisphere (Sanders and Minnich 2000) and remained relatively unnoticed except in the Coachella and Imperial Valleys of California deserts where it was observed to be locally established (Musil 1948, 1950; Robbins et al. 1951). Herbaria records track its spread to coastal California in the late 1950s and to Tucson, Arizona and Sonora, Mexico in the early 1970s. The species apparently saw a population boom beginning in the 1980s when it spread rapidly throughout the southwest (Sanders and Minnich 2000).

Field sampling & common garden design

We collected seeds from 20 individual maternal plants from ten locations spanning a $\sim 10^\circ$ latitudinal and $\sim 15^\circ$ longitudinal gradient across the Sahara mustard's invaded US range (Fig. 4.1, Table 4.1). Sites ranged from coastal Mediterranean to hot desert ecosystems. Desert ephemerals, like Sahara mustard, have been shown to require exposure to summer temperatures to break dormancy and queue seeds for germination (Clauss and Venable 2000; Huang et al. 2016). Thus, we over-summered field seeds in coin envelopes in the UCI greenhouses (daily

mean temperatures ranged from 26–32°C) and then stored them in our laboratory before growth experiments began. Sahara mustard seeds can remain viable 4–5 years after collection when stored in a dark drawer as we have done (Chauhan et al. 2006).

We grew field-collected seeds for two generations to account for the influence of maternal effects. From each maternal line, we randomly selected three seeds and sowed them into the top 1 cm of soil in 3-gallon containers following results from Chauhan et al. (2006). We used a custom desert soil mix of 85% unwashed sand, 10% perlite, and 5% cactus mix (Scotts Miracle-Gro Inc., Marysville, OH) and placed containers randomly on greenhouse benches but grouped by sampling site to prevent fertilization across sites. Sampling sites were randomly rotated weekly to control for potential location effects in the greenhouse. Greenhouse temperatures were automated and kept above freezing and below 24 °C, averaging 15–20 °C for the duration of the experiment (GEM Link, QCOM, Irvine, CA). Soils were watered regularly to keep seeds moist and encourage germination. Pots were surveyed daily for germination and any additional germinants beyond the first were removed. It is possible this introduces a bias towards earlier germinating plants in our study but we tested for variation in the number of germinants beyond the first and found no difference across sites (Fig. S4.1). Plants were given liquid fertilizer (Peter's 20-20-20 solution, Scotts Miracle-Gro Inc., Marysville, OH) weekly once individuals produced two true leaves. Populations were grouped on the same benches and rotated weekly to control for site effects within the greenhouse. Outcrossing rates are incredibly low (< 10%; Winkler 2017) and plants were allowed to self-pollinate or cross-pollinate within sites. Seeds from this first common garden generation (F2 seeds) were harvested once seedpods had visibly ripened but before pods burst. F2 seeds were then weighed and this second-generation seed was sown as above. In total, we sowed 2,000 F1 field-collected seeds from 115 maternal

lines from our ten sampling sites. Not all seeds were viable and this resulted in 1,600 F2 seeds from 87 maternal lines from across our ten sites.

Measurements

To assess whether Sahara mustard populations vary in functional traits in our common garden experiment, we measured phenotypes of F2 individuals. Functional trait measurements included phenological, morphological, and physiological traits. We chose traits that have been shown to be important for invasives or shown to respond strongly to environmental conditions typical of the biomes invaded in North America (Leishman et al. 2007, Tecco et al. 2010, Huxman et al. 2013, Kimball et al. 2014, Murphy et al. 2016). Phenological traits included time to germination, leaf expansion, and flowering. We recorded germination day for each individual. Leaf phenology was tracked daily during the first month and switched to observations alternating every other day thereafter. We recorded the date of emergence for the first five leaves each individual produced. Time to flowering was recorded as the number of days from germination until anthesis.

Morphological traits included relative growth rate, aboveground biomass including stem, leaf, and reproductive structure dry weights, and leaf area. Three individual plants were randomly selected from each location and plants were harvested biweekly beginning one month after initial germination and until seeds ripened approximately three months later ($n = 6$ harvests total). Aboveground parts were cut at the soil surface and sorted into leaves, stems, and reproductive structures. Leaves were counted, weighed, and scanned using a Canon MF8200C printer (Canon, Tokyo, Japan). Leaf area was calculated for up to 25 of the largest individual leaves using ImageJ (Schneider et al. 2012). All remaining leaves were grouped for area

measurements. Samples were then dried for 48 hours at 60 °C and dry weights were subsequently obtained. Relative growth rates were estimated for each population as the slope of separate linear regressions between log-transformed aboveground biomass and time (c.f., Angert et al. 2007). We also calculated mean leaf area as the sum of the area of all leaves divided by the total number of leaves. Last, we calculated the percentage of biomass allocated to reproductive structures (% repro) as the dry weight of reproductive structures divided by the total aboveground biomass of each plant.

Physiological traits included water-use efficiency, leaf carbon (C_{mass}) and nitrogen content (N_{mass}), and relative water content of leaves. Leaf tissues were collected prior to flowering during the third harvest for isotopic analysis. Leaf ^{13}C , C_{mass} , and N_{mass} were analyzed at the University of California, Davis Stable Isotope Facility via an elemental analyzer interfaced to a mass spectrometer (PDZ Europa, ANCA-GSL and PDZ Europa 20-20, Secron Ltd., UK). Carbon isotope ratios were converted to discrimination values (Δ , per mil ‰—a time-integrated measure of water-use efficiency; Dawson et al. 2002) by the equation:

$$\Delta = \delta a - \delta p / (1 + \delta p) \times 0.0001,$$

Where δa is the carbon isotope ratio of atmospheric CO_2 (assumed to be -8 ‰) and δp is the measured carbon isotope ratio of the leaf tissue (Farquhar et al. 1989). Lower values of Δ indicate higher intrinsic water-use efficiency values (Dawson et al. 2002). Relative water content of leaves was measured at peak productivity as an indirect measurement of leaf turgor (Smart and Bingham 1974). Three individuals were sampled from each site and we used leaf punches from the healthiest, fully emerged leaf from each plant. We obtained fresh weights of leaf

punches and then floated leaves in distilled water in a Petri dish for 6 h in a dark room to allow for rehydration. The assumed turgid weights were then obtained and leaves were dried for 24 hours at 60 °C to obtain dry weights. Relative water content (RWC) was then calculated (Weatherley 1950; González and González-Vilar 2001),

$$\text{RWC} = w_f - w_d / w_t - w_d,$$

where w_f , w_d , and w_t are fresh weight, dry weight, and turgid weight.

Environmental data

We used BioClim climate variables from each sampling site to test for local adaptation. These climate variables represent annual and seasonal trends, as well as extremes in temperature and precipitation, which are often useful in describing species distributions. We evaluated 19 BioClim climate variables (BIO1–19; Hijmans et al., 2005) at a 30 arc-second resolution (ca. 1 km²) for inclusion in our models. We tested all variables for pairwise correlation across the study area using the Raster package in R (Hijmans & van Etten, 2012). We retained 7 of the 19 BioClim layers that had correlation coefficients under |0.70, four of which related to temperature and three of which related to precipitation. Temperature variables included isothermality (temp_{iso} ; or the mean monthly range divided by the annual range in temperatures; BIO3), temperature seasonality ($\text{temp}_{\text{seasonality}}$; standard deviation \times 100; BIO4), and mean temperatures of the wettest ($\text{temp}_{\text{wetqtr}}$) and coldest ($\text{temp}_{\text{coldqtr}}$) quarters (BIO8 & 9, respectively). Precipitation variables

included precipitation during the wettest ($\text{precip}_{\text{wetmonth}}$) and driest ($\text{precip}_{\text{drymonth}}$) months (BIO13 & 14, respectively), and precipitation of the coldest quarters ($\text{precip}_{\text{coldqtr}}$; BIO19).

Statistical analyses

We measured key functional traits associated with phenology, physiology, and morphology important to plant success in the invaded context and first calculated summary statistics by population (Table S4.1). We then performed Pearson's correlations on our targeted traits to test for autocorrelation that could produce false positives. In total, nine key functional traits were retained with correlation coefficients under $|0.70|$. These functional traits were joined with the subsetting geographic and bioclimatic variables in subsequent analyses. All of the remaining correlation coefficients are reported in supplementary materials (Tables S4.2–S4.3).

We used nested ANOVA to examine potential effects of site and maternal lineages as sources of variation in functional traits. We included age of the plant as a covariate for non-phenology traits to account for potential effects of plant age from different harvests. We then used hierarchical partitioning to examine the relative contribution of geographic and bioclimatic temperature and precipitation variables in explaining variation in functional traits. We did this for the functional traits where population was determined to be significant from the nested ANOVA models. Hierarchical partitioning enables better estimation of the relative importance of each variable while also accounting for potential collinearity of explanatory variables (Chevan and Sutherland 1991; Murray and Conner 2009). First, we accounted for correlated error structures that arise from repeated measures (within populations) and used linear mixed effects models with each functional trait as the response variable and maternal lineage nested within

population as random effects and related the residuals to geographic and bioclimatic variables using hierarchical partitioning. We then performed randomization tests on each functional trait (1000 iterations each using a r^2 goodness-of-fit measure) to assess the significances of each geographic and bioclimatic variable. We computed Z -scores to determine significance of each explanatory variable. Last, we used linear regression to test relationships between population means for each functional trait and the geographic and bioclimatic variables identified as significant by randomization tests. All statistical analyses were carried out in R 3.3.2 (R Core Team 2014), and models were evaluated using the `piecewiseSEM` and `effects` packages (Lefcheck 2015, Fox 2003).

Results

There were strong differences among populations for 9 of the functional traits (Table 4.2), suggesting potential local adaptation across the invaded range of Sahara mustard. This was evidenced most strongly in phenological and morphological traits relating to germination (days to germination: $F = 7.84$, $P < 0.001$; seed weight: $F = 3.70$, $P < 0.001$) and early growth (1st leaf: $F = 4.97$, $P < 0.001$; 1st flower: $F = 5.41$, $P < 0.001$). These traits varied by 105, 57, and 145 percent, respectively, across the 10 localities evaluated from the invaded range in North America. However, populations also exhibited differences in additional morphological traits including mean leaf area ($F = 3.49$, $P = 0.002$) and proportion of biomass allocation to reproduction (% repro; $F = 5.19$, $P < 0.001$), which varied by 1,143 and 1,300 percent respectively. They also were differentiated by 16, 9, and 121 percent for physiological traits

related to water stress and nutrient allocation—RWC ($F = 2.55$, $P = 0.045$), WUE ($F = 51.68$, $P < 0.001$), and N_{mass} ($F = 19.87$, $P < 0.001$), respectively.

Variation within populations was observed for some but not all traits (Table 4.2). Significant effects of maternal lineage were detected for days to germination ($F = 5.92$, $P < 0.001$), 1st leaf ($F = 4.97$, $P < 0.001$), 1st flower ($F = 2.12$, $P < 0.001$), mean leaf area ($F = 1.58$, $P = 0.05$), WUE ($F = 84.16$, $P < 0.001$), and N_{mass} ($F = 11.63$, $P < 0.001$). We successfully controlled for ontogenetic effects with plant age in nearly all models (Table 4.2).

Based on hierarchical partitioning models, the most important bioclimatic variables that explained variation in plant traits differed across the traits tested but precipitation during the growing season ($\text{precip}_{\text{wetmonth}}$) explained four of the nine tested traits—primarily phenological (germination time and days to first leaf) and morphological features (seed weight, allocation to reproduction; Fig. 4.2, Table S4.4). Elevation, compared to the other geographic and bioclimatic variables, explained the largest percentage of variation for any traits, accounting for nearly 40% of the variation in timing of 1st leaf and RWC (a phenological and physiological trait respectively). In both cases, elevation likely correlates with additional environmental variables that drive leaf phenology and potential cellular water deficit as indicated by RWC (a physiological trait). $\text{temp}_{\text{seasonality}}$ also explained a substantial proportion of variation, but only did so for the timing of the 1st flower produced (phenology). For physiological traits, no bioclimatic descriptor explained dynamics, except temperature seasonality for leaf nitrogen content.

All other geographic and bioclimatic variables explained less than 30% of the variation in functional traits. However, $\text{precip}_{\text{wetmonth}}$ significantly explained a percentage of variance for the most number of functional traits, including days to germination, timing of the 1st leaf, seed weight, and percentage of biomass allocated to reproductive structures (% repro), all functional

traits related to issues associated with aligning biological activity to conditions during germination and early stages of growth. A significant portion of variation of timing to 1st flower was explained by longitude, following typical phenology patterns from the coastal to interior sites associated with growing season constraints arising from aridity. Similarly, mean leaf area variance was significantly explained by latitude. Last, a significant percentage of variation in seed weights was explained by $\text{precip}_{\text{drymonth}}$, likely indicating a shift in investing in heavier seeds that need to survive hot summers in relatively dry environments.

The number of days to germination decreased with increasing winter season precipitation ($\text{precip}_{\text{wetmonth}}$; $r^2 = 0.49$, $P = 0.03$; Fig. 4.3). Time to 1st leaf increased with longitude ($r^2 = 0.50$, $P = 0.05$), again, likely following typical phenology patterns as sites become more inland. Time to 1st leaf also increased with increasing winter season precipitation ($\text{precip}_{\text{wetmonth}}$; $r^2 = 0.56$, $P = 0.03$). Similar to hierarchical partitioning results, seed weights decreased with increasing winter season precipitation and precipitation during the driest months ($\text{precip}_{\text{wetmonth}}$; $r^2 = 0.62$, $P = 0.01$; $\text{precip}_{\text{drymonth}}$; $r^2 = 0.52$, $P = 0.03$). This trend was also observed with percentage of biomass allocated to reproductive structures (% repro) decreasing with increasing winter season precipitation ($\text{precip}_{\text{wetmonth}}$; $r^2 = 0.80$, $P < 0.01$; Fig. 4.3). Last, signs of adapting to water stress were evidenced by the lowest RWC (i.e., highest cellular water deficit although marginally significant; $r^2 = 0.43$, $P = 0.06$) at the lowest elevations in desert sites and, at the same time, the highest water-use efficiency at these low elevation sites ($r^2 = 0.68$, $P = 0.01$) that also followed $\text{precip}_{\text{drymonth}}$ (marginally significant $r^2 = 0.47$, $P = 0.06$; Fig. 4.3).

Discussion

Invasive populations may encounter unique selective pressures and limitations across their ranges, including differences in abiotic conditions such as drought, day length, and seasonality. By identifying the plant traits and their expression that have contributed to, and may predispose invasives to spread into novel environments, we can understand the mechanisms driving invasion and provide targets for management (Funk et al. 2008). Here we demonstrate significant variation in key functional traits in Sahara mustard in a common garden environment, which suggests that this species has responded to variable selection pressures with different phenological, physiological, and morphological traits across a broad range of environmental conditions in its invaded range in the US southwest. Further, we quantified the relative contributions of geographic and bioclimatic factors in explaining variation in observed phenotypes, showing that growth strategies of Sahara mustard correspond with local variation in seasonally available precipitation. The shifts in phenological, morphological, and physiological traits observed among populations of Sahara mustard is likely to have facilitated the successful invasion across the region. With this, the species likely altered its water-use efficiency to tolerate drought stress, adjusted key timings of biological events within the context of aridity, and invested heavily in reproduction to ensure future success. These patterns are consistent with the generalized strategies associated with ephemeral plant strategies associated with desert adaptation (e.g., Smith et al. 1997; Huxman et al. 2013), suggesting that invasive species exploit trait-relationships associated with native species success in these environments, but likely employ enhanced performance in several attributes to achieve success (e.g., Kimball et al. 2014).

While the ecological and evolutionary patterns in arid systems like those occupied by Sahara mustard are similar overall, regional environmental contexts vary significantly, especially that of rainfall variability and seasonal temperature covariance (Loik et al. 2004). Functional trait approaches relating species performance to environmental variation (Angert et al. 2007, Tecco et al. 2010, Chapman et al. 2014, Wolkovich et al. 2013) have proven useful for determining effects of contemporary climate change (e.g., Kimball et al. 2010) and can be powerful in elucidating the mechanisms promoting invasive species success (Colautti and Barrett 2013, Funk 2013, Gilbert et al. 2017). However, a grand challenge in ecology and evolutionary biology is understanding how invasive species respond to and leverage environmental variation during establishment. This challenge is made more urgent by the need to forecast ecological and evolutionary dynamics in the face of climate change and future invasions.

Although many invasions arise as a result of accidental introductions (Lehan et al. 2013), range expansions of invasives already established may be possible under future climate scenarios (Novy et al. 2012, Nguyen et al. 2016). Overall, its range of reproductive investment and water-use efficiency strategies in multiple environments coupled with it being a self-compatible species makes it a strong contender for continued invasion under future scenarios (DeFalco et al. 2002, Nguyen et al. 2016). Sahara mustard has colonized multiple ecoregions of North America in less than 100 years and appears well poised to continue to dominate arid environments and spread. Part of this success has been attributed to its reproductive strategies and its ability to self-fertilize (Schemske 1984, Barrett et al. 2008, Marushia et al. 2012, Pannell 2015), but here we identify that shifting phenologies, investment in leaves and reproductive structure, and water-use efficiencies to match environmental drivers are critical for establishment, survival, and reproduction in the invaded range. Sahara mustard responded to decreasing winter season

precipitation ($\text{precip}_{\text{wetmonth}}$) by increasing allocation to reproductive structures and assuring that seeds were prepared to tolerate drought stress in the driest sites. Together, these reproductive and functional traits likely allow Sahara mustard to overcome recruitment barriers that challenge species in novel environments.

Species invasions result from a combination of ecological, evolutionary, and organismal processes that interact with specific traits such that rapid evolution of phenological and morphological traits associated with establishment niche and changes in growing season length (e.g., germination timing, seed size) are likely necessary for most invasives to succeed (Muth and Pigliucci 2006, Wolkovich and Cleland 2011, Novy et al. 2012, Nguyen et al. 2016). Other invasive species have rapidly adapted to invaded environments over short periods of time (e.g., less than 100 years; Novy et al. 2012, Nguyen et al. 2016). We show that Sahara mustard exhibits several genotypes that initiate germination and growth in response to local, seasonal precipitation. In doing so, Sahara mustard is able to synchronize its growth to local conditions, thereby ensuring a high level of fitness and, potentially, a shift in its phenology in competition with native species (Powell et al. 2011, Wolkovich and Cleland 2011). Our common garden results suggest a strong genetic component to the variation in functional traits in Sahara mustard, which likely contributes to the continued success of the populations we sampled and, given their variability across the range, may enable it to spread into additional semi-arid, or pulse-driven systems if the ideal genotype is introduced (Drenovsky et al. 2012). Thus, the level of trait divergence observed in our common gardens is consistent with local adaptation given the unlikely alternative that many distinct Sahara mustard genotypes invaded. Our results strongly suggest that local adaptation of functional traits have enabled or followed Sahara mustard's successful colonization across its invaded range.

Nonetheless, phenotypic plasticity is often important for successful establishment of invasives early on in the invasion process (Richards et al. 2006, Funk 2008, Murphy et al. 2016). In doing so, plasticity can eventually promote local adaptation by enabling a population to persist in a novel environment, in which they experience new selection pressures and potentially lose plasticity through time (Parker et al. 2003, Franks et al. 2007, Ghalambor et al. 2007, Crispo 2008). In this analysis, we controlled for the effects of trait plasticity and ontogenetic drift to understand microevolutionary dynamics associated with this species success and it remains likely that Sahara mustard also exhibits a plasticity associated with the invasion and the large range that contains many similar environments (Tecco et al. 2010, Drenovsky et al. 2012). An open question is how the multiple processes interact to influence plant behavior, encompassing the complex system of multiple traits and how plasticity, population divergence and ontogenetic dynamics are combined across the range.

Sahara mustard exhibits tremendous variability in functional traits that align with multiple environments across its invaded range in the US southwest and suggest that it is a dangerous invasive with a still unknown invasion potential. Overall, our study demonstrates that Sahara mustard has responded to thousands of kilometers of environmental variability by locally adapting its phenotypes to fit the drivers at each site. In doing so, we show that linking life history strategies, functional traits, and their responses to environmental variation can assist in producing a mechanistically-based predictive framework for ecologists to understand population dynamics (Rees et al. 2001). Future studies should address the degree to which plasticity has enabled Sahara mustard's successful invasion, the potential for it to continue its range expansion, and its ability to respond to further climate change.

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Table 4.1. Site codes, names, geographic locations, and elevation.

code	location	latitude, longitude	elevation (m)
CA1	Nipomo	35.048, -120.512	128.22
CA4	In-Ko-Pah Park Road	32.647, -116.106	905.68
CA3	Coachella Valley Preserve	33.772, -116.304	24.64
CA2	Mojave National Preserve	34.803, -115.612	1193.11
NV	Las Vegas	36.090, -115.233	726.88
UT	Red Cliffs National Conservation Area	37.225, -113.406	985.23
AZ2	Saguaro National Park	32.177, -110.739	962.64
AZ1	Dateland	32.801, -113.541	130.86
NM	Mesquite	32.184, -106.678	1195.52
TX	Fort Hancock	31.299, -105.832	1114.53

Table 4.2. Nested ANOVA results testing for effects of population, maternal lineage, and individual plant age on functional traits.

trait	population				maternal line				plant age				error	
	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>SS</i>
days to germ	8.00	12.61	7.88	< 0.001	33.00	34.26	5.19	< 0.001	-	-	-	-	501.00	100.19
1 st leaf	8.00	10.15	4.97	< 0.001	33.00	37.52	4.46	< 0.001	-	-	-	-	489.00	124.79
1 st flower	8.00	907.00	5.41	< 0.001	31.00	1378.70	2.12	< 0.001	-	-	-	-	332.00	6958.30
seed wt	9.00	0.72	3.70	< 0.001	9.00	0.25	1.29	0.25	1.00	0.60	27.74	< 0.001	119.00	2.59
mean leaf area	7.00	7.60	3.48	0.00	27.00	13.34	1.59	0.05	1.00	20.47	65.67	< 0.001	113.00	35.31
% repro	8.00	0.13	5.20	< 0.001	26.00	0.12	1.57	0.06	1.00	0.24	80.00	< 0.001	89.00	0.27
RWC	8.00	3.30	2.55	0.05	15.00	2.87	1.18	0.36	1.00	0.10	0.60	0.45	19.00	3.07
WUE (Δ)	7.00	0.01	51.68	< 0.001	9.00	0.03	84.16	< 0.001	1.00	0.00	59.11	< 0.001	9.00	0.00
N_{mass}	7.00	1.14	19.87	< 0.001	9.00	0.85	11.63	< 0.001	1.00	0.33	40.05	< 0.001	9.00	0.07

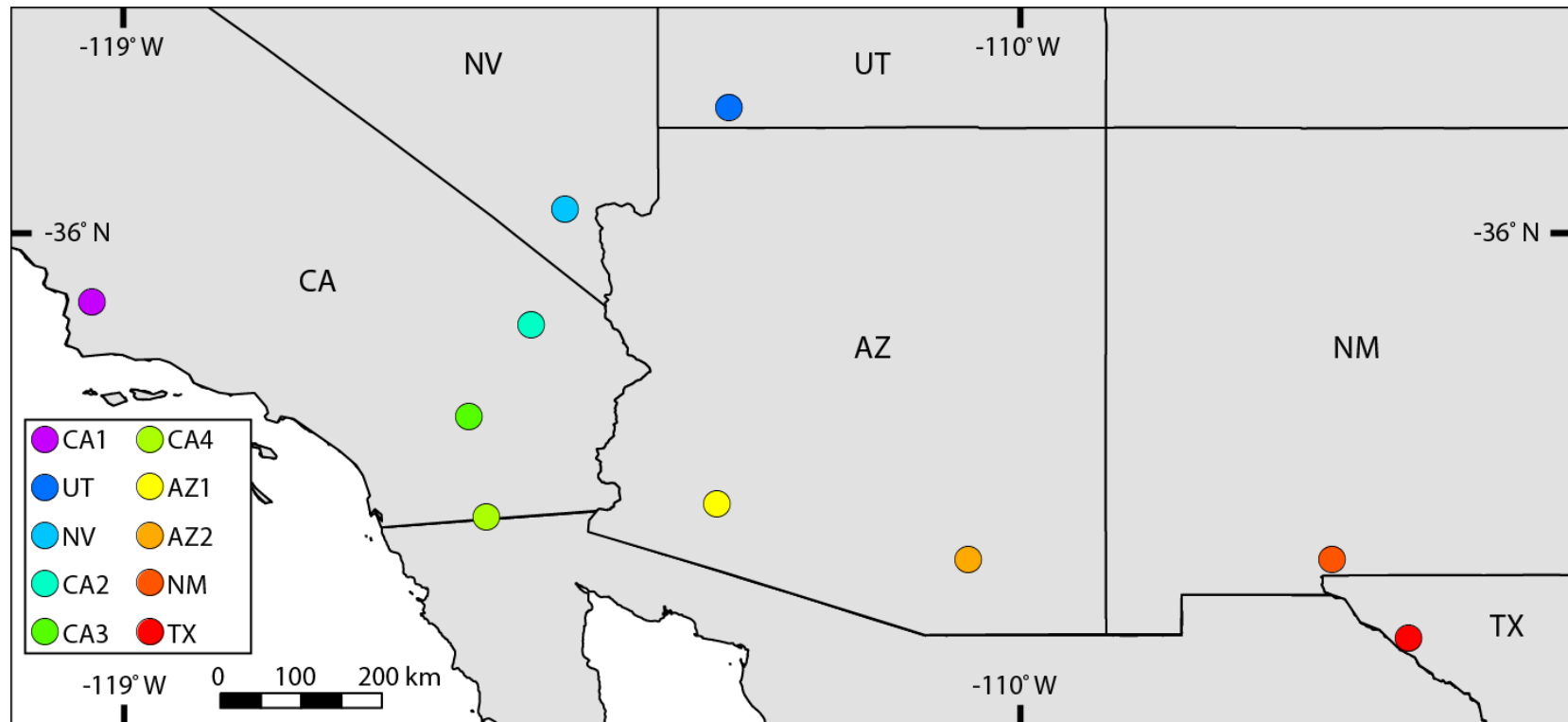


Fig. 4.1. Sampling sites across the invaded range of Sahara mustard (*Brassica tournefortii*).

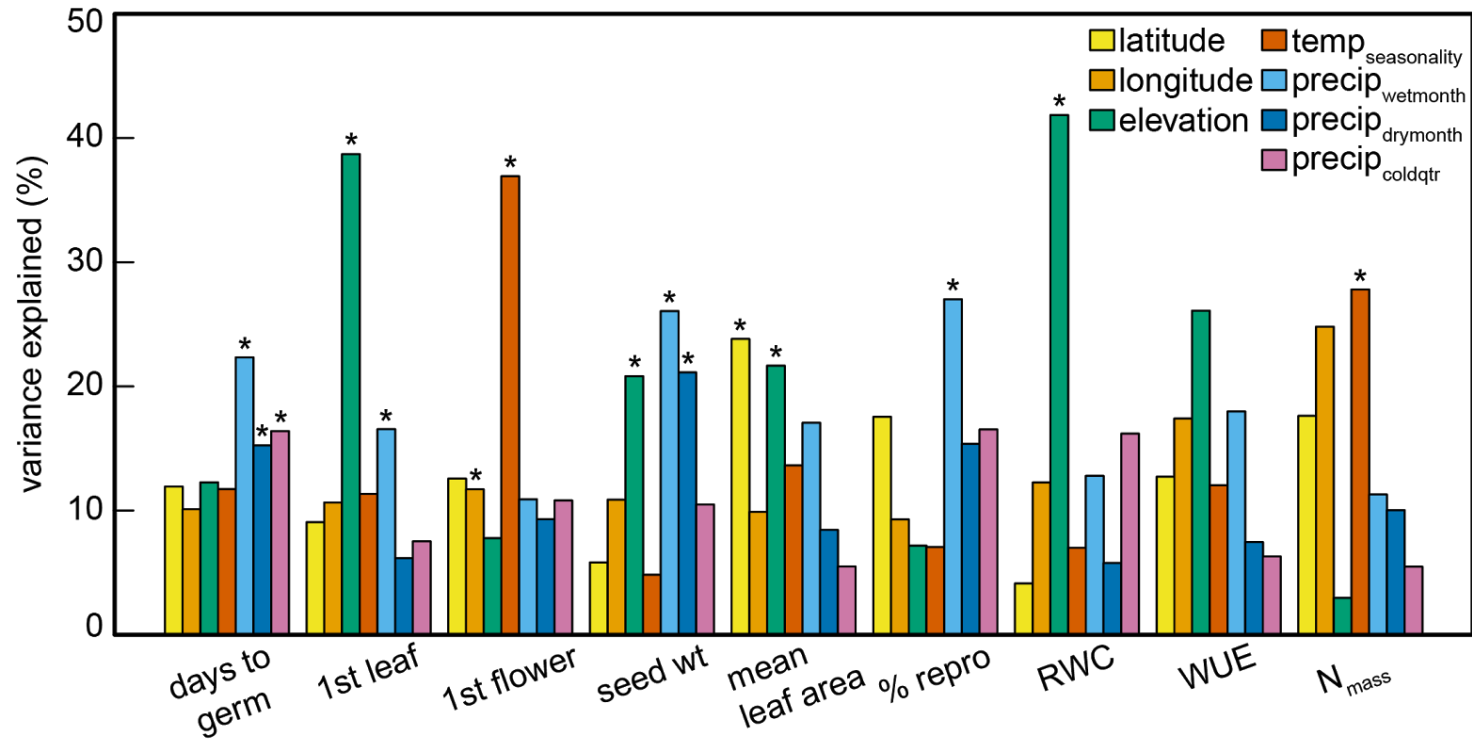


Fig. 4.2. Hierarchical partitioning results showing percentage of variance explained by individual geographic and bioclimatic variables. Asterisks (*) indicate which variables explained a significant amount of variance based on randomization tests for hierarchical partitioning with an upper 0.95 confidence limit ($Z \geq 1.65$; statistical results reported in Table 3).

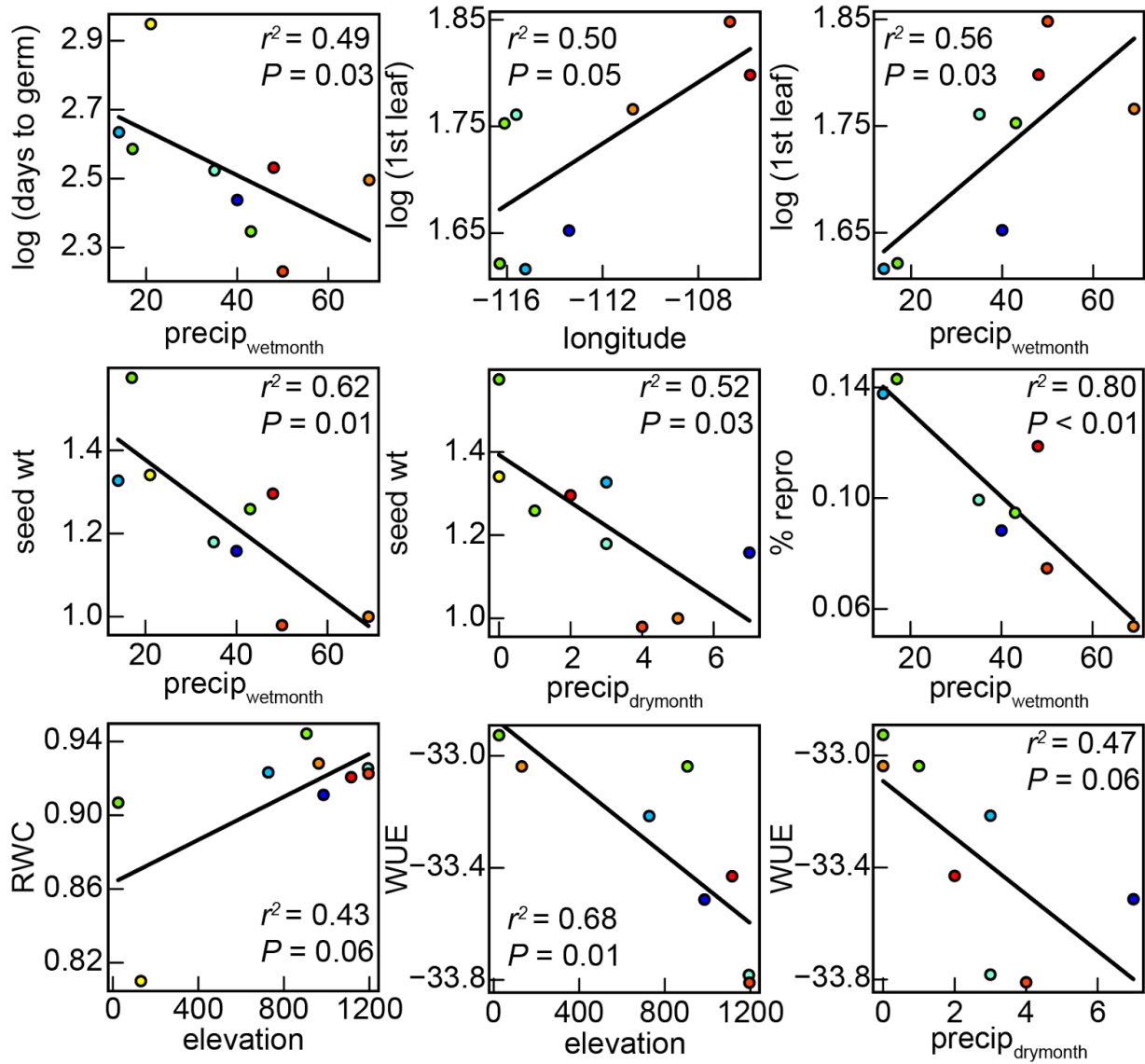


Fig. 4.3. Linear regressions of functional traits and geographic and bioclimatic variables identified as significant in hierarchical partitioning analyses and with significant linear regressions. r^2 and P values are reported in each graph.

Appendix S4

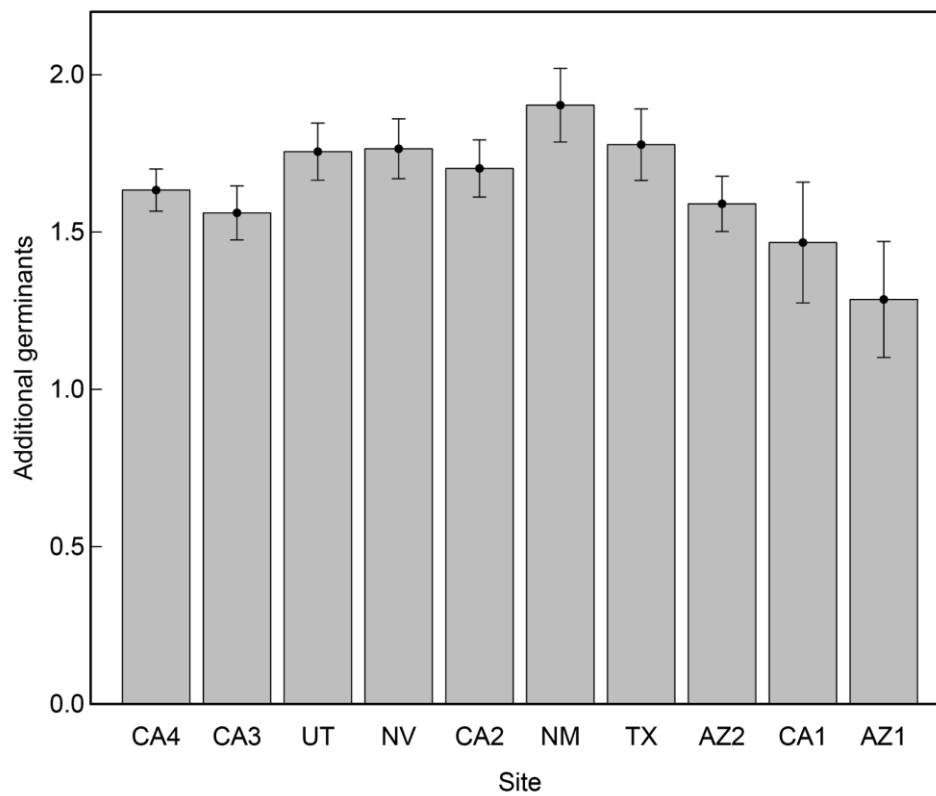


Fig. S4.1. Mean (\pm SEM) number of secondary and tertiary germinants removed from each pot after the first seedling emerged. ANOVA indicate no significant different between sites ($F_{1,353} = 0.034$, $P = 0.854$).

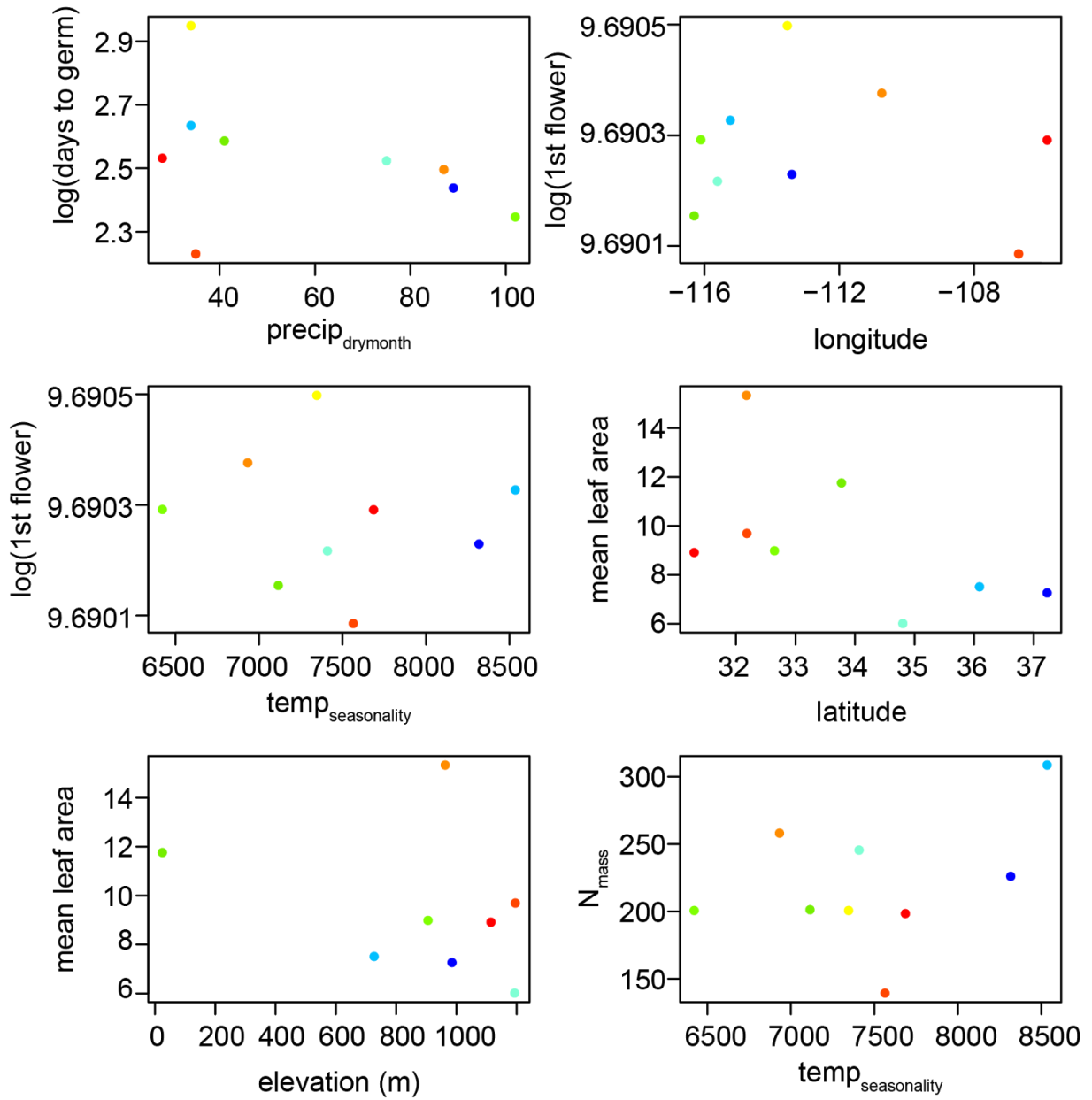


Fig. S4.2. Non-significant relationships between traits and geographic and bioclimatic variables based on linear regressions.

Table S4.1. Mean \pm SEM for functional traits by population.

	population									
trait	CA4	CA3	UT	NV	CA2	NM	TX	AZ2	CA1	AZ1
days to germ	10.45 \pm 0.58	13.28 \pm 0.75	11.45 \pm 0.64	13.94 \pm 1.03	12.47 \pm 0.77	9.3 \pm 0.74	12.57 \pm 0.8	12.13 \pm 0.82	8.94 \pm 1.1	19.08 \pm 1.58
1st leaf	5.77 \pm 0.27	5.06 \pm 0.24	5.22 \pm 0.25	5.03 \pm 0.32	5.82 \pm 0.27	6.35 \pm 0.29	6.04 \pm 0.32	5.85 \pm 0.42	8.19 \pm 0.43	3.64 \pm 0.4
2nd leaf	6.95 \pm 0.26	7.06 \pm 0.23	7.33 \pm 0.27	6.49 \pm 0.34	8.07 \pm 0.47	7.67 \pm 0.27	7.54 \pm 0.29	7.6 \pm 0.43	11.38 \pm 0.47	6.6 \pm 0.59
3rd leaf	10.46 \pm 0.31	10.36 \pm 0.25	10.59 \pm 0.25	10.42 \pm 0.35	11.89 \pm 0.62	10.72 \pm 0.42	11.27 \pm 0.32	11.04 \pm 0.43	14.38 \pm 0.57	10 \pm 0.41
4th leaf	13.25 \pm 0.36	13.81 \pm 0.24	13.43 \pm 0.21	12.92 \pm 0.35	14.47 \pm 0.57	13.74 \pm 0.37	14.02 \pm 0.33	13.68 \pm 0.44	18.75 \pm 0.83	13.52 \pm 0.64
5th leaf	16.33 \pm 0.36	16.96 \pm 0.28	17.24 \pm 0.29	15.98 \pm 0.53	18.28 \pm 0.54	16.25 \pm 0.37	16.75 \pm 0.43	17.26 \pm 0.47	22.64 \pm 0.96	16.92 \pm 0.63
1st flower	16159.96	16157.74	16158.95	16160.53	16158.75	16156.63	16159.95	16161.32	16156	16163.29

nleaves	42.67 ± 4.21	35.83 ± 3.5	40 ± 3	28 ± 1.22	34 ± 8	40 ± 4.58	37 ± 2.31	30.25 ± 4.11	29 ± 6.51	37.5 ± 0.5
seed wt	1.26 ± 0.07	1.57 ± 0.08	1.16 ± 0.07	1.33 ± 0.08	1.18 ± 0.05	0.98 ± 0.09	1.3 ± 0.14	1 ± 0.14	1.19 ± 0.07	1.34 ± 0.07
leaf area	8.99 ± 1.11	11.76 ± 1.63	7.26 ± 1.45	7.51 ± 0.92	6.01 ± 1.17	9.69 ± 1.35	8.91 ± 1.67	15.33 ± 2.46	17.25 ± 4.92	74.69 ± 17.15
mean area	46.73 ± 3.66	50.74 ± 4.49	58.03 ± 14.34	44.17 ± 1.81	50.07 ± 1.61	53.86 ± 4.27	41.52 ± 2.51	66.18 ± 4.34	69.19 ± 7.28	59.41
sla	0.25 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.27 ± 0	0.28 ± 0	0.22 ± 0.01	0.27 ± 0.02	0.22 ± 0.02	0.2 ± 0.04	0.17
leaf dry wt	2251.18 ± 218.4	2502.18 ± 381.32	2971.03 ± 826.23	1607.92 ± 80.88	1717.05 ± 114.05	3188.6 ± 543.49	2267.2 ± 221.84	3533.57 ± 444.95	4935.5 ± 367.41	4185.23 ± 426.13
stem dry wt	3790.07 ± 488.51	2958.28 ± 338.66	2949.33 ± 1530.93	3044.38 ± 221.38	4157.9 ± 756.5	3739.37 ± 586.33	2333.57 ± 680.63	2980.31 ± 533.79	2864.3 ± 842.77	5921.93 ± 1144.58
repro dry wt	869.92 ± 233.88	1338.55 ± 159.75	704.03 ± 149.17	1100.36 ± 89.68	1342.2 ± 286.1	578.43 ± 308.79	1466.87 ± 845.37	381.38 ± 139.08	187.73 ± 70.16	257.2 ± 58.8
agb	6911.17 ± 603.41	6799.01 ± 470.08	6624.4 ± 2208	5752.66 ± 391.94	7217.15 ± 928.55	7506.4 ± 1201.44	6067.63 ± 759.39	6895.26 ± 682.14	7987.53 ± 691.93	10364.35 ± 777.25

% repro	0.09 ± 0.02	0.14 ± 0.02	0.09 ± 0.01	0.14 ± 0.02	0.1 ± 0.02	0.07 ± 0.02	0.12 ± 0.04	0.05 ± 0.01	0.04 ± 0.01	0.01 ± 0
rgr	0.11	0.13	0.12	0.09	0.13	0.12	0.12	0.12	0.14	0.15
rwc	0.94 ± 0.01	0.91 ± 0.02	0.91 ± 0.02	0.92 ± 0.01	0.93 ± 0.02	0.92 ± 0.01	0.92 ± 0.01	0.93 ± 0.02	0.91 ± 0.02	0.81 ± 0.01
WUE	-33.04 ± 0.69	-32.93 ± 0.51	-33.51 ± 0.03	-33.21 ± 0.01	-33.78 ± 0.22	-33.81 ± 0	-33.43	-30.96 ± 2.52	-32.34 ± 0.36	-33.04 ± 0.69
15N	1.13 ± 0.48	1.42 ± 0.21	1.26 ± 0.38	2 ± 0.44	1.43 ± 0.28	2.65 ± 0.51	1.81 ± 0.77	1.77 ± 0.44	-0.29 ± 1.42	-2.39 ± 0.95
C_{mass}	1639.8 ± 51.58	1620.53 ± 76.82	1718.48 ± 4.03	1781.38 ± 66.54	1669.46 ± 71.14	1666.11 ± 0	1702.36	1493.08 ± 245.59	1414.08 ± 59.69	1639.8 ± 51.58
N_{mass}	200.68 ± 38.04	201.29 ± 23.29	226.04 ± 17.58	308.58 ± 25.42	245.5 ± 21.37	139.42 ± 0	198.43	258.06 ± 13.18	242.37 ± 33.89	200.68 ± 38.04
C:N	8.88 ± 0.94	8.66 ± 1.13	7.71 ± 0.67	5.85 ± 0.32	6.97 ± 0.74	11.95 ± 0	8.58	5.75 ± 0.66	5.99 ± 1.08	8.88 ± 0.94

Table S4.2. Reduced pairwise correlation coefficients of functional traits.

	mean leaf area	% repro	1st flower	days to germ	1st leaf	RWC	WUE	N _{mass}	seed wt
mean leaf area	1.00	-0.67	0.59	-0.02	-0.53	0.00	0.12	-0.16	0.20
% repro		1.00	-0.20	-0.13	-0.11	0.56	-0.38	0.17	0.46
1st flower			1.00	0.44	-0.18	-0.49	0.23	0.26	0.14
days to germ				1.00	-0.68	-0.38	-0.02	0.16	0.49
1st leaf					1.00	0.57	0.18	-0.01	-0.42
RWC						1.00	0.03	0.16	-0.30
WUE							1.00	0.36	-0.19
N _{mass}								1.00	0.10
seed wt									1.00

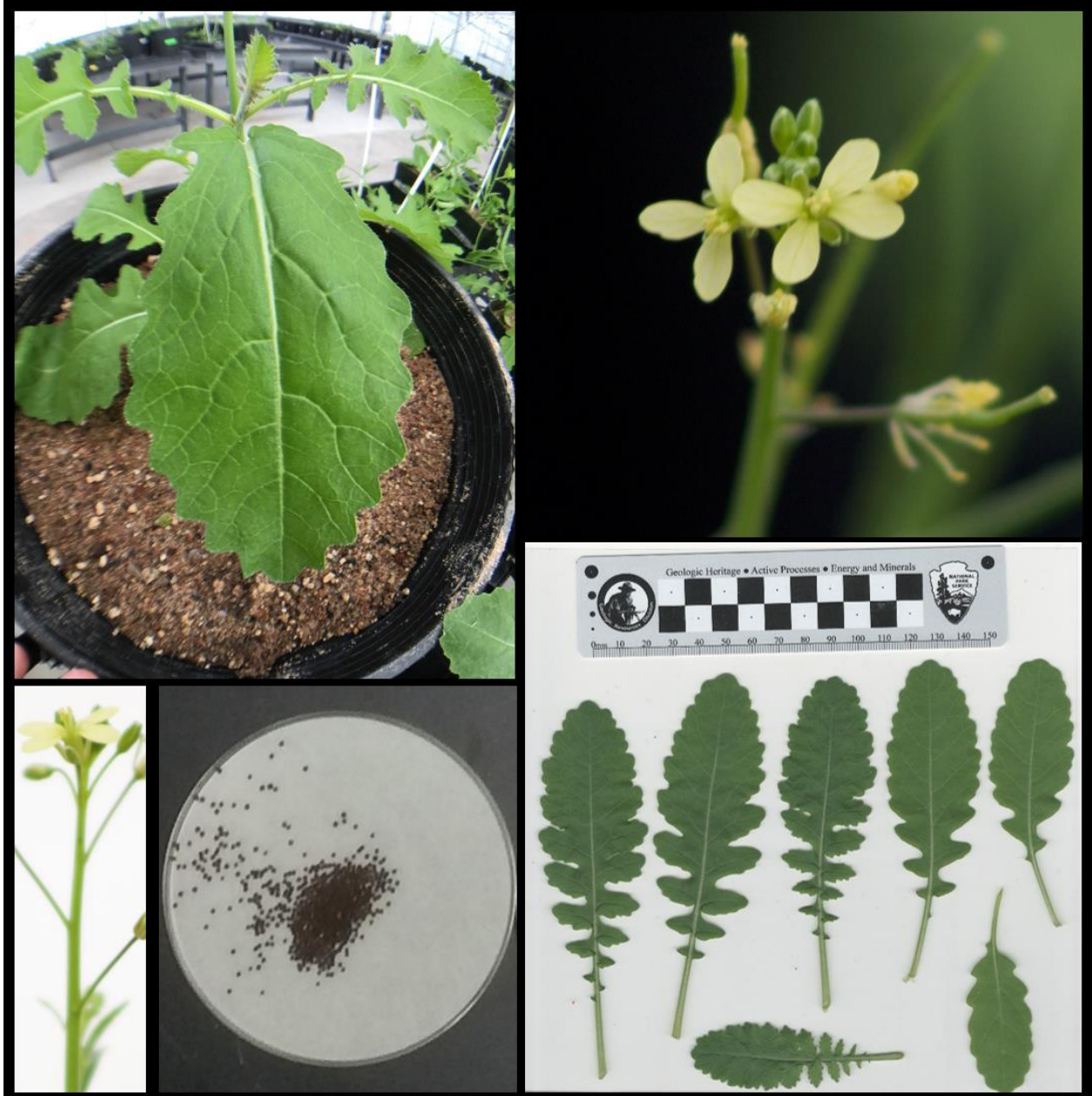
Table S4.3. Reduced pairwise correlation coefficients of geographic and bioclimatic temperature and precipitation variables.

	lat	long	elevation	bio4	bio13	bio14	bio19
lat	1.00	-0.59	-0.14	0.02	-0.18	0.32	0.30
long		1.00	0.61	0.56	-0.01	0.42	-0.64
elevation			1.00	0.47	0.12	0.66	-0.28
bio4				1.00	-0.40	0.51	-0.45
bio13					1.00	0.10	0.39
bio14						1.00	-0.17
bio19							1.00

Table S4.4. Hierarchical partitioning results sorted by percent variance explained by predictors.

trait	predictor	percent	i	j	z
days to germ	precip _{wetmonth}	22.33331	0.010774	0.000678	3.66*
	precip _{coldqtr}	16.39392	0.007908	-0.00078	2.18*
	precip _{drymonth}	15.23772	0.007351	-0.00539	2.13*
	elevation	12.2706	0.005919	0.004815	1.52
	latitude	11.93069	0.005755	-0.00322	1.32
	temp _{seasonality}	11.73034	0.005686	-0.00167	1.36
	longitude	10.10342	0.004874	-0.00176	1.21
1st leaf	elevation	38.70466	0.019511	0.01099	6.93*
	precip _{wetmonth}	16.55427	0.008345	0.016762	2.31*
	temp _{seasonality}	11.34308	0.005718	-0.00357	1.61
	longitude	10.64976	0.005368	0.009063	1.37
	latitude	9.065235	0.00457	0.002272	1.14
	precip _{coldqtr}	7.517792	0.00379	-0.00183	0.75
	precip _{drymonth}	6.165201	0.003108	0.001108	0.42
1st flower	temp _{seasonality}	36.92858	0.029434	-0.02932	6.12*
	latitude	12.5697	0.010019	-0.00946	1.55
	longitude	11.70989	0.009333	-0.0092	1.66*
	precip _{wetmonth}	10.90378	0.008691	-0.00585	1.27
	precip _{coldqtr}	10.81251	0.008618	-0.0068	1.57
	precip _{drymonth}	9.303888	0.007416	-0.00738	1.34
	elevation	7.771661	0.006194	-0.00537	0.86
seed weight	precip _{wetmonth}	26.06705	0.040244	0.078625	4.33*
	precip _{drymonth}	21.12127	0.032609	0.042498	2.74*
	elevation	20.82324	0.032148	0.070359	2.53*
	longitude	10.87213	0.016785	0.025587	0.92
	precip _{coldqtr}	10.48352	0.016185	0.024624	0.89
	latitude	5.813619	0.008976	-0.00849	0.21
	temp _{seasonality}	4.819164	0.00744	-0.00721	0.02
leaf area	latitude	23.81601	0.028787	3.15E-02	2.81*
	elevation	21.66549	0.026187	-1.81E-02	2.02*
	precip _{wetmonth}	17.06151	0.020622	6.96E-03	1.57
	temp _{seasonality}	13.63332	0.016479	2.12E-02	0.91
	longitude	9.893345	0.011958	1.96E-05	0.49
	precip _{drymonth}	8.441111	0.010203	-5.46E-03	0.35
	precip _{coldqtr}	5.489212	0.006635	-6.63E-03	-0.05
% repro	precip _{wetmonth}	27.01662	0.026351	2.99E-02	1.65*
	latitude	17.54532	0.017113	-3.44E-05	0.89
	precip _{coldqtr}	16.52914	0.016122	2.24E-03	0.7
	precip _{drymonth}	15.37672	0.014998	-2.67E-03	0.86

RWC	longitude	9.287351	0.009058	1.22E-02	0.08
	elevation	7.174239	0.006997	3.52E-03	-0.11
	temp _{seasonality}	7.070607	0.006896	3.69E-03	-0.13
	elevation	41.85411	0.083073	1.90E-02	1.95*
	precip _{coldqtr}	16.18754	0.03213	5.10E-02	0.31
	precip _{wetmonth}	12.79676	0.025399	2.99E-02	0.17
	longitude	12.26263	0.024339	-2.37E-02	0.02
	temp _{seasonality}	6.990634	0.013875	6.06E-03	-0.29
WUE	precip _{drymonth}	5.781819	0.011476	5.96E-03	-0.37
	latitude	4.126515	0.00819	-6.54E-03	-0.47
	elevation	26.09191	0.079502	-0.06106	0.92
	precip _{wetmonth}	17.97861	0.05478	-0.0126	0.32
	longitude	17.4144	0.053061	-0.05248	0.4
	latitude	12.71875	0.038754	0.005198	0
	temp _{seasonality}	12.03475	0.03667	0.001382	-0.07
	precip _{drymonth}	7.460905	0.022733	-0.02255	-0.32
N_{mass}	precip _{coldqtr}	6.30068	0.019198	-0.00826	-0.5
	temp _{seasonality}	27.8065	0.132076	0.01173	2.08*
	longitude	24.80226	0.117806	-0.07187	1.5
	latitude	17.62589	0.08372	0.0905	0.87
	precip _{wetmonth}	11.29352	0.053642	-0.02047	0.27
	precip _{drymonth}	10.02854	0.047634	-0.0035	0.24
	precip _{coldqtr}	5.480733	0.026033	-0.02533	-0.28
	elevation	2.962556	0.014072	-0.0094	-0.51



Above images: *Brassica tournefortii* leaves, flowers, and seeds. Photos by D. E. Winkler.

“There are three stages of scientific discovery: first people deny it is true; then they deny it is important; finally they credit the wrong person.”

—widely attributed to Alexander von Humboldt