University of Nevada, Reno

Multi-empirical investigations on the population genetic structure, ecological niche, and regeneration of *Ivesia webberi* with conservation implications

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in
Ecology, Evolution and Conservation Biology

by

Israel Temitope Borokini

Dr. Mary M. Peacock – Advisor
Dr. Peter J. Weisberg – Co-advisor

May, 2021

THE GRADUATE SCHOOL

We recommend that the dissertation
prepared under our supervision by

Israel Temitope Borokini

entitled

Multi-empirical investigations on the population genetic structure, ecological niche, and regeneration of Ivesia webberi with conservation implications

be accepted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

Mary M. Peacock, Ph.D.
Advisor

Peter J. Weisberg, Ph.D.
Co-advisor

Elizabeth A. Leger, Ph.D.
Committee Member

Thomas L. Parchman Ph.D.
Committee Member

Ana de Bettencourt Dias, Ph.D.
Graduate School Representative

David W. Zeh, Ph.D., Dean
Graduate School

May, 2021

ABSTRACT

Ecosystems often contain a few cosmopolitan species and a large number of rare species. Despite their relative low abundance and biomass, rare species support the multifunctionality and resilience of ecosystems. Therefore, empirical studies on rare and range-restricted species can increase our understanding of eco-evolutionary underpinnings of species and ecosystem persistence, and generate sufficient knowledge to design effective conservation programs. These research studies can also benefit conservation programs for rare and range-restricted species, which are often prioritized. This research focuses on *Ivesia webberi*, a federally threatened perennial forb and the vegetative communities that harbor the species. Specifically, empirical studies investigated the following: (1) species-environment relationship of *I. webberi* using iterative and multi-year ecological niche modeling with complementary model-guided sampling, to describe and predict suitable habitats; (2) the relationship between soil seed bank and aboveground vegetation in plant communities where *I. webberi* is found, to understand the regeneration niche of *I. webberi* and assess ecological resilience of the vegetative communities; (3) genetic diversity, structure, and functional connectivity among *I. webberi* populations in order to characterize genetic resources and therefore evolutionary potential; (4) the relationships between genome size variation and bioclimatic variables within *I. webberi* and among *Ivesia* taxa; and (5) seed viability of *I. webberi*, including spatiotemporal variability and storage behavior.

Findings from the 5-year iterative niche modeling study resulted in the discovery of seven novel populations, an expansion of the known species distribution range, and identification of important environmental drivers of the ecological niche of *I. webberi*.

Native species richness was higher in aboveground vegetation in the sampled sites where *I. webberi* occurs while the soil seed bank is dominated by invasive annual grasses. This resulted in low floristic similarity between the aboveground vegetation and the soil seed bank, and highlights the importance of seeding with native plants and control of invasive plant species to maintain the ecological legacies of these sites in the Great Basin Desert. Genetic diversity is relatively low across *I. webberi* populations and exhibited significant spatial genetic structure; functional connectivity was influenced by synergistic effects of geographic distance and landscape features. However, *I. webberi* exhibits a significant temporal, not geographical, variation in seed viability, and seed viability potentially reduces with storage time suggesting a recalcitrant behavior. Seed viability can be reliably estimated and monitored using non-destructive x-ray imagery and multispectral imaging techniques.

An 8-fold variation in genome size of 31 *Ivesia* taxa was observed, ranging from 0.73 pg/2C in *I. baileyi* var. *beneolens* to 5.91 pg/2C in *I. lycopodioides* ssp. *megalopetala*. This genome size variation significantly correlated with actual evapotranspiration and seed size. Inference from genome size suggest that all sampled *Ivesia* are diploid with 28 chromosomes. Similar significant correlations between intraspecific genome size variation in *I. webberi* and evapotranspiration and seed size were observed; genome size was larger in *I. webberi* populations closer to the species' range center and smaller towards the margin. Relatively small genome sizes and their correlations with functional trait and energy availability indicate that genome size has adaptive significance for these desert-adapted species. Overall, the findings of these studies have advanced scientific knowledge on the eco-evolutionary processes in a range-restricted plant species in the Great Basin Desert, and provide useful information to design effective conservation programs.

ACKNOWLEDGEMENTS

This dissertation is dedicated to my parents, Pastor Ayodele Borokini and Dr. (Mrs.) Olufunke Borokini, who are constantly on their knees praying for my career success and for their unflinching support and encouragement. I am grateful for the immense support from my wife, Sylvia Borokini, and my daughter, Victoria. I count myself very fortunate to have Dr. Mary Peacock and Dr. Peter Weisberg as my advisors. Their experience, expertise, mentoring, and guidance is unparalleled. I am also grateful to the rest of my academic committee – Dr. Elizabeth Leger, Dr. Ana de Bettencourt Dias, and Dr. Thomas Parchman for their contribution and encouragement throughout the journey. My work has benefited immensely from the support and advice of alumni and faculty in the University of Nevada, Reno (UNR) community - Dr. Julie Allen, Dr. Kevin Shoemaker, Dr. Ken Nussear, Dr. Scott Bassett, Dr. Kelly Klingler, Dr. Sarah Barga, Dr. Phillip Street, Dr. Jane Dell, Dr. Frank Amankonah, Dr. Jacob Francis, Dr. Alfred Schissler, and Mr. Tom Dilts. I am also grateful to my peers in the Ecology, Evolution and Conservation Biology program including Joshua Hallas, Adriana Parra, James Simmons, and Anjana Parandhaman, as well as Charles Edwards of the UNR Social Psychology graduate program, and Gary Sterle of the Hydrology graduate program. I cannot forget other scholars beyond the University who have contributed to data analysis aspects of my research, including Dr. Pierre Legendre, Dr. Town Peterson, Dr. William Peterman, Dr. Javan Bauder, Dr. Scott Buchanan, Dr. Andrew Shirk, Dr. James Birchler, Dr. Georges Damien, and Dr. Samuel Cushman.

I am also grateful to several local botanists, citizen scientists, and conservation managers who contributed to my field surveys, collections, collection permits, and other expert advice. This include, but not limited to Kathryn Birgy (UNLV Herbarium), Elizabeth Scott (Portland State University), Tianxiao Hao (University of Melbourne, Australia), Charlene Duncan, and Arnold Tiehm (UNR), Sarah Kulpa (U.S. Fish and Wildlife Service, Reno Field Office), Valda Lockie (Bureau of Land Management, Susanville CA Office), Janel Johnson and Dr. James Morefield (Nevada Natural Heritage Program), Prof. Tina Ayers (Northern Arizona University), Dr. Duncan Bell and Dr. Naomi Fraga (Rancho Santa Ana Botanical Garden), Dr. Jennifer Wilkening (USFWS Southern Nevada office), Dr. Jeanette Perry and Dr. Patricia Hardesty (Nevada National Security Site), John Christopherson, Gary Reese, and Cayenne Engel (Nevada Department of Forestry), Jordan Brown (Oregon Native Plant Conservation Program, and Department of Botany, Oregon State University), Lindsey Wise (Oregon Biodiversity Information Center), Michael Hagebusch, John Dittes, Dr. Margaret Widdowson, Derek Antonelli, Greg Gust, Tara de Queiroz, and Maura Olivos.

The dissertation research was funded through a grant from the U.S. Fish and Wildlife Service, with additional support from the following research grants – UNR EECB summer stipends, UNR Biology Department, California Native Plant Society (CNPS) San Diego chapter mini-grant, CNPS Stebbins fund, Native Plant Society of Oregon field research grant, Utah Native Plant Society grant-in-aid program, CNPS Shasta chapter Oak tree grant, Arizona Native Plant Society Doug Green research grant, and Idaho Native Plant Society education, research and inventory grant, Northern California Botanists (NCB) Botany research scholarship program, the Nevada Native

Plant Society Margaret Williams research grant, and the Society for Conservation Biology (SCB) graduate student research fellowship.

TABLE OF CONTENTS

List of Tables

List of Figures

INTRODUCTION

Israel T. Borokini

Program in Ecology, Evolution and Conservation Biology, Department of Biology, University of Nevada, Reno

Life on earth is supported by plant species and vegetation provides the foundation for all terrestrial ecosystems. Therefore, local and global loss of plant diversity will severely impact other groups of species and human well-being (Díaz et al., 2007; Brummitt et al., 2015a). About 600 plant species have gone extinct (Humphreys et al., 2019; Lughadha et al., 2020) and one-third of all angiosperms are threatened (Brummitt et al., 2015b; Pimm and Joppa, 2015; Corlett, 2016). Global assessment of the International Union of Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2020) as well as national biodiversity risk assessments in Canada and the United States show that habitat loss and fragmentation are the biggest threat to terrestrial plant species (Wilcove et al., 1998; Venter et al., 2006; Venter et al., 2016; Tilman et al., 2017; Díaz et al., 2019; Figueiredo et al., 2019; Lughadha et al., 2020). Habitat loss and fragmentation are attributed to growth in human population and economic development (Losfeld et al., 2015; Tilman et al., 2017), with reports showing that over 50% of terrestrial ecosystems have been modified by human activities (Millennium Ecosystem Assessment, 2005; Lander et al., 2019). Furthermore, deforestation, illegal trade and overharvest make overexploitation the second biggest threat to biodiversity (Goettsch et al., 2015; Food and Agriculture Organization [FAO], 2016). Invasive species are linked with local extirpation and reduction of native plant diversity, as well as alterations in wildfire regimes and soil nutrients (Pyšek et al., 2012; Thomas and Palmer, 2015). Despite an increase of 1 °C in global average temperature (IPCC, 2014), empirical support for anthropogenic climate change driven plant extinction is limited (Le Roux et al., 2019; Lughadha et al., 2020), but climate change may impact plants synergistically with other drivers of biodiversity loss (Oliver and Morecroft, 2014; Bidartondo et al., 2018). Moreover, studies have shown shifts in plant species phenology, community composition, and species ranges to higher latitudes and elevation (Hijioka et al., 2014; Morueta-Holme et al., 2015; Panetta et al., 2018; Diez et al., 2020), in addition to predicted future species range contraction and increased wildfires (Warren et al., 2018; Gomes et al., 2019).

Habitat loss and fragmentation reduce the population habitat area, create edge effects, and increase interactions with the surrounding landscape matrix, resulting in variations in species richness and abundance in the fragmented habitats (Fontúrbel and Murúa, 2014; Aguilar et al., 2019). Furthermore, habitat fragmentation alters among-population gene flow patterns (Rymer et al., 2015), impacts plant reproduction and reduces seed dispersal (Cheptou et al., 2017; Lander et al., 2019), and potentially reduces available resources (Herrera et al., 2011; Fontúrbel and Murúa, 2014). Additionally, landscape alterations resulting in demographic stochasticity can severely impact plant-animal interactions, especially obligate pollination and dispersal mutualisms (Ollerton et al., 2011; Rossetti et al., 2017). Reduced gene flow due to habitat fragmentation can result in spatial genetic structure, inbreeding and genetic drift, which can cause reduced neutral and adaptive genetic diversity (Aguilar et al., 2008; Lander et al., 2019). Inbreeding depression in plant populations can reduce seed set and seedling survival, ultimately resulting in reduced population viability and persistence (Byrne et al. 2007; Brudvig et al., 2015).

Biogeography theory predicts that when faced with climate changes, plant species can acclimate (through physiological adjustment and phenological plasticity), adapt (by directional selection and local adaption), move to new sites with suitable conditions, or go extinct (Morueta-Holme et al., 2015; Corlett, 2016; Panetta et al., 2018). In support of these predictions, studies have demonstrated the impacts of anthropogenic climate change resulting in shifts in plant phenology, species range, genetic diversity, physiology and behavior (Franks et al., 2014; Lenoir and Svenning; 2015; Thackeray et al., 2016; Yu et al., 2017). Migration rates and dispersal capacity for many plant species are inherently low (Aitken et al., 2008), which is further inhibited by natural and anthropogenic barriers to movement (Mills and Schwartz 2005; Partel et al., 2005) and the decline in the abundance of animal-dispersers due to overexploitation or habitat modifications (Harrison et al., 2013). Different plant species in a vegetative community may respond to climate change differently resulting in phenological mismatches that can impact species interactions and coevolutionary processes, leading to novel plant communities (Enquist et al., 2014; Morellato et al., 2016). Further, climate change may favor early regeneration of many invasive plant species, giving them access to environmental resources and facilitating their colonization, a concept known as priority effect (Zhang et al., 2011; Alexander and Levine, 2019; Grainger et al., 2019). This gives many invasive species competitive advantage over native seedlings; thus, they reduce recruitments into native populations and become ecosystem engineers (Porté et al., 2011; Dickson et al., 2012).

Generalists and widely distributed plant species may adapt quickly to environmental changes due to their wide climatic niche breadth, reduced dependence on specific ecological interactions, and greater ability for phenological and physiological shifts (Sheth and Angert, 2014; Yu et al., 2017). Conversely, many range-restricted species and species with small and isolated populations are more prone to stochastic demographic and environmental processes, inbreeding depression and random genetic drift (Reed, 2004; Harnik et al., 2012). Many studies have established the strong relationships between geographic range size and climatic niche breadth, and predict greater extinction risks for range-restricted species under climate change (Cardillo et al., 2019; Vincent et al., 2020). Roughly 36% of the known 435,000 plant species are endemic to a small region (Enquist et al., 2019), while over 62% of the North American flora are range-restricted (Mills and Schwartz, 2005). Further, the western United States have a disproportionately high species richness of endemic flora (Mills and Schwartz, 2005; Kraft et al., 2010) and endemic lineages of evolutionary significance (Thornhill et al., 2017; Mishler et al., 2020), including 151 endemic plants in the Great Basin Desert (Ricketts et al., 1999; Nachlinger et al., 2001). Many hypotheses have been proposed to explain differences in the geographic range sizes among plant species, focusing on climatic history (Enquist et al., 2019), geological history (Jansson, 2003), evolutionary history (Gaston, 1996), dispersal and colonization ability (Lowry and Lester, 2006), ecological specializations (Wamelink et al., 2014; Cardillo et al., 2019), environmental heterogeneity (Baldwin 2005; Kraft et al., 2010), and niche breadth (Gaston and Spicer, 2001), among others. The richness of rare and endemic flora in the Great Basin Desert and the adjacent California Floristic Province has been attributed to the region’s particular geological history, topographic complexity, and significant microclimatic gradients (Lesica et al., 2006; Cassel et al., 2009; Kraft et al., 2010).

In light of the current and predicted future environmental pressures on biodiversity, it is important to investigate the effect of contemporary environmental changes (that is, habitat loss) on threatened plant species (Montoya et al., 2010; Rybicki et al., 2020). Equally important are empirical investigations that assess the capacity of populations and species to respond to future changes (e.g., Segan et al., 2016; Razgour et al., 2019). Such studies could involve comparative approaches to assess the response of congenerics sharing the same evolutionary history, but varying climatic niche breadth and geographical distributions (e.g., Sáyago et al., 2018; Fortunel et al., 2020). Furthermore, such studies can be expanded to evaluate the ecological resilience of ecosystems to habitat fragmentation, biological invasion, and climate change (e.g., Rogora et al., 2018; Angeler et al., 2019). Such studies will advance scientific understanding to identify important functional traits that can ensure the persistence of species and multifunctionality of ecosystems under several environmental pressures. Findings from such studies can also inform the design of effective conservation programs for threatened species.

The Great Basin Desert is a cold desert receiving most of its annual precipitation in the winter (Comstock and Ehleringer, 1992). Most of the pre-European settlement changes in vegetation cover were attributed to historical climate change (Grayson, 2011), but human activities in the last 150 years have resulted in rapid land cover changes that impacted wildfire regimes and facilitated colonization by invasive and nonnative annual grasses (Wisdom et al., 2005; Morris and Rowe, 2014). Furthermore, a 0.7 to 1.4 °C increase in temperature has been reported for the Great Basin Desert (Wagner, 2003; Snyder et al., 2019), which may be associated with the decline in snowpack (Mote et al., 2005), early arrival of spring season, and dramatic interannual variation in precipitation (Baldwin et al., 2003; Chambers, 2008). A 2 to 5 °C warming is predicted for the region over the next 100 years, which may increase the colonization and invasion success of the

C_4 invasive grasses and further impact wildfire regimes in the Great Basin Desert (Smith et al., 2000; Cubashi et al., 2001; Westerling et al., 2006; Chambers, 2008). For these reasons, the *Artemisia* spp. (sagebrush) ecosystem of the Great Basin Desert is one of the most critically endangered habitats in the United States (Noss et al., 1995; Stein et al., 2000), with over 600 native plants considered species of conservation concern (Nachlinger et al., 2001).

Ivesia webberi A. Gray (Webber's ivesia or wire mousetail) is one of the native plant species of conservation concern in the Great Basin Desert. The perennial forb, belonging to the Rosaceae family, grows on shallow rocky clay soils in sparsely vegetated low sagebrush (*Artemisia arbuscula*) communities in mid-elevation areas, near the northern Sierra Nevada eastern foothills and the western fringes of the Great Basin Desert (Witham, 2000; US Fish and Wildlife Service [USFWS], 2014). Throughout its range, the species is threatened by invasive non-native plant species, including *Bromus tectorum* (cheatgrass), *Taeniatherum caput-medusae* (medusa head) and *Poa bulbosa* (bulbous bluegrass), which have been observed in 12 *I. webberi* sites (USFWS, 2014). Most of the known locations for this species experienced wildfires over the past 40 years, and 11 *I. webberi* sites are adjacent to and/or intersected by road development and off-highway vehicle (OHV) use (USFWS 2014). The main concern, however, is the increased economic development and urbanization in Northern Nevada, which threatens many of the adjacent *I. webberi* locations.

Identification of species-specific threats remains critical to conservation efforts (Visconti et al., 2016), especially for range-restricted and threatened species that are already vulnerable to genetic, environmental, and demographic stochasticity (Schwartz et al., 2006). An empirical assessment of how species respond to environmental changes will advance scientific knowledge on species-environment relationship and provide adequate information required for effective conservation of susceptible populations and species (Pacifici et al., 2015; Yu et al., 2017). Despite conservation research and prioritization on rare species (Myers et al. 2000, Sadler and Bradfield, 2010), conservation is challenging due to the lack of knowledge on biology, reproduction and distribution for many of these species (Smart et al., 2015). This is particularly true of *I. webberi*. Although it is listed on the U.S. Endangered Species Act (e.g., Federal Register 2014a, 2014b), the development of strategic management plans for this species is impeded by a lack of basic knowledge on reproductive biology, population viability, regeneration niche, genetic diversity and structure, pollinators and dispersers, and species-environment relationships.

The federal listing of the species as “threatened” has facilitated protection of its most vulnerable habitat patches by fencing. However, it is not enough to protect the habitats of imperiled species, especially those with small populations, such as observed in *I. webberi*. Conservation actions must include empirical studies and management to optimize gene flow and maintain genetic diversity among and within the populations. *I. webberi* produces dry, indehiscent achenes, which are not adapted for long-range dispersal. Furthermore, dispersal is suspected to be impeded by the north-south mountain ranges and increasing urbanization, therefore *I. webberi* response to future environmental change may be limited to phenological shifts, local adaptation, or extirpation (Berg et al., 2010). Empirical studies have shown that the persistence of plant populations under rapid climate change could be achieved with phenotypic plasticity (Vitasse et al., 2010; Nicotra et al., 2010). However, studies have also demonstrated a positive relationship between genetic diversity and phenotypic plasticity in plant populations in response to changing environments (van Kleunen and Fischer, 2005; Gratani, 2014). Therefore, the evolutionary potential of plant populations to adapt to environmental changes is dependent on their genetic diversity.

Species niches and distributions are determined by species interactions, dispersal capacity, and environmental requirements (Costa et al., 2018). Ecological theory predicts unimodal species-environment relationships whereby niches are constrained at the extremes of environmental gradients (Austin, 1999; Costa et al., 2018). Therefore, species distribution models (SDM) are used to investigate species-environment relationships, quantify niche breadth, and predict rarity, extinction risks, and species responses to future environmental changes (Kotiaho et al., 2005; Broennimann et al., 2006; Jiménez-Valverde et al., 2011). However, SDMs are fitted with the assumption that the species range is known and all biologically relevant environmental variables were included (Wisz et al., 2008; Elith and Leathwick, 2009). This is often not the case with range-restricted species that may not be in equilibrium with their environment, whose occurrence records are based on geographically biased sampling, and which are less well studied and hence whose biologically relevant environmental conditions are at best assumed (Lomba et al., 2010; Jarnevich et al., 2015). Predictions from poorly-fitted models can misinform management decisions for rare species (Ramesh et al., 2020). Species distribution models were fitted for *Ivesia webberi* in chapter 1, using an iterative and multiyear modeling approach, accompanied by model-guided field surveys to the predicted suitable sites for field validation. The iterative niche modeling approach and field surveys generate additional novel presence and absence points that can refine scientific understanding of the species-environment relationships and improve the predictive ability of the ecological niche models.

Land use changes caused by anthropogenic habitat fragmentation remains the greatest threat to global biodiversity (Danneyrolles et al., 2019; Lughadha et al., 2020), and in particular to biodiversity in the Great Basin Desert (Morris and Rowe, 2014). The local and global impacts of habitat loss and fragmentation on biodiversity are severe to the extent that many conservation programs now focus on ecological restoration to reverse the biodiversity loss (Haapalehto et al., 2017). Therefore, it is not surprising that 2021-2030 was dubbed the “UN Decade on Ecosystem Restoration” (United Nations Environment Agency, 2019). However, effective ecological restoration of degraded and disturbed landscapes to historical legacies partly depends on the ecological resilience of the ecosystem, which in turn, relies on the preservation and germination of the soil seed bank of native plant communities, post-disturbance (Korb et al., 2005; Ma et al., 2019; Mndela et al., 2020). The Great Basin Desert plant communities are dominated by perennial species (Allen and Nowak, 2008; Martyn et al., 2016), many of which are lithophytes, chasmophytes and long-lived plants that undergo clonal reproduction or have a mixed strategy of seed and vegetative reproduction, have low and infrequent seed production, poor dispersal mechanism, and have seeds that form a transient seed bank (Goodwillie et al., 2005; Pekas and Schupp, 2013). Therefore, the focus of chapter 2 was on the quantification of the soil seed bank and the relationship with aboveground vegetation in the sites that harbor *I. webberi*. Such information can contribute to an understanding of the regeneration niche of *I. webberi* and predict secondary succession events in the native plant communities, post-disturbance. This is particularly important for *I. webberi* that has been observed to regenerate both from dormant vegetative structures and seed germination.

Chapter 3 assesses the neutral genetic diversity and gene flow rates among the known populations of *I. webberi* using Euclidean distance, environmental variables, land cover, and the predicted habitat suitability maps, to test for isolation by distance, by environment, and by landscape resistance. This allows us to evaluate spatial genetic structure in *I. webberi*, from which we can make inference on the effect of habitat fragmentation and disturbances on gene flow and species' capacity to adapt to future environmental changes. Additionally, inferences can be drawn on the mating system exhibited by this species. Field observations indicate that *I. webberi* primarily regenerates annually from dormant underground vegetative structures, but seed regeneration has also been observed. Vegetative reproduction may support population persistence under habitat fragmentation, disturbance, and climate change (Bellingham, 2000; Lander et al., 2019), but pollination and seed-based regeneration are necessary to maintain genetic diversity, dispersal, and establishment in new sites (Lander et al., 2019). However, previous studies show that species with a long history of self-compatibility or mixed mating strategies can persist in fragmented habitats because detrimental recessive alleles and products of deleterious mutations can be purged from the populations (Husband and Schemske, 1996; Aguilar et al., 2019).

Identifying the functional traits related to phenotypic plasticity and niche breadths can be used to assess plants' response to environmental change (Berg et al., 2010; Gratani, 2014; Costa et al., 2018). Genome size, also known by nuclear DNA content and C-value, is the total amount of DNA in an unreplicated haploid nucleus of an organism (Greilhuber et al., 2005). Previous studies have reported positive correlations between genome size and nucleus weight, RNA and protein content, cell size, flower and fruit size, and minimum generation time in herbaceous plants (Bennett, 1987). Additional empirical evidence shows a positive correlation between genome size and seed size (Chung et al., 1998; Beaulieu et al., 2007), guard cell and epidermal cell sizes (Beaulieu et al., 2008; Hoang et al., 2019), leaf length and width (Chung et al., 1998), and mitotic cell cycle duration (Francis et al., 2008; Hodgson et al., 2010). The correlation of genome size with these physiological plant traits suggests that genome size may have adaptive significance by influencing the life history strategy, plant distribution and phenology of plants (Knight and Ackerly, 2002; Hodgson et al., 2010; Dušková et al., 2010). Within a species, genome size variations have also been reported and attributed to differing selection and evolutionary processes that occur in the genetically isolated populations (Šmarda and Bureš, 2010; Bilinski et al., 2018). Therefore, chapter 4 investigates the adaptive significance of genome size variations among 31 *Ivesia* taxa and 11 *I. webberi* locations.

Post-disturbance restoration projects of plant communities in the Great Basin Desert rely on accurate knowledge of species regeneration biology, including seed viability and storage behavior. Moreover, *ex situ* conservation, via seed banking and other methods, is recommended as a management strategy for nationally or globally threatened plant species under the Global strategy for plant conservation (GSPC) of the United Nations' Convention on Biological Diversity (CBD) (CBD, 2019). However, there is limited empirical knowledge on the seed storage behavior for most of the threatened plant species, the focus of the GSPC (Hay and Probert, 2013; Wyse and Dickie, 2017). The seeds of many plant species can be dried and stored for longer periods of time without losing their viability, but others lose their seed viability with storage time and desiccation, hence they are not suitable for seed banking (Roberts, 1973; Hay and Probert, 2013). Therefore, chapter 5 of this dissertation focuses on investigating the storage behavior of *I. webberi* seeds, identifying populations that produce seeds with higher viability, interannual variations in seed viability and the potentials of discriminating between viable and nonviable seeds using non-destructive seed testing methods. These investigations will inform the decisions on populations to select for seed harvesting, strategies to ensure effective seed banking, and approaches for reducing the cumulative loss of stored seeds from periodic seed viability monitoring.

These studies have conservation applications for the management of *Ivesia webberi* and the ecosystem that harbors it. The gene flow patterns, genetic diversity and structure in *I. webberi* populations can help managers assess how the species is responding to the effects of current anthropogenic and natural landscape features on gene flow, and how the species would respond to future environmental changes. Given lack of species-specific empirical studies on many Great Basin Desert plant species, findings from this study can be extrapolated to other threatened and range-restricted plant species with niche breadth, mating systems, and functional traits similar to *I. webberi*. Furthermore, the iterative niche modeling approach explained in this research can be applied to other rare and native plants with few occurrence records. Model-guided vegetative sampling can lead to the discovery of new locations and an expansion of their geographic range. A characterization of the soil seed bank in the mid-elevation sites where *I. webberi* is located and its relationship with the aboveground vegetation can help managers predict future secondary succession in these sites and design ecological restoration programs that can decimate the populations of the invasive plant species and facilitate the recovery of the resident native flora. Moreover, post-disturbance ecological restoration programs in the Great Basin Desert such as native plant seeding and the *ex-situ* conservation can benefit immensely from an understanding of the seed storage behavior, while seed banking practices can be modified to use nondestructive methods for seed viability testing.

REFERENCES

Aguilar R., Cristóbal-Pérez E.J., Balvino-Olvera F.J., Aguilar-Aguilar M.J., Aguirre-Acosta N., Ashworth L., Lobo J.A., Martén-Rodríguez S., Fuchs E.J., Sanchez-Montoya G., Bernardello G., and Quesada M. (2019). Habitat fragmentation reduces plant progeny quality: a global synthesis. *Ecology Letters* 22(7): 1163-1173. https://doi.org/10.1111/ele.13272

Aguilar R., Quesada M., Ashworth L., Herrerias-Diego Y., and Lobo J. (2008). Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Molecular Ecology* 17(24): 5177–5188. https://doi.org/10.1111/j.1365-294X.2008.03971.x

Aitken S.N., Yeaman S., Holliday J.A., Wang T., and Curtis-McLane S. (2008). Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* 1(1): 95-111. https://doi.org/10.1111/j.1752-4571.2007.00013.x

Alexander J.M., and Levine J.M. (2019). Earlier phenology of a nonnative plant increases impacts on native competitors. *Proceedings of the National Academy of Science of the United States of America* 116(13): 6199-6204. https://doi.org/10.1073/pnas.1820569116

Allen E.A., and Nowak R.S. (2008). Effect of pinyon-juniper tree cover on the soil seed bank. *Rangeland Ecology and Management* 61(1): 63–73. https://doi.org/10.2111/07-007R2.1

Angeler D.G., Fried-Petersen H., Allen C.R., Garmestani A., Twidwell D., Birgé H.E., Chuang W., Donovan V.M., Eason T., Roberts C.P., Sundstrom S.M., and Wonkka C.L. (2019). Adaptive capacity in ecosystems. *Advances in Ecological Research* 60: 1–24. https://doi.org/10.1016/bs.aecr.2019.02.001

Austin, M. P. (1999). The potential contribution of vegetation ecology to biodiversity research. *Ecography* 22(5): 465– 484. https://doi.org/10.1111/j.1600-0587.1999.tb01276.x

Baldwin B.G. (2005). Origin of the serpentine-endemic herb *Layia discoidea* from the widespread *L. glandulosa* (Compositae). *Evolution* 59(11): 2473–2479. https://doi.org/10.1554/05-147.1

Baldwin C.K., Wagner F.H., and Lall U. (2003). Water Resources. In: Wagner F.H. (ed.), *Rocky Mountain/Great Basin Regional Climate-Change Assessment*. Report of the U.S. Global Change Research Program. Utah State University, Logan, UT: IV, pp 79-112.

Beaulieu J.M., Leitch I.J., Patel S., Pendharkar A., and Knight C.A. (2008). Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist* 179(4): 975–986. https://doi.org/10.1111/j.1469-8137.2008.02528.x

Beaulieu J.M., Moles A.T., Leitch I.J., Bennett M.D., Dickie J.B., and Knight C.A. (2007). Correlated evolution of genome size and seed mass. *New Phytologist* 173(2): 422–437. https://doi.org/10.1111/j.1469-8137.2006.01919.x

Bellingham P.J. (2000). Resprouting as a life history strategy in woody plant communities. *Oikos* 89(2): 409–416. https://doi.org/10.1034/j.1600-0706.2000.890224.x

Bennett M.D. (1987). Variation in genomic form in plants and its ecological implications. *New Phytologist* 106: 177-200. https://doi.org/10.1111/j.1469-8137.1987.tb04689.x

Berg M.P., Kiers E.T., Driessen G., Van Der Heijden M. Kooi B.W., Kuenen F., Liefting M., Verhoef H.A., and Ellers J. (2010). Adapt or disperse: understanding species persistence in a changing world. *Global Change Biology* 16(2): 587-598. https://doi.org/10.1111/j.1365-2486.2009.02014.x

Bidartondo M., Ellis C., Kauserud H., Kennedy P., Lilleskov E., Suz L., and Andrew C. (2018). Climate change: Fungal responses and effects. In K. J. Willis (Ed.), *State of the World's Fungi*, Richmond: Royal Botanic Gardens, Kew, pp. 62– 69.

Bilinski P., Albert P.S., Berg J.J., Birchler J.A., Grote M.N., Lorant A., Quezada J., Swarts K., Yang J., Ross-Ibarra J. (2018). Parallel altitudinal clines reveal trends in adaptive evolution of genome size in *Zea mays*. *PLoS Genetics* 14: e1007162. https://doi.org/10.1371/journal.pgen.1007162

Broennimann O., Thuiller W., Hughes G., Midgley G.F., Alkemade J.M.R., and Guisan A. (2006). Do geographic distribution, niche property and life form explain plants' vulnerability to global change? *Global Change Biology* 12(6): 1079-1093. https://doi.org/10.1111/j.1365-2486.2006.01157.x

Brudvig L.A., Damschen E.I., Haddad N.M., Levey D.J., and Tewksbury J.J. (2015). The influence of habitat fragmentation on multiple plant–animal interactions and plant reproduction. *Ecology* 96(10): 2669–2678. https://doi.org/10.1890/14-2275.1

Brummitt N., Bachman S.P., Aletrari E., Chadburn H., Griffiths-Lee J., Lutz M., Moat J., Rivers M.C., Syfert M.M., and Nic Lughadha E.M. (2015b). The Sampled Red List Index for Plants, phase II: Ground-truthing specimen-based conservation assessments. *Philosophical Transactions of the Royal Society B: Biological Sciences* 370(1662): 20140015. https://doi.org/10.1098/rstb.2014.0015

Brummitt N., Bachman S.P., Griffiths-Lee J., Lutz M., Moat J.F., Farjon A., Donaldson J.S., Hilton-Taylor C., Meagher T.R., Albuquerque S., Aletrari E., Andrews A.K., Atchison G., Baloch E., Barlozzini B., Brunazzi A., Carretero J., Celesti M., Chadburn H., Cianfoni E., Cockel C., Coldwell V., Concetti B., Contu S., Crook V., Dyson P., Gardiner L., Ghanim N., Greene H., Groom A., Harker R., Hopkins D., Khela S., Lakeman-Fraser P., Lindon H., Lockwood H., Loftus C., Lombrici D., Lopez-Poveda L., Lyon J., Malcolm-Tompkins P., McGregor K., Moreno L., Murray L., Nazar K., Power E., Tuijtelaars M.Q., Salter R., Segrott R., Thacker

H., Thomas L.J., Tingvoll S., Watkinson G., Wojtaszekova K., and Nic Lughadha E.M. (2015a). Green plants in the red: A baseline global assessment for the IUCN sampled red list index for plants. *PLoS ONE* 10(8): e0135152. https://doi.org/10.1371/journal.pone.0135152

Byrne M., Elliot C.P., Yates C., and Coates D.J. (2007). Extensive pollen dispersal in a bird-pollinated shrub, *Calothamnus quadrifidus*, in a fragmented landscape. *Molecular Ecology* 16(6): 1303–1314. https://doi.org/10.1111/j.1365-294X.2006.03204.x

Cardillo M., Dinnage R., and McAlister W. (2019). The relationship between environmental niche breadth and geographic range size across plant species. *Journal of Biogeography* 46(1): 97-109. https://doi.org/10.1111/jbi.13477

Cassel E.J., Calvert A.T., and Graham S.A. (2009). Age, geochemical composition, and distribution of Oligocene ignimbrites in the northern Sierra Nevada, California: implications for landscape morphology, elevation, and drainage divide geography of the Nevadaplano. *International Geology Review* 51(7-8): 723–742. https://doi.org/10.1080/00206810902880370

Chambers J.C. (2008). Climate Change and the Great Basin. In: Chambers J.C., Devoe N., and Evenden A. (eds.), *Collaborative management and research in the Great Basin - examining the issues and developing a framework for action*. Gen. Tech. Rep. RMRS-GTR-204. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. p. 29-32

Cheptou P.-O., Hargreaves A., Bonte D., and Jacquemyn H. (2017). Adaptation to fragmentation: evolutionary dynamics driven by human influences. *Philosophical Transactions of the Royal Society B Biological Sciences* 372(1712): 20160037. https://doi.org/10.1098/rstb.2016.0037

Chung J., Lee J.-H., Arumuganathan K., Graef G.L., and Specht J.E. (1998). Relationships between nuclear DNA content and seed and leaf size in soybean. *Theoretical and Applied Genetics* 96: 1064-1068. https://doi.org/10.1007/s001220050840

Comstock J.P., and Ehleringer J.R. (1992). Plant adaptations in the Great Basin and Colorado Plateau. *Great Basin Naturalist* 52(3): 195–215

Convention on Biological Diversity [CBD] (2019) Global strategy for plant conservation: targets 2011–2020 [Internet]. [Cited 30 Dec 2019]. Available from: http://www.cbd.int/gspc/targets.shtml

Corlett R.T. (2016). Plant diversity in a changing world: Status, trends, and conservation needs. *Plant Diversity* 38(1): 10–16. https://doi.org/10.1016/j.pld.2016.01.001

Costa D.S., Gerschlauer F., Kiese R., Fischer M., Kleyer M., and Hemp A. (2018). Plant niche breadths along environmental gradients and their relationship to plant functional traits. *Diversity and Distributions* 24(12): 1869-1882. https://doi.org/10.1111/ddi.12815

Cubashi U., Meehl G.A., and Boer G.J. (2001). Projections of future climate change. In: Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Winden PJ and Dai X (eds.), *Climate Change 2001: The Scientific Basis. Contribution of the Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC)*. Cambridge University Press, pp 525-582.

Danneyrolles V., Dupuis S., Fortin G., Leroyer M., de Römer A., Terrail R., Vellend M., Boucher Y., Laflamme J., Bergeron Y., and Arseneault D. (2019). Stronger influence of anthropogenic disturbance than climate change on century-scale compositional changes in northern forests. *Nature Communications* 10: 1265. https://doi.org/10.1038/s41467-019-09265-z

Díaz S., Fargione J., Chapin F.S. III, and Tilman D. (2007). Biodiversity loss threatens human well-being. *PLoS Biology* 4(8): e277. https://doi.org/10.1371/journal.pbio.0040277

Díaz S., Settele J., Brondízio E., Ngo H.T., Guèze M., Agard J., and Zayas C. (2019). *Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Bonn, Germany*. https://ipbes.net/global-assessment-report-biodiversity-ecosystem-services

Dickson T.L., Hopwood J.L., and Wilsey B.J. (2012). Do priority effects benefit invasive plants more than native plants? An experiment with six grassland species. *Biological Invasions* 14: 2617–2624. https://doi.org/10.1007/s10530-012-0257-2

Diez J., Kauserud H., Andrew C., Heegaard E., Krisai-Greilhuber I., Senn-Irlet B., Høiland K., Egli S., and Büntgen U. (2020). Altitudinal upwards shifts in fungal fruiting in the Alps. *Proceedings of the Royal Society B: Biological Sciences* 287(1919): 20192348. https://doi.org/10.1098/rspb.2019.2348

Dušková E., Kolář F., Sklenář P., Rauchová J., Kubešová M., Fer T., Suda J., and Marhold K. (2010). Genome size correlates with growth form, habitat and phylogeny in the Andean genus *Lasiocephalus* (*Asteraceae*). *Preslia* 82: 127-148

Elith J., and Leathwick J.R. (2009). Species Distribution Models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics* 40: 677-697. https://doi.org/10.1146/annurev.ecolsys.110308.120159

Enquist B.J., Feng X., Boyle B., Maitner B., Newman E.A., Jørgensen P.M., Roehrdanz P.R., Thiers B.M., Burger J.R., Corlett R.T., Couvreur T.L.P., Dauby G., Donoghue J.C., Foden W., Lovett J.C., Marquet P.A., Merow C., Midgley G., Morueta-Holme N., Neves D.M., Oliveira-Filho A.T., Kraft N.J.B., Park D.S., Peet R.K., Pillet M., Serra-Diaz J.M., Sandel B., Schildhauer M., Šímová I., Violle C., Wieringa J.J., Wiser S.K., Hannah L., Svenning J.-C., and McGill B.J.

(2019). The commonness of rarity: Global and future distribution of rarity across land plants. *Science Advances* 5(11): EAAZ0414. doi: 10.1126/sciadv.aaz0414

Enquist C.A.F., Kellermann J.L., Gerst K.L., and Miller-Rushing A.J. (2014). Phenology research for natural resource management in the United States. *International Journal of Biometeorology* 58: 579–589. https://doi.org/10.1007/s00484-013-0772-6

Federal Register (2014a). Endangered and Threatened Wildlife and Plants; Threatened Species Status for *Ivesia webberi*; Final Rule. 50 CFR Part 17. Volume 79 (106): 31878 – 31883

Federal Register (2014b). Endangered and Threatened Wildlife and Plants; Designation of critical habitat for *Ivesia webberi*; Final Rule. 50 CFR Part 17. Volume 79 (106): 32125 – 32155

Figueiredo L., Krauss J., Steffan-Dewenter I., and Sarmento Cabral J. (2019). Understanding extinction debts: Spatio–temporal scales, mechanisms and a roadmap for future research. *Ecography* 42(12): 1973–1990. https://doi.org/10.1111/ecog.04740

Fontúrbel F.E., and Murúa M.M. (2014). Microevolutionary effects of habitat fragmentation on plant-animal interactions. *Advances in Ecology* 2014: 379267. https://doi.org/10.1155/2014/379267

Food and Agriculture Organization [FAO] (2016). *State of the world's forests 2016. Forests and agriculture: Land-use challenges and opportunities*. Rome, Italy: Food and Agriculture Organization of the United Nations.

Fortunel C., Stahl C., Heuret P., Nicolini E., and Baraloto C. (2020). Disentangling the effects of environment and ontogeny on tree functional dimensions for congeneric species in tropical forests. *New Phytologist* 226(2): 385–395. https://doi.org/10.1111/nph.16393

Francis D., Davies M.S., and Barlow P.B. (2008). A strong nucleotypic effect of DNA C-value on the cell cycle regardless of ploidy level. *Annals of Botany* 101(6): 747–757. https://doi.org/10.1093/aob/mcn038

Franks S.J., Weber J.J., and Aitken S.N. (2014). Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications* 7(1): 123-139. https://doi.org/10.1111/eva.12112

Gaston K.J. (1996). Species-range-size distributions: patterns, mechanisms and implications. *Trends in Ecology & Evolution* 11(5): 197–201. https://doi.org/10.1016/0169-5347(96)10027-6

Gaston K.J., and Spicer J.I. (2001). The relationship between range size and niche breadth: a test using five species of *Gammarus* (Amphipoda). *Global Ecology and Biogeography* 10(2): 179–188. https://doi.org/10.1046/j.1466-822x.2001.00225.x

Goettsch B., Hilton-Taylor C., Cruz-Piñón G., Duffy J.P., Frances A., Hernández H.M., Inger R., Pollock C., Schipper J., Superina M., Taylor N.P., Tognelli M., Abba A.M., Arias S., Arreola-Nava H.J., Baker M.A., Bárcenas R.T., Barrios D., Braun P., Butterworth C.A., Búrquez A., Caceres F., Chazaro-Basañez M., Corral-Díaz R., del Valle Perea M., Demaio P.H., de Barros W.A.D., Durán R., Yancas L.F., Felger R.S., Fitz-Maurice B., Fitz-Maurice W.A., Gann G., Gómez-Hinostrosa C., Gonzales-Torres L.R., Griffith M.P., Guerrero P.C., Hammel B., Heil K.D., Hernández-Oria J.G., Hoffmann M., Ishihara M.I., Kiesling R., Larocca J., León-de la Luz J.L., Loaiza C.R., Lowry M., Machado M.C., Majure L.C., Ávalos J.G.M., Martorell C., Maschinski J., Méndez E., Mittermeier R.A., Nassar J.M., Negrón-Ortiz V., Oakley L.J., Ortega-Baes P., Ferreira A.B.P., Pinkava D.J., Porter J.M., Puente-Martinez R., Gamarra J.R., Pérez P.S., Martínez E.S., Smith M., Sotomayor J.M., Stuart S.N., Muñoz J.L.T, Terrazas T., Terry M., Trevisson M., Valverde T., Van Devender T.R., Véliz-Pérez M.E., Walter H.E., Wyatt S.A., Zappi D., Zavala-Hurtado J.A., and Gaston K.J. (2015). High proportion of cactus species threatened with extinction *Nature Plants* 1: 15142. https://doi.org/10.1038/nplants.2015.142

Gomes V.H.F., Vieira I.C.G., Salomão R.P., and ter Steege H. (2019). Amazonian tree species threatened by deforestation and climate change. *Nature Climate Change* 9(7): 547–553. https://doi.org/10.1038/s41558-019-0500-2

Goodwillie C., Kalisz S., and Eckert C.G. (2005). The evolutionary enigma of mixed mating systems in plants: Occurrence, theoretical explanations, and empirical evidence. *Annual Reviews on Ecology, Evolution and Systematics* 36: 47-79. https://doi.org/10.1146/annurev.ecolsys.36.091704.175539

Grainger T.N., Letten A.D., Gilbert B., and Fukami T. (2019). Applying modern coexistence theory to priority effects. *Proceedings of the National Academy of Science of the United States of America* 116(13): 6205-6210. https://doi.org/10.1073/pnas.1803122116

Gratani L. (2014). Plant phenotypic plasticity in response to environmental factors. *Advances in Botany* 2014: 208747. https://doi.org/10.1155/2014/208747

Grayson D.K. (2011). *The Great Basin: a natural prehistory*. University of California Press, Berkeley

Greilhuber J., Doležel J., Lysák M.A., and Bennett M.D. (2005). The origin, evolution and proposed stabilization of the terms “Genome size” and “C-value” to describe nuclear DNA contents. *Annals of Botany* 95(1): 255–260. https://doi.org/10.1093/aob/mci019

Haapalehto T., Juutinen R., Kareksela S., Kuitunen M., Tahvanainen T., Vuori H., and Kotiaho J. S. (2017). Recovery of plant communities after ecological restoration of forestry-drained peatlands. *Ecology and Evolution* 7(19): 7848–7858. https://doi.org/10.1002/ece3.3243

Harnik P.G., Simpson C., and Payne J.L. (2012). Long-term differences in extinction risk among the seven forms of rarity. *Proceedings of the Royal Society B Biological Sciences* 279(1749): 4969–4976. http://doi.org/10.1098/rspb.2012.1902

Harrison R.D., Tan S., Plotkin J.B., Slik F., Detto M., Brenes T., Itoh A., and Davies S.J. (2013). Consequences of defaunation for a tropical tree community. *Ecology Letters* 16(5): 687-694. https://doi.org/10.1111/ele.12102

Hay F.R., and Probert R.J. (2013). Advances in seed conservation of wild plant species: a review of recent research. *Conservation Physiology* 1(1): 1–11. https://doi.org/10.1093/conphys/cot030

Herrera J.M., Morales J.M., and García D. (2011). Differential effects of fruit availability and habitat cover for frugivore-mediated seed dispersal in a heterogeneous landscape. *Journal of Ecology* 99(5): 1100–1107. https://doi.org/10.1111/j.1365-2745.2011.01861.x

Hijioka Y., Lin E., Pereira J.J., Corlett R.T., Cui X., Insarov G.E., Lasco R.D., Lindgren E., and Surjan A. (2014). Asia. In: *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Barros V.R., Field C.B., Dokken D.J., Mastrandrea M.D., Mach K.J., Bilir T.E., Chatterjee M., Ebi K.L., Estrada Y.O., Genova R.C., Girma B., Kissel E.S., Levy A.N., MacCracken S., Mastrandrea P.R., and White L.L. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1327-1370.

Hoang P.T.N., Schubert V., Meister A., Fuchs J., and Schubert I. (2019). Variation in genome size, cell and nucleus volume, chromosome number and rDNA loci among duckweeds. *Scientific Reports* 9: 3234. https://doi.org/10.1038/s41598-019-39332-w

Hodgson J.G., Sharafi M., Jalili A., Díaz S., Montserrat-Martí G., Palmer C., Cerabolini B., Pierce S., Hamzehee B., Asri Y., Jamzad Z., Wilson P., Raven J.A., Band S.R., Basconcelo S., Bogard A., Carter G., Charles M., Castro-Díez P., Cornelissen J.H., Funes G., Jones G., Khoshnevis M., Pérez-Harguindeguy N., Pérez-Rontomé M.C., Shirvany F.A., Vendramini F., Yazdani S., Abbas-Azimi R., Boustani S., Dehghan M., Guerrero-Campo J., Hynd A., Kowsary E., Kazemi-Saeed F., Siavash B., Villar-Salvador P., Craigie R., Naqinezhad A., Romo-Díez A., de Torres Espuny L., and Simmons E. (2010). Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? *Annals of Botany* 105(4): 573–584. https://doi.org/10.1093/aob/mcq011

Humphreys A.M., Govaerts R., Ficinski S.Z., Nic Lughadha E., and Vorontsova M.S. (2019). Global dataset shows geography and life form predict modern plant extinction and rediscovery. *Nature Ecology & Evolution* 3(7): 1043–1047. https://doi.org/10.1038/s41559-019-0906-2

Husband B.C., and Schemske D.W. (1996). Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50(1): 54–70. https://doi.org/10.1111/j.1558-5646.1996.tb04472.x

International Union for Nature Conservation [IUCN] (2020). The IUCN red list of threatened species. Version 2020-1. Retrieved from https://www.iucnredlist.org

IPCC (2014). *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Barros V.R., Field C.B., Dokken D.J., Mastrandrea M.D., Mach K.J., Bilir T.E., Chatterjee M., Ebi K.L., Estrada Y.O., Genova R.C., Girma B., Kissel E.S., Levy A.N., MacCracken S., Mastrandrea P.R., and White L.L. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 688.

Jansson R. (2003). Global patterns in endemism explained by past climatic change. *Proceedings of the Royal Society B Biological Sciences* 270(1515): 583–590. https://doi.org/10.1098/rspb.2002.2283

Jarnevich C.S., Stohlgren T.J., Kumar S., Morisette J.T., and Holcombe T.R. (2015). Caveats for correlative species distribution modeling. *Ecological Informatics* 29: 6-15. https://doi.org/10.1016/j.ecoinf.2015.06.007

Jiménez-Valverde A., Peterson A.T., Soberón J., Overton J.M., Aragón P., and Lobo J.M. (2011). Use of niche models in invasive species risk assessments. *Biological Invasions* 13: 2785-2797. https://doi.org/10.1007/s10530-011-9963-4

Joppa L.N., Roberts D.L., Myers N., and Pimm S.L. (2011). Biodiversity hotspots house most undiscovered plant species. *Proceedings of the National Academy of Sciences of the United States of America* 108(32): 13171–13176. https://doi.org/10.1073/pnas.1109389108

Knight C.A., and Ackerly D.D. (2002). Genome size variation across environmental gradients in the California flora. *Ecology Letters* 5(1): 66–76. https://doi.org/10.1046/j.1461-0248.2002.00283.x

Korb J.E., Springer J.D., Powers S.R., and Moore M.M. (2005). Soil seed banks in Pinus ponderosa forests in Arizona, clues to site history and restoration potential. *Applied Vegetation Science* 8(1): 103–112. https://doi.org/10.1111/j.1654-109X.2005.tb00634.x

Kotiaho J.S., Kaitala V., Komonen A., and Päivinen J. (2005). Predicting the risk of extinction from shared ecological characteristics. *Proceedings of the National Academy of Sciences of the United States of America* 102(6): 1963–1967. https://doi.org/10.1073/pnas.0406718102

Kraft N.J.B., Baldwin B.G., and Ackerly D.D. (2010). Range size, taxon age and hotspots of neoendemism in the California flora. *Diversity and Distributions* 16(3): 403–413. https://doi.org/10.1111/j.1472-4642.2010.00640.x

Lander T.A., Harris S.A., Cremona P.J., and Boshier D.H. (2019). Impact of habitat loss and fragmentation on reproduction, dispersal and species persistence for an endangered Chilean tree. *Conservation Genetics* 20: 973–985. https://doi.org/10.1007/s10592-019-01187-z

Le Roux J.J., Hui C., Castillo M.L., Iriondo J.M., Keet J.-H., Khapugin A.A., Médail F., Rejmánek M., Theron G., Yannelli F.A., and Hirsch H. (2019). Recent anthropogenic plant extinctions differ in biodiversity hotspots and coldspots. *Current Biology* 29(17): 2912–2918.e2912. https://doi.org/10.1016/j.cub.2019.07.063

Lenoir J., and Svenning J.C. (2015). Climate-related range shifts - a global multidimensional synthesis and new research directions. *Ecography* 38(1): 15–28. https://doi.org/10.1111/ecog.00967

Lesica P., Yurkewycz R., and Crone E.E. (2006). Rare plants are common where you find them. *American Journal of Botany* 93(3): 454-459. https://doi.org/10.3732/ajb.93.3.454

Lomba A., Pellissier L., Randin C., Vicente J., Moreira F., Honrado J., and Guisan A. (2010). Overcoming the rare species modelling paradox: A novel hierarchical framework applied to an Iberian endemic plant. *Biological Conservation* 143(11): 2647-2657. https://doi.org/10.1016/j.biocon.2010.07.007

Losfeld G., L'Huillier L., Fogliani B., Jaffre T., and Grison C. (2015). Mining in New Caledonia: environmental stakes and restoration opportunities. *Environmental Science and Pollution Research* 22: 5592-5607. https://doi.org/10.1007/s11356-014-3358-x

Lowry E., and Lester S.E. (2006). The biogeography of plant reproduction: potential determinants of species' range sizes. *Journal of Biogeography* 33(11): 1975–1982. https://doi.org/10.1111/j.1365-2699.2006.01562.x

Lughadha E.N., Bachman S.P., Leão T.C.C., Forest F., Halley J.M., Moat J., Acedo C. Bacon K.L., Brewer R.F.A., Gâteblé G., Gonçalves S.C., Govaerts R., Hollingsworth P.M., Krisai-Greilhuber I., de Lirio E.J., Moore P.G.P., Negrão R., Onana J.M., Rajaovelona L.R., Razanajatovo H., Reich P.B., Richards S.L., Rivers M.C., Cooper A., Iganci J., Lewis G.P., Smidt E.C., Antonelli A., Mueller G.M., and Walker B.E. (2020). Extinction risk and threats to plants and fungi. *New Phytologist* 2(5): 389-408. https://doi.org/10.1002/ppp3.10146

Ma M., Baskin C.C., Li W., Zhao Y., Zhao Y., Zhao L., Chen N., and Du G. (2019). Seed banks trigger ecological resilience in subalpine meadows abandoned after arable farming on the Tibetan Plateau. *Ecological Applications* 29(7): e01959. https://doi.org/10.1002/eap.1959

Martyn T.E., Bradford J.B., Schlaepfer D.R., Burke I.C., and Lauenroth W.K. (2016). Seed bank and big sagebrush plant community composition in a range margin for big sagebrush. *Ecosphere* 7(10): e01453. https://doi.org/10.1002/ecs2.1453

Millennium Ecosystem Assessment (2005). *Ecosystems and Human Well-being: Synthesis.* Island Press, Washington, DC.

Mills M.H. and Schwartz M.W. (2005). Rare plants at the extremes of distribution: broadly and narrowly distributed species. *Biodiversity and Conservation* 14: 1401-1420. https://doi.org/10.1007/s10531-004-9666-6

Mishler B.D., Guralnick R., Soltis P.S., Smith S.A., Soltis D.E., Barve N., Allen J.M., and Laffan S.W. (2020). Spatial phylogenetics of the North American flora. *Journal of Systematics and Evolution* 58(4): 393-405. https://doi.org/10.1111/jse.12590

Mndela M., Madakadze C.I., Nherera-Chokuda F., and Dube S. (2020). Is the soil seed bank a reliable source for passive restoration of bush-cleared semi-arid rangelands of South Africa? *Ecological Processes* 9: 1. https://doi.org/10.1186/s13717-019-0204-6

Montoya D., Alburquerque F.S., Rueda M., and Rodríguez M.A. (2010). Species' response patterns to habitat fragmentation: do trees support the extinction threshold hypothesis? *Oikos* 119(8): 1335-1343. https://doi.org/10.1111/j.1600-0706.2010.18280.x

Morellato L.P.C., Alberton B., Alvarado S.T., Borges B., Buisson E., Camargo M.G.G., Cancian L.F., Carstensen D.W., Escobar D.F.E., Leite P.T.P., Mendoza I., Rocha N.M.W.B., Soares N.C., Silva T.S.F., Staggemeier V.G., Streher A.S., Vargas B.C., and Peres C.A. (2016). Linking plant phenology to conservation biology. *Biological Conservation* 195: 60–72. https://doi.org/10.1016/j.biocon.2015.12.033

Morris L.R., and Rowe R.J. (2014). Historical land use and altered habitats in the Great Basin. *Journal of Mammalogy* 95(6): 1144–1156. https://doi.org/10.1644/13-MAMM-S-169

Morueta-Holme N., Engemann K., Sandoval-Acuña P., Jonas J.D., Segnitz R.M., and Svenning J.-C. (2015). Strong upslope shifts in Chimborazo's vegetation over two centuries since Humboldt. *Proceedings of the National Academy of Sciences of the United States of America* 112(41): 12741– 12745. https://doi.org/10.1073/pnas.1509938112

Mote P.W., Hamlet A.F., Clark M.P., and Lettenmaier D.P. (2005). Declining mountain snowpack in western North America. *Bulletin of the American Meteorological Society* 86: 39-49. https://doi.org/10.1175/BAMS-86-1-39

Myers N., Mittermeier R.A., Mittermeier C.G., Fonseca G.A.B., and Kent J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858. https://doi.org/10.1038/35002501

Nachlinger J., Sochi K., Comer P., Kittel G., and Dorfman D. (2001). *Great Basin: an ecoregion-based conservation blueprint*. The Nature Conservancy, Reno, Nevada, USA.

Nicotra A.B., Atkin O.K., Bonser S.P., Davidson A.M., Finnegan E.J., Mathesius U., Poot P., Purugganan M.D., Richards C.L., Valladares F., and Van Kleunen M. (2010). Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* 15(12): 684–692. https://doi.org/10.1016/j.tplants.2010.09.008

Noss R.F., LaRoe E.T., and Scott J.M. (1995). Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. *United States Department of the Interior National Biological Service Biological Report* 28: 1–95. http://biology.usgs.gov/pubs/ecosys.htm

Oliver T.H., and Morecroft M.D. (2014). Interactions between climate change and land use change on biodiversity: Attribution problems, risks, and opportunities. *WIREs Climate Change* 5(3): 317– 335. https://doi.org/10.1002/wcc.271

Ollerton F., Winfree R., and Tarrant S. (2011). How many flowering plants are pollinated by animals? *Oikos* 120(3): 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Pacifici M., Foden W.B., Visconti P., Watson J.E.M., Butchart S.H.M, Kovacs K.M., Scheffers B.R., Hole D.G., Martin T.G., Akçakaya H.R., Corlett R.T., Huntley B., Bickford D., Carr J.A., Hoffmann A.A., Midgley G.F., Pearce-Kelly P., Pearson R.G., Williams S.E., Willis S.G., Young B., and Rondinini C. (2015). Assessing species vulnerability to climate change. *Nature Climate Change* 5: 215-224. https://doi.org/10.1038/nclimate2448

Panetta A.M., Stanton M.L., and Harte J. (2018). Climate warming drives local extinction: Evidence from observation and experimentation. *Science Advances* 4(2): eaaq1819. doi: 10.1126/sciadv.aaq1819

Pärtel M., Kalamees R., Reier U., Tuvi E.L., Roosaluste E., Vellack A., and Zobel M. (2005). Grouping and prioritization of vascular plant species for conservation: combining natural rarity and management need. *Biological Conservation* 123(3): 271-278. https://doi.org/10.1016/j.biocon.2004.11.014

Pekas K.M., and Schupp E.W. (2013). Influence of aboveground vegetation on seed bank composition and distribution in a Great Basin Desert sagebrush community. *Journal of Arid Environments* 88: 113–120. https://doi.org/10.1016/j.jaridenv.2012.08.013

Pimm S.L., and Joppa L.N. (2015). How many plant species are there, where are they, and at what rate are they going extinct? *Annals of the Missouri Botanical Garden* 100(3): 170-176. https://doi.org/10.3417/2012018

Porté A.J., Lamarque L.J., Lortie C.J., Michalet R., and Delzon S. (2011). Invasive *Acer negundo* outperforms native species in non-limiting resource environments due to its higher phenotypic plasticity. *BMC Ecology* 11: 28. https://doi.org/10.1186/1472-6785-11-28

Pyšek P., Jarošík V., Hulme P.E., Pergl J., Hejda M., Schaffner U., and Vilà M. (2012). A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Global Change Biology* 18: 1725-1737. https://doi.org/10.1111/j.1365-2486.2011.02636.x

Ramesh V., Gopalakrishna T., Barve S., and Melnick D.J. (2017). IUCN greatly underestimates threat levels of endemic birds in the Western Ghats. *Biological Conservation* 210(A): 205– 221. https://doi.org/10.1016/j.biocon.2017.03.019

Razgour O., Forester B., Taggart J.B., Bekaert M., Juste J., Ibáñez C., Puechmaille S.J., Novella-Fernandez R., Alberdi A., and Manel S. (2019). Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proceedings of the National Academy of Sciences of the United States of America* 116(21): 10418-10423. https://doi.org/10.1073/pnas.1820663116

Reed D.H. (2004). Extinction risk in fragmented habitats. *Animal Conservation* 7(2): 181-191. https://doi.org/10.1017/S1367943004001313

Ricketts T.H., Dinerstein E., Olson D.M., Loucks C.J., Eichbaum W., DellaSala D., Kavanagh K., Hedao P., Hurley P.T., Carney K.M., Abell R., and Walters S. (1999). *Terrestrial ecoregions of North America: a conservation assessment*. Island Press, Washington, D.C., USA

Roberts E.H. (1973). Predicting the storage life of seeds. *Seed Science and Technology* 1: 499-514.

Rogora M, Frate L., Carranza M.L., Freppaz M., Stanisci A., Bertani I., Bottarin R., Brambilla A., Canullo R., Carbognani M., Cerrato C., Chelli S., Cremonese E., Cutini M., Di Musciano M., Erschbamer B., Godone D., Iocchi M., Isabellon M., Magnani A., Mazzola L., Morra di Cella U., Pauli H., Petey M., Petriccione B., Porro F., Psenner R., Rossetti G., Scotti A., Sommaruga R., Tappeiner U., Theurillat J.-P., Tomaselli M., Viglietti D., Viterbi R., Vittoz P., Winkler M., and Matteucci G. (2018). Assessment of climate change effects on mountain ecosystems through a cross-site analysis in the Alps and Apennines. *Science of The Total Environment* 624: 1429-1442. https://doi.org/10.1016/j.scitotenv.2017.12.155

Rossetti M.R., Tscharntke T., Aguilar R., and Batary P. (2017). Responses of insect herbivores and herbivory to habitat fragmentation: a hierarchical meta-analysis. *Ecology Letters* 20(2): 264–272. https://doi.org/10.1111/ele.12723

Rybicki J., Abrego N., and Ovaskainen O. (2020). Habitat fragmentation and species diversity in competitive communities. *Ecology Letters* 23(3): 506-517. https://doi.org/10.1111/ele.13450

Rymer P., Sandiford M., Harris S., Billingham M., and Boshier D. (2015). Remnant *Pachira quinata* pasture trees have greater opportunities to self and suffer reduced reproductive success due to inbreeding depression. *Heredity* 115: 115–124. https://doi.org/10.1038/hdy.2013.73

Sadler K.D., and Bradfield G.E. (2010). Ecological facets of plant species rarity in rock outcrop ecosystems of the Gulf Islands, British Columbia. *Botany* 88(4): 429-434. https://doi.org/10.1139/B10-011

Sáyago R., Quesada M., Aguilar R., Ashworth L., Lopezaraiza-Mikel M., and Martén-Rodríguez S. (2018). Consequences of habitat fragmentation on the reproductive success of two *Tillandsia* species with contrasting life history strategies. *AoB PLANTS* 10(4): ply038. https://doi.org/10.1093/aobpla/ply038

Schwartz M.W., Iverson L.R., Prasad A.M., Matthews S.N., and O'Connor R.J. (2006). Predicting extinctions as a result of climate change. *Ecology* 87(7): 1611–1615. https://doi.org/10.1890/0012-9658(2006)87[1611:PEAARO]2.0.CO;2

Segan D.B., Murray K.A., and Watson J.E.M (2016). A global assessment of current and future biodiversity vulnerability to habitat loss–climate change interactions. *Global Ecology and Conservation* 5: 12-21. https://doi.org/10.1016/j.gecco.2015.11.002

Sheth S.N., and Angert A.L. (2014). The evolution of environmental tolerance and range size: a comparison of geographically restricted and widespread *Mimulus*. *Evolution* 68(10): 2917–2931. https://doi.org/10.1111/evo.12494

Šmarda P., and Bureš P. (2010). Understanding intraspecific variation in genome size in plants. *Preslia* 82: 41-61

Smart S.M., Jarvis S., Walker K.J., Henrys P.A., Pescott O.L., and Marrs R.H. (2015). Common plants as indicators of habitat suitability for rare plants; quantifying the strength of the association between threatened plants and their neighbours. *New Journal of Botany* 5(2): 72-88. https://doi.org/10.1179/2042349715Y.0000000011

Smith S.D., Huzman T.E., Zitzer S.F., Charlet T.N., Housman D.C., Coleman J.S., Fenstermaker L.K., Seeman J.R., and Nowak R.S. (2000). Elevated CO_2 increases productivity and invasive species success in an arid system. *Nature* 408(6808): 79-82. https://doi.org/10.1038/35040544

Snyder K.A., Evers L., Chambers J.C., Dunham J., Bradford J.B., and Loik M.E. (2019). Effects of Changing Climate on the Hydrological Cycle in Cold Desert Ecosystems of the Great Basin and Columbia Plateau. *Rangeland Ecology & Management* 72(1): 1-12. https://doi.org/10.1016/j.rama.2018.07.007.

Stein B., Kutner L.S., and Adams J.S. (2000). *Precious Heritage: the Status of Biodiversity in the United States*. Oxford University Press, New York.

Thackeray S.J., Henrys P.A., Hemming D., Bell J.R., Botham M.S., Burthe S., Helaouet P., Johns D.G., Jones I.D., Leech D.I., Mackay E.B., Massimino D., Atkinson S., Bacon P.J., Brereton T.M., Carvalho L., Clutton-Brock T.H., Duck C., Edwards M., Elliott J.M., Hall S.J.G., Harrington R., Pearce-Higgins J.W., Høye T.T., Kruuk L.E.B., Pemberton J.M., Sparks T.H., Thompson P.M., White I., Winfield I.J., and Wanless S. (2016). Phenological sensitivity to climate across taxa and trophic levels. *Nature* 535: 241–245. https://doi.org/10.1038/nature18608

Thomas C.D., and Palmer G. (2015). Non-native plants add to the British flora without negative consequences for native diversity. *Proceedings of the National Academy of Sciences of the United States of America* 112: 4387-4392. https://doi.org/10.1073/pnas.1423995112

Thornhill A.H., Baldwin B.G., Freyman W.A., Nosratinia S., Kling M.M., Morueta-Holme N., Madsen T.P., Ackerly D.D., and Mishler B.D. (2017). Spatial phylogenetics of the native California flora. *BMC Biology* 15: 96. https://doi.org/10.1186/s12915-017-0435-x

Tilman D., Clark M., Williams D., Kimmel K., Polasky S., and Packer C. (2017). Future threats to biodiversity and pathways to their prevention. *Nature* 546: 73–81. https://doi.org/10.1038/nature22900

United Nations Environment Agency (2019). Resolution 73/284: United Nations Decade on Ecosystem Restoration (2021–2030). https://undocs.org/A/RES/73/284 (2019)

United States Fish and Wildlife Service [USFWS] (2014). *Species Report for Ivesia webberi (Webber's ivesia)*. Nevada Fish and Wildlife Office, Reno. United States Fish and Wildlife Services, 49 pp.

Van Kleunen M., and Fischer M. (2005). Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* 166(1): 49–60. https://doi.org/10.1111/j.1469-8137.2004.01296.x

Venter O., Brodeur N.N., Nemiroff L., Belland B., Dolinsek I.J., and Grant J.W.A. (2006). Threats to endangered species in Canada. *Bioscience* 56(11): 903-910. https://doi.org/10.1641/0006-3568(2006)56[903:TTESIC]2.0.CO;2

Venter O., Sanderson E.W., Magrach A., Allan J.R., Beher J., Jones K.R., Possingham H.P., Laurance W.F., Wood P., Fekete B.M., Levy M.A., and Watson J.E.M. (2016). Sixteen years of change in the global terrestrial human footprint and implications for biodiversity conservation. *Nature Communications* 7(1): 12558. https://doi.org/10.1038/ncomms12558

Vincent H., Bornand C.N., Kempel A., and Fischer M. (2020). Rare species perform worse than widespread species under changed climate. *Biological Conservation* 246: 108586. https://doi.org/10.1016/j.biocon.2020.108586

Visconti P., Bakkenes M., Baisero D., Brooks T., Butchart S.H., Joppa L., Alkemade R., Di Marco M., Santini L., Hoffmann M., Maiorano L., Pressey R.L., Arponen A., Boitani L., Reside A.E., Van Vuuren D.P., and Rondinini C. (2016). Projecting global biodiversity indicators under future development scenarios. *Conservation Letters* 9(1): 5–13. https://doi.org/10.1111/conl.12159

Vitasse Y, Bresson C.C., Kremer A., Michalet R., and Delzon S. (2010). Quantifying phenological plasticity to temperature in two temperate tree species. *Functional Ecology* 24(6): 1211–1218. https://doi.org/10.1111/j.1365-2435.2010.01748.x

Wagner F.H. (ed.) (2003). *Preparing for a changing climate- the potential consequences of climate variability and change. Rocky Mountain/Great Basin regional climate-change assessment*. A report of the Rocky Mountain/Great Basin Regional Assessment Team for the U.S. Global Change Research Program. Logan, UT: Utah State University; 240 pp.

Wamelink G.W.W., Goedhart P.W., and Frissel J.Y. (2014). Why some plant species are rare. *PLoS ONE* 9(7): e102674. https://doi.org/10.1371/journal.pone.0102674

Warren R., Price J., Graham E., Forstenhaeusler N., and VanDerWal J. (2018). The projected effect on insects, vertebrates, and plants of limiting global warming to 1.5°C rather than 2°C. *Science* 360(6390): 791–795. doi: 10.1126/science.aar3646

Westerling A.L., Hidalgo H.G., Cayan D.R., and Swetnam T.W. (2006). Warming and earlier spring increase U.S. forest wildfire activity. *Science* 313(5789): 940-943. doi: 10.1126/science.1128834

Wilcove D.S., Rothstein D., Dubow J., Philips A. and Losos E. (1998). Quantifying threats to imperiled species in the United States -- assessing the relative importance of habitat destruction, alien species, pollution, overexploitation and disease. *Bioscience* 48(8): 607-615. https://doi.org/10.2307/1313420

Wisdom M.J., Rowland M.M., and Suring L.H. (eds.) (2005). *Habitat threats in the sagebrush ecosystem: methods of regional assessment and applications in the Great Basin*. Alliance Communications Group Lawrence, Kansas.

Wisz M.S., Hijmans R.J., Li J., Peterson A.T., Graham C.H., Guisan A., and NCEAS Predicting Species Distributions Working Group (2008). Effects of sample size on the performance of species distribution models. *Diversity and Distributions* 14(5): 763-773. https://doi.org/10.1111/j.1472-4642.2008.00482.x

Witham C.W. (2000). *Current Knowledge and Conservation Status of Ivesia webberi A. Gray (Rosaceae), the Webber ivesia, in Nevada*. Status Report prepared for the Nevada Natural Heritage Program, Nevada Department of Conservation and Natural Resources, Carson City, NV. 52pp.

Wyse S.V., and Dickie J.B. (2017) Predicting the global incidence of seed desiccation sensitivity. *Journal of Ecology* 105 (4): 1082-1093. https://doi.org/10.1111/1365-2745.12725

Yu F., Groen T.A., Wang T., Skidmore A.K., Huang J., and Ma K. (2017). Climatic niche breadth can explain variation in geographical range size of alpine and subalpine plant. *International Journal of Geographical Information Science* 31(1): 190-212. https://doi.org/10.1080/13658816.2016.1195502

Zhang R., Jongejans E., and Shea K. (2011). Warming increases the spread of an invasive thistle. *PLoS ONE* 6: e21725. https://doi.org/10.1371/journal.pone.0021725

CHAPTER 1 Geographic predictions of iterative ecological niche modeling result in the discovery of novel populations of a rare cold desert perennial*

Israel T. Borokini[1], Kenneth Nussear[2], Tom E. Dilts[3], Peter J. Weisberg[3]

[1]Ecology, Evolution and Conservation Biology Graduate Program, Department of Biology, University of Nevada, Reno, Reno NV 89557

[2]Department of Geography, University of Nevada, Reno, Reno NV 89557

[3]Department of Natural Resources and Environmental Science, University of Nevada, Reno, Reno NV 89557

*Formatted for *Diversity and Distributions*

ABSTRACT

Despite the immense benefit of niche modeling to the management of rare and threatened species, it is often challenging to fit these models with high predictive accuracy for species with occurrence records that are commonly few, spatially biased, and incomplete. In this study, we applied an iterative niche modeling approach to guide prospective field sampling, improve niche model formulation, and better understand ecological influences on the distribution of *Ivesia webberi*. Six of the 72 assembled variables selected from three successive reduction analyses were used to fit preliminary niche models, from which the top three predictor variables were used for final niche modeling in each iteration. The iterative niche models were fitted using Boosted Regression Trees, Random Forests, Maximum Entropy, Artificial Neural Networks, Generalized Additive Models and Generalized Linear Models, and evaluated using true skill statistic, area under curve of the receiver operating characteristic, specificity, and the continuous Boyce index. Weighted average ensembles of the single models were used to produce geographic habitat suitability projection maps, which were used to design field sampling. New spatial data from the field sampling were added to the dataset for subsequent modeling. The environmental conditions of both the original and novel species occurrences were plotted in principal component analysis (PCA) ordination space. The degree of similarity among the geographical predictions of the iterative niche modeling was assessed using niche overlap analysis. Iterative niche modeling and model-guided field surveys resulted in the discovery of seven novel locations and expanded the northern reach of the distribution range by 65 km. Perennial herbaceous layer and topographic position index were the two most important ecological drivers of *I. webberi* niche across all four modeling iterations. PCA ordination showed that the new occurrence points filled the realized niche space for *I. webberi* and slightly expanded climatic and topographic breadth of the species in ordination space. This study demonstrated that model-guided sampling and iterative niche modeling can advance scientific understanding of the species-environment interactions, ecological niche, and geographic distributions of rare species, which is crucial for effective conservation.

KEYWORDS: species distribution models, habitat suitability, iterative niche modeling, BIOMOD2, niche breadth, *Ivesia webberi*, Great Basin Desert

INTRODUCTION

Species with restricted geographical distributions, small population sizes and narrow ecological tolerance ranges are more vulnerable to future environmental change and thus, are commonly prioritized for conservation (Lavergne et al. 2005, Lomba et al. 2010, Sousa-Silva et al. 2014). Ecological niche modeling (ENM), also referred to as species distribution modeling (SDM) or climatic envelope modeling, has become an important conservation tool for the management of species at risk because it can be used to identify environmental factors that support the persistence of the species (Jiménez-Valverde, Lobo, & Hortal, 2008; Jiménez-Valverde, Peterson, Soberón, Overton, Aragón, & Lobo, 2011), and limiting factors that restrict their geographical distributions (Gorban, Pokidysheva, Smirnova, & Tyukina, 2011). ENM can also be used to evaluate degree of rarity of species (Broennimann, Thuiller, Hughes, Midgley, Alkemade, & Guisan, 2006), and identify areas that may serve as future climatic refugia (Petitpierre, Kueffer, Broennimann, Randin, Daehler, & Guisan, 2012; Sousa-Silva et al., 2014). Furthermore, niche models are often integrated into models of landscape connectivity, population genetic and metapopulation dynamics (e.g., Ikeda, Max, Allan, Lau, Shuster, & Whitham, 2016; Keeley, Beier, Keeley, & Fagan, 2017; Banerjee, Mukherjee, Guo, Ng, & Huang, 2019), or used for mapping the phylogenetic diversity of an area (e.g., Scherson, Thornhill, Urbina-Casanova, Freyman, Pliscoff, & Mishler, 2017).

Understanding the distribution of rare species is critical for effective conservation planning, but with incomplete and spatially biased occurrence records, modeling the niches of rare species with high predictive accuracy can be challenging (Hernandez, Graham, Master, & Albert, 2006; Wisz, Hijmans, Li, Peterson, Graham, Guisan, &

NCEAS Predicting Species Distributions Working Group, 2008), a condition referred to as the rare species modeling paradox (Lomba et al., 2010). Furthermore, correlative species distribution models (SDM) are fitted with an underlying assumption that species are in equilibrium with their environment (i.e., temporal and spatial stationarity) and that all important and biologically-relevant variables have been included in the niche model (Elith & Leathwick, 2009). This presents challenges to modeling rare species because the inclusion of many predictors when the number of occurrences are few can lead to model overfitting (Stockwell & Peterson, 2002; Wisz et al., 2008; Jarnevich, Stohlgren, Kumar, Morisette, & Holcombe, 2015). Moreover, limited knowledge on species-environment relationships for rare species makes predictor variable selection challenging and subjective (Aranda & Lobo, 2011). Consequently, poorly fit models and misjudgements of model predictions can lead to over- or underestimation of the rare species' niche, resulting in poor management decisions (Ramesh, Gopalakrishna, Barve, & Melnick, 2017; Burns, Clemann, & White, 2020). Therefore, niche models for rare species are recommended only for hypothesis testing for further sampling and possible discovery of novel populations (Stockwell & Peterson, 2002; Wisz et al., 2008; Jarnevich et al., 2015).

Predicted habitat suitability maps from preliminary niche modeling can be used to stratify and optimize sampling efficiency (Chiffard, Marciau, Yoccoz, Mouillot, Duchateau, Nadeau, …, Besnard, 2020). This niche-based sampling approach (Guisan, Broennimann, Engler, Vust, Yoccoz, Lehmann, & Zimmermann, 2006) can reduce spatial bias in the presence/true absence points (Singh, Yoccoz, Bhatnagar, & Fox, 2009), and result in the discovery of novel locations of the studied taxa and thus the correction of species range boundaries to encompass larger areas (de Siqueira, Durigan, de Marco

Junior, & Peterson, 2009; Deb, Jamir, & Kikon, 2017; Burns, Clemann, & White, 2020). Integrating the new spatial data from niche-based sampling in subsequent iterations can help increase the predictive accuracy of niche models for rare species and facilitates identification of biologically-relevant environmental factors (Rinnhofer, Roura-Pascual, Arthofer, Dejaco, Thaler-Knoflach, Wachter, …, Schlick-Steiner, 2012).

However, niche model performance can vary widely among different algorithms (Pearson et al. 2006, Thuiller et al. 2009, Marmion et al. 2009, Oppel et al. 2012, Li and Wang 2013), and this inter-model variation and uncertainty can be significant when fitting niche models for species with fewer occurrences (Araújo et al. 2005, Hernandez et al. 2006, Wisz et al. 2008). Therefore, comparative niche modeling with different algorithms (Stockwell and Peterson 2002, Araújo et al. 2005, Wisz et al. 2008), and combining models from different algorithms into an ensemble model (Araújo and New 2007, Thuiller et al. 2009) are recommended. This is because ensemble predictions minimize the prediction errors and uncertainties while retaining the true signals of species-environment relationships in each algorithm (Araújo and New 2007, Marmion et al. 2009, Dormann et al., 2018).

The aim of this study was to evaluate the ability of niche-based sampling and an iterative modeling approach to predict the niche of a rare plant (*Ivesia webberi* A. Gray), relative to an effort that used only the limited presence/absence data available at the outset of our study. Therefore, we asked the following questions: (1) How does additional spatial data from niche-based sampling alter our understanding of the species-environment relationships for *I. webberi*? (2) To what extent did additional spatial data alter the habitat suitability map projections from the modeling iterations? (3) Do modeling iterations in tandem with model-guided sampling significantly improve the predictive accuracy of niche models for *Ivesia webberi*? (4) How did the performance of modeling algorithms change with additional spatial data across iterative niche models? (5) Did ensemble models perform better than single niche models across iterative niche models?

METHODS

Species and occurrence data

Ivesia webberi is a perennial forb restricted to the eastern foothills of the Sierra Nevada and the adjacent western edge of the Great Basin Desert. At the outset of our study, it had been recorded from 21 locations, most of which are spatially aggregated and have been under human and biological invasion pressures. The species was estimated to have originated between 1.3 and 3.8 million years ago (Töpel, Antonelli, Yesson, & Eriksen, 2012), and may be one of the many Great Basin Desert neoendemics and phylogenetically young taxa that have not had enough time to fully colonize their range (Kraft, Baldwin, & Ackerly, 2010; Thornhill, Baldwin, Freyman, Nosratinia, Kling, Morueta-Holme, …, Mishler, 2017).

Initial niche modeling started in 2015 with 21 occurrence points and 758 absence points obtained from the Nevada Natural Heritage Program (NNHP). Absence points were collected from historical surveys by NNHP botanists and citizen scientists in areas where *I. webberi* was not detected. Additional spatial points were added following iterative modeling and field validation (described below) to predicted suitable habitats.

The absence points were thinned using a distance of 7.5 km in *spThin* R package (Aiello-Lammens, Boria, Radosavljevic, Vilela, & Anderson, 2015) to reduce the effect of spatial aggregation, and were merged with the presence points for niche modeling (Table 1). Additionally, absence points within 5 km of an occurrence point were removed to avoid false negatives. In all modeling iterations, the absence points were more than the presence points in order to reduce prevalence in the spatial data set, and avoid an overprediction of the geographical distribution for the rare *I. webberi* (Jiménez-Valverde, Lobo, & Hortal, 2009).

Study extent

The study extent was defined by a 60 km buffer from marginal ranges of known populations as of 2015. Field surveys indicate that this species occurs in or near ephemeral washes and forest dry meadow gaps in mostly gentle slope areas (Witham, 2000). Also, the species produces relatively heavy achene seeds which are not adapted for long-range dispersal. Therefore, the study area was restricted within this narrow range, excluding the expansive adjacent unsuitable areas of playas in the central Great Basin Desert.

Predictor variables

A total of 72 predictor variables describing edaphic, topographic, land cover, vegetative cover, and climatic factors were assembled for fitting niche models for *Ivesia webberi* (see Table S1 in Supporting Information). To avoid overfitting and maintain a 1:10 predictor variable to occurrence points ratio (Harrell, Lee, & Mark, 1996), the full set of predictor variables were reduced to six uncorrelated predictors (Table 2, Figure S1) using a combination of the Kendall *r* correlation coefficient (*r* > 0.6), feature selection runs in *Boruta* R package (Kursa & Rudnicki, 2010), and recursive feature elimination algorithm in *caret* R package (Kuhn, 2008).

The climatic variables (actual evapotranspiration, minimum annual temperature and annual precipitation) were downsampled from the PRISM climatic data (1971-2000) normals (Daly, Halbleib, Smith, Gibson, Doggett, Taylor, … Pasteris, 2008) from 800 m to 30 m spatial resolution using ordinary kriging and the Climatic Water Deficit Toolbox (Dilts, 2014; Dilts, Weisberg, Dencker, & Chambers, 2015; see Table S1). The cosine aspect was derived from slope using the formula: $\theta \times \cos(\alpha)$, where θ is slope (in percentage), and α is aspect (in radians), while slope was calculated from the 1 arc-second digital elevation models (DEM; USGS, 2017). Perennial herbaceous vegetative cover is considered both a biotic and land cover layer in this modeling framework. It is a vegetation type raster layer obtained from the Multi-Resolution Land Characteristics (MRLC) development of the 2016 U.S. National Land Cover Database (NLCD; Xian, Homer, Meyer, & Granneman, 2013). Topographic Position Index (TPI) was calculated from the DEM as in Weiss (2001).

Modeling algorithms

Six algorithms were used for *Ivesia webberi* niche modeling: Generalized linear models (GLMs; McCullagh and Nelder 1989), Generalized additive models (GAMs; Hastie and Tibshirani 1990), boosted regression trees (BRTs; Elith et al. 2008), random forests (RF; Breiman 2001), maximum entropy (MAXENT; Phillips et al. 2006), and artificial neural networks (ANN; Ripley 1996). Preliminary models also included surface resistance envelopes (SRE) which were dropped from the final modeling due to poor model performance.

Generalized linear models (GLMs)

GLMs are extensions of linear models assuming a parametric relationship between species distributions and the environmental variables. By using the appropriate link function, GLMs allow a non-Gaussian transformed species occurrence to be modeled as a linear function of environmental variables (Guisan et al. 2002). Unlike linear models, GLMs allow for the distribution of errors in different families, including binomial. In GLMs-based niche modeling, polynomial terms are recommended (Aguirre-Gutiérrez et al. 2013, Shabani et al. 2016).

Generalized Additive Models (GAMs)

GAMs are non-parametric extensions of the GLMs that allow for flexible modeling of species' non-linear response to environmental variables using smoothing functions computed independently for each of variable and used to build the final model (Guisan et al. 2002, Segurado and Araújo 2004). This is useful because species-environment relationships are often skewed and non-linear (Austin 2002, 2007).

Boosted Regression Trees (BRTs)

BRT is a machine learning algorithm comprising classification and regression trees, and boosting algorithm to produce a final model with high performance from many weak, simpler models (De'ath 2007, Elith et al. 2008). BRTs make recursive splits using values of the variables or variable interactions that minimize prediction errors in a stepwise manner and weighting subsequent trees by the residuals of the first trees, all decision trees are then combined by collapsing the weakest splits to optimize model performance based on specific rules of tree complexity and regularization (De'ath 2007, Elith et al. 2008). Many studies have reported superior performance of BRTs to regression models (e.g., Moisen et al. 2006, Leathwick et al. 2006, García-Callejas et al. 2016), and BRTs are shown to be less sensitive to species with fewer and clumped distribution in environment space (Elith et al. 2008).

Maximum Entropy (MAXENT)

MAXENT is a machine learning algorithm that estimates the suitability of a site for a particular species by fitting the unknown probability distribution of maximum entropy to the predictors (Phillips et al. 2006). It does this iteratively by comparing values of predictor variables at species occurrence points to predictor variable values in the random background points, within different modeling functions (e.g., linear, quadratic, etc.), at the same time avoiding overfitting by regularization mechanisms (Phillips et al. 2006, Phillips and Dudík 2008). It is robust to modeling the niche of species with fewer and incomplete occurrence points and it does not require absence points, therefore it is often used to fit niche models for rare species (Moreno et al. 2011, Merow et al. 2013).

Random Forests (RF)

RF, another tree-based machine-learning algorithm, uses the classification and regression trees to produce hundreds of random trees with a bootstrapped subset of the predictor variables at each node iteratively (Breiman 2001, Prasad et al. 2006, De'ath 2007). The resulting trees are averaged to produce the final model, using bootstrap aggregating (or “bagging”) method (Cutler et al. 2007). Optimizing parameters such as the number of variables used and number of trees was recommended to help minimize the generalization (“out-of-bag”) error (Prasad et al. 2006, Gromping 2009). Fitted trees in the forest used fewer predictors, therefore there is low correlation with the predictors used, while all of the trees were averaged to produce the final tree thereby reducing chances of overfitting, bias and uncertainty (Prasad et al. 2006, Wiesmeier et al. 2011).

Artificial Neural Networks (ANN)

ANN development was inspired by brain structure, comprising artificial neurons (processors), organized into three layers that compute species-environment relationship through a network of links and their associated functions (Li and Wang 2013). ANN is composed of three layers that represent the predictor variables, intermediate (hidden) layer and the output layer (Lek and Guegan, 1999). The intermediate layer is like a black box that processes predictor variable values using a function which can operate as regression or classification tree depending on the predictor variable (Li and Wang 2013). ANN was included in this modeling based on earlier studies that reported its superior model performance (e.g., Segurado and Araújo 2004).

Niche modeling, projections, and field validation

The niche models were fitted iteratively in 2015, 2018, 2019 and 2020, hereafter called iterative niche models (Fig. 1). For each model iteration, the six predictor variables were used to fit preliminary models, from which the top three predictors were selected for the final modeling. All preliminary, final and iterative niche modeling was implemented in BIOMOD2 package in R (Thuiller et al. 2009, R Core Team 2020). We used default

BIOMOD modeling settings as recommended by Thuiller et al. (2009). GLMs were generated using quadratic terms in a stepwise approach, and using the Akaike information Criterion (AIC) for model selection. BRT models were generated by fitting 2500 trees and three cross-validations, while GAMs were analyzed with a spline smoothing function. We used five cross-validations to select the optimal hidden layers in ANN models, we fitted 500 random forest models, and ran MAXENT models with 10,000 background points, using linear, quadratic and product features but with logistic model output (Phillip and Dudík 2008, Thuiller et al. 2009, Aguirre-Gutiérrez et al. 2013). Due to the small number of occurrence points, spatial data was not partitioned to independent training and test data; rather all iterative niche models were fitted with 10 replicates each for the six algorithms using 80% of the data for cross-validation (Araújo et al. 2005, Thuiller et al. 2009). Ensembles of best single models (TSS > 0.7) were also fitted for all model iterations.

An initial niche model was fitted in 2015 with 21 occurrence points and 53 spatially thinned true absence points (Table 1) from which habitat suitability map projections were generated. The original 758 absence points were overlaid on the predicted habitat map in ArcGIS software and suitable areas that had not been previously surveyed were selected for field validation, however the field sampling was limited to public lands and sites under protection. Additionally, since many of the occurrence points were spatially aggregated, predicted suitable sites adjacent to existing locations of *I. webberi* (that is, adaptive cluster sampling; Thompson 1990) were also surveyed. This resulted in the generation of about 900 true absence points and three new locations, which were added to existing data for modeling iteration in 2018.

Niche modeling in 2018 used 26 occurrence and spatially thinned absence points. The 26 occurrence points included the original 21 points, the three new locations and spatial adjustment of existing locations. For example, the USFWS population designations clustered multiple locations into one population. The disaggregation of these subpopulations resulted in three additional presence points. All absence points were overlaid on the resulting habitat suitability map projection from which sites for field validation were selected for sampling. Ground truthing resulted in the discovery of a new location and 229 absence points, which were added to the dataset for modeling iterations in 2019. Predicted habitat maps from the 2019 modeling iteration were used to select sampling sites for field validation in 2020. Absence points were overlaid on the prediction map, and predicted suitable sites in close proximity to known occurrences (i.e., adaptive cluster sampling) were selected for field validation. This resulted in the discovery of four new locations, which increased the spatial points to 31 occurrences and 2289 absence points (Table 1), which were used for the most recent modeling iteration.

In all final modeling iterations, replicates of all single models with TSS ≥ 0.7 were averaged into an ensemble model (Araújo & New, 2007; Marmion, Parviainen, Luoto, Heikkinen, & Thuiller, 2009; Thuiller et al., 2009), which was also evaluated using TSS and AUC. The ensemble model with the highest TSS was used to generate maps of projected habitat suitability that were used for the model-guided field sampling. On the predicted habitat maps, areas with a ≥ 0.5 probability of occurrence were considered suitable, based on the recommendation for fitting niche models for species with few occurrence points (Araújo & Peterson, 2012). All model-guided field surveys were conducted between May and June of each year when the plants are in flower to ensure optimal detection.

We used a different niche modeling approach to assess overfitting in the iterative models where overfitting is indicated by a large difference between observed accuracies for a fitted model using the training data set, and accuracies for models developed using spatially independent test data. The same spatial dataset used for the iterative niche modeling each year were partitioned into three spatial blocks, two of which were used for training while the third block was used for testing. Blocking is a nonrandom allocation of spatial data to reduce the effect of spatial bias and autocorrelation in the resulting models (Valavi, Elith, Lahoz-Monfort, & Guillera-Arroita, 2019). In this study, we partitioned the entire study area into six latitudinal and longitudinal bins, which were then clustered into three spatial blocks. Due to the data requirements of different algorithms, we limited the niche modeling to random forest, maximum Entropy, and Artificial Neural Networks. Overfitting was assessed as a difference between the training and test AUC (Warren & Siefert, 2011).

Assessment of niche model performance

Multi-metric model comparison is recommended for evaluating models generated from different algorithms, because each metric assesses a different aspect of model performance (Elith and Graham 2009, Escober et al. 2018). In order to produce model predictions with maximal accuracy and minimal omission errors, true statistic skill (TSS; Allouche et al. 2006), area under the curve (AUC) of the receiver operating characteristics (ROC) plot (Hanley and McNeil 1982), specificity and Continuous Boyce index (CBI; Boyce et al. 2002) were used to evaluate the performance of the fitted niche models.

TSS is computed as TSS = sensitivity + specificity – 1, thus considering both commission and omission errors, and accuracy (Allouche et al. 2006). The main strength of TSS is that it is not sensitive to species prevalence (Allouche et al. 2006, Beaumount et al. 2016). Specificity measures the probability of a model correctly classifying an absence point (Allouche et al. 2006). However, both TSS and specificity are threshold-based metrics, using a default or defined threshold to predict the suitability of a grid based on the confusion matrix, while AUC is a threshold-independent metric. Sensitivity values are plotted against specificity values to produce the ROC plot, from which the AUC score is obtained (Fielding and Bell, 1997), therefore it could be interpreted as the average sensitivity across all values of specificity, or vice versa (Liu et al. 2011). Experts have cautioned that AUC weights commission and omission errors equally, and it is sensitive to overfitting when the test data is not independent (Lobo et al. 2008, Warren and Seifert 2011). In spite of this, AUC is the most commonly used model performance metric (Lobo et al. 2008). The Continuous Boyce Index (CBI) is the Spearman rank correlation between model predictions and the occurrence points used in the modeling (Boyce et al. 2002, Hirzel et al. 2006). It was developed originally for assessing the accuracy of presence-only models, but can be used for all niche modeling frameworks (Breiner et al. 2015).

Assessment of differences among iterative niche models

Model performance was assessed for each year of iterative niche modeling. Generalized Linear mixed effect models (GLMM) were used to account for the random effect of years of iterative modeling on the difference in predictive performance of each of the six algorithms, using the scores of the performance metrics. In the GLMM, values of the performance metric (response variable) were regressed against the six algorithms, while the years of iterative modeling was included as a random effect.

Furthermore, niche overlap analysis was performed on the ensemble predictions of habitat suitability for each modeling iteration, using the *I* similarity metric, which is based on the Hellinger distance (Warren, Glor, & Turelli, 2008). Niche overlap analysis is a cell-by-cell comparison of geographical predictions of occurrence and randomization tests to quantify niche differences and assess their statistical significance (Warren, Glor, & Turelli, 2008). The similarity metric of the niche overlap analysis, as implemented in the *nicheOverlap* function of the dismo R package, ranges from 0 to 1 representing the degree of similarity between two niche model projections (Warren, Glor, & Turelli, 2008; Hijmans, Phillips, Leathwick, & Elith, 2017). Additionally, the number of predicted suitable raster cells (≥ 0.5 probability of occurrence) was counted.

Additionally, we conducted principal component analysis (PCA) to assess changes in the ecological niche of *I. webberi* due to the novel locations and additional absence points. This complements the niche overlap analysis and corrects for a possible overestimation of the similarities among the predicted niches (Broennimann, Fitzpatrick, Pearman, Petitpierre, Pellissier, Yoccoz, …, Guisan, 2012). PCA was implemented in FactoMineR R package (Lê, Josse, & Husson, 2008), which uses a singular value decomposition method that examines covariances and correlations among individuals (Wall, Rechtsteiner, & Rocha, 2003). We conducted PCA using the six uncorrelated predictor variables and *I. webberi* occurrence data for the 2015 iterative modeling and all new presence points derived as from the 2020 spatial data. The PCA bivariate plot of the first two PCA axes included two convex hulls representing original and new presence points. In addition, we also plotted boxplots of the six environmental predictors between the original *I. webberi* points and the new locations discovered during the model-guided surveys.

RESULTS

Does iterative niche modeling improve our understanding of species-environment relationships for *I. webberi*?

The iterative niche modeling and model-guided field surveys resulted in the discovery of seven new locations of *I. webberi* and also expanded the northern distribution range by 65 km. The positions of the new occurrence points on the PCA bivariate plot in relation to the original presence points show that the new locations filled the realized niche space and slightly expanded the climatic breadth for the *I. webberi* ecological niche (Fig. 2). The first two axes together explained 49% of the variation in the data. Axis 1 represents climatic and topographic gradients, while axis 2 represents gradients in the available energy and cosine aspect (Table 3). The boxplots show that some of the novel locations expanded the range for actual evapotranspiration, summer precipitation, and monthly minimum temperature and (Fig. 3a, d, f, respectively), but the sites were within the range of the original occurrence for the remaining three predictors (Fig. 3b, c, and e).

The variable importance plots showed that the perennial herbaceous layer and topographic position index (TPI) consistently contributed the most to *I. webberi* niche across all niche modeling iterations (Fig. 4a-d). AET, indicating the available energy, was the third predictor in the 2015 niche model iteration (Fig. 4a), cosine aspect layer, illustrating exposure to sunlight, came third in both the 2018 and 2019 modeling iterations (Fig. 4c-d), while mean summer precipitation was the third predictor for the 2020 niche modeling (Fig. 4d).

The response plots show that the probability of *I. webberi* occurrence was optimized between 25- and 33-mm summer precipitation, sites with ≥20% native perennial herbaceous occurrence, and topographic positions that are either gentle lateral valleys or gentle lateral ridges, but never in flat areas (Fig. 5).

To what extent did additional presence and absence data alter the habitat suitability map projections?

Niche overlap analysis showed a slight but gradual dissimilarity among the ensemble habitat suitability map projections across the modeling iterations (Fig. 6a-d; Table 4). Furthermore, the percentage of the ensemble predicted suitable raster cells (using 0.5 threshold) decreased from 5.98% in 2015, to 3.10% in 2018, 3.06% in 2019, but slightly increased to 3.34% in 2020. Together, both results indicate that additional presence and absence points slightly altered the geographic projections of the iterative niche modeling.

Does iterative modeling improve the niche of *Ivesia webberi*?

Both TSS and AUC scores decreased from 2015 to 2019 iterative niche models, and slightly increased in the final niche model iteration (Fig. 7a and b). Tukey post-hoc comparison test on the MMLR analysis for both the TSS and AUC values showed that 2015 niche models were significantly different from 2019 and 2020 modeling iterations ($p<0.05$; Table 5). The 2018 iterative models were also significantly different from 2019 ($p<0.05$; Table 5). Furthermore, CBI values decreased between 2015 and 2018 niche models, but slightly increased in 2019 and 2020 iterations (Fig. 7c). Specificity values increased consistently with years of iterative modeling (Fig. 7d). However, Tukey post-hoc comparison test on the MMLR of both CBI and specificity metrics were not significantly different (Table 5). The difference in the training and test AUC values from the niche modeling with spatial blocks shows overfitting in both the 2015 and 2019 iterative niche models, while the 2018 and 2020 iterative niche models did not exhibit overfitting (Table 6).

How did the performance of modeling algorithms change across iterative niche models?

Across all four model performance metrics used, RF and GLM had the best and worst performance respectively across the years of iterative modeling (Fig. 8a-d), but the difference was not greater than expected by chance (MLM; $p>0.05$). However, when accounting for the effect of years of iterative modeling, Tukey post-hoc test on the GLMM analysis showed that TSS values from GLM niche models were significantly lower than for GBM (adjusted $p<0.01$), RF (adjusted $p=0.01$), and MAXENT models (adjusted *p*=0.03). Similar results were obtained using AUC values showing that AUC values for GLM models was significantly lower than GBM (adjusted *p*=0.01) and RF models (adjusted *p*=0.01).

For CBI, while accounting for the year effect using GLMM, MAXENT was significantly different (*p*=0.03), but the difference was lost in the Tukey post-hoc correction. For specificity, RF and ANN had the highest and lowest specificity respectively. Tukey post-hoc test on the multivariate multiple regression showed that ANN niche models were significantly lower to GAM (adjusted *p*=0.04), GBM (adjusted *p*=0.01), GLM (adjusted *p*=0.01), and RF (adjusted *p*=0.01). Similar results were obtained in GLMM where the effect of iterative modeling was accounted for: ANN was significantly lower than the remaining algorithms - GAM (adjusted *p*=0.01), GBM (adjusted *p*=0.01), GLM (adjusted *p*=0.01), MAXENT (adjusted *p*=0.03), and RF (adjusted *p*=0.01).

Did ensemble modeling perform better than single algorithms?

Based on TSS and AUC, the default BIOMOD metrics for ensemble models, ensemble models performed better than all single models (Fig. 9a-b), however this difference was only significant between ensemble and GLM niche models from the Tukey post-hoc test on a linear regression (adjusted *p*=0.01) on the TSS metric, but not for the AUC metric. While accounting for the year effect, Tukey post-hoc test on the GLMM showed significant difference between ensemble models and ANN (adjusted *p*=0.02), and GLM (adjusted *p*=0.01) for the TSS metric, and only GLM (adjusted *p*=0.01) for the AUC metric.

DISCUSSION

Strengths and weaknesses of the iterative modeling approach

The discovery of seven novel localities and an overall expansion of *Ivesia webberi*'s known geographical range underscores the importance of iterative niche modeling to guide field surveys, identify biological relevant ecological factors that support species persistence, and accurately map their geographical distributions. Previous studies have reported the discovery of new localities from niche model guided field surveys (e.g., Aitken, Roberts, & Schultz, 2007; Williams, Seo, Thorne, Nelson, Erwin, O'Brien, & Schwartz, 2009; de Siqueira, Durigan, de Marco Jr., & Peterson, 2009; Newbold, Reader, El-Gabbas, Berg, Shohdi, Zalat, … Gilbert, 2010; Rebelo & Jones, 2010; Le Lay, Engler, Franc, & Guisan, 2010; Menon, Choudhury, Khan, & Peterson, 2010; Särkinen, Gonzáles, & Knapp, 2013; Groff, Marks, & Hayes, 2014; McCune, 2016; Deb, Jamir, & Kikon, 2017; Burns et al., 2020). This study and other similar studies demonstrate that the discovery of new locations can result in an expansion of the known distribution range of the study species and lead to increased understanding of the niche and species-environment relationships (Rinnhofer et al., 2012; Young Fairchild, Belcher, Evangelista, Verdone, & Stohlgren, 2019; Chiffard et al., 2020). Iterative ecological modeling is not limited to niche models, but has also been recommended for accurate forecasting of plant response to climate change (White, Yenni, Taylor, Christensen, Bledsoe, Simonis, & Ernest, 2018).

When occurrence points are few, the chances of model prediction errors are high because of an inflation of the influence of spatial bias and high risk of violating stationarity assumptions in the niche models (Wisz et al., 2008; Lobo & Tognelli, 2011; Galante, Alade, Muscarella, Jansa, Goodman, & Anderson, 2018). Moreover, the ability of models to reliably identify relevant drivers of a species distribution is reduced (Wisz et al., 2008; Galante et al. 2018). Therefore, commission errors do not necessarily mean a failure of the model to characterize suitability accurately, but may highlight potential suitable but unsampled sites or an incomplete model due to the absence of important variables (Araújo & Peterson, 2012). Therefore, when modeling the niches of rare species with few occurrences, locations of commission errors should be considered potential suitable habitats which can be validated through an adaptive sampling strategy.

Novel locations of a species have both conservation and ecological significance. In this study, we observed slight dissimilarities among the geographic projections of the iterative niche models for *I. webberi*, which highlights the role of additional spatial data in the model predictions. However, niche overlap metrics do not indicate the directionality or type of difference in the niche, and do not provide a quantitative indication of the position and breadth of the niches, thus, they cannot tell us if the modeled niches have expanded, shrunk or remained the same (Broennimann et al., 2012). Both the boxplots and the PCA bivariate plot show that the novel presence points expanded the climatic breadth of *I. webberi* ecological niche. Previous studies have reported similar ecological significance of additional occurrence in niche modeling and predictions (Rinnhofer et al., 2012; Chiffard et al., 2020). Overall, the niche model projections are congruent with field observations in that *I. webberi* is spatially distributed in climatically suitable sites, on gentle slopes, dominated by mixed perennial herbaceous and shrub vegetation and gravely bare ground (see Figures S2-14).

Conversely, absence points can have significant effects on model predictions. In this study, we observed decreasing performance in the iterative niche models which can be attributed to an increase in the number of absence points. Absence points in niche models can represent sites with unsuitable climatic conditions, abiotically suitable sites but the species cannot naturally reach due to limited dispersal or the species is competitive excluded, and sites where the species was present but not detected (Lobo, Jiménez-Valverde, & Hortal, 2010). Sites that are environmentally unsuitable for a species represent true absence while the other two absence types can introduce noise into bioclimatic envelopes and reduce the reliability of potential niche predictions (Lobo, 2008; Lauzeral, Grenouillet, & Brosse, 2012). Different algorithms have varying statistical artefacts to deal with noisy absences, inevitably resulting in uncertainty in the prediction of spatial distributions (Lauzeral, Grenouillet, & Brosse, 2012). Therefore, absence points generated from field sampling of locations of commission errors should be treated with caution because their inclusion in iterative niche models can result in the underprediction of the potential niche. Conversely, additional absence points resulting from iterative niche modeling will reduce the absence ratio and increase specificity in the model predictions. This trend was observed in our study and has also been observed in previous studies (Chiffard et al., 2020).

A common limitation of ecological niche modeling for rare species is the lack of true absence points; therefore, many modelers are restricted to generating and using pseudo-absence points (Barbet-Massin, Jiguet, Albert, & Thuiller, 2012). However, niche models that used true absence points performed better than those that used pseudo-absence points (Elith et al. 2006, Elith et al. 2011, Drake 2014). However, many state and federal conservation agencies in the United States have repositories of historical vegetative sampling that can help overcome the lack of true absence points for niche modeling. We recommend the development of open-source flora databases from these site-specific plant surveys which can be used to generate true absence points for ecological niche modeling.

Considerations regarding the choice of predictor variables

The perennial herbaceous layer, which is a spatial distribution of native grasses, forbs and cacti (Wickham et al, 2014), consistently contributed the most to all niche models. This may be partly due to that fact that biotic variables help reduce commission errors by predicting suitability only in areas with favorable abiotic and biotic conditions (de Araújo et al. 2014), which can only be captured in the fine-scale modeling used in this study (Heikkinen et al. 2007a, Muñoz-Mas et al. 2017). The niche models showed that *I. webberi* are located in sites with moderate to high perennial herb cover, which may reflect the abundance and coexistence of *I. webberi* and native shrubs such as *A. arbuscula* observed in known sites. The significant contribution of the perennial herb layer to *I. webberi* niche models validates previous studies that showed the importance of biotic interactions in niche models (e.g., Araújo and Luoto 2007, Heikkinen et al. 2007b, Meier et al. 2010, Bateman et al. 2012). It is widely believed that climatic factors influence species distributions at coarse scale, and the effect of biotic interactions are more appropriately captured at smaller spatial resolutions (Whittaker et al. 2001, Pearson and Dawson 2003, McGill 2010, Ashcroft et al. 2011, but see Meier et al. 2010, Bateman et al. 2012, de Araújo et al. 2014), a concept formally described as the Eltonian noise hypothesis (Soberón and Nakamura 2009).

In this study, we incorporated biotic interactions in five ways: a) modeling at fine scale (30 m) to capture the floristic heterogeneity, since some of *I. webberi* locations are in forest gaps, b) inclusion of forest cover to illustrate competitive biotic interaction since *I. webberi* does not grow under the trees, c) inclusion of remotely sensed modified soil-adjusted vegetation index (MSAVI) and the normalized difference vegetation index (NDVI), d) inclusion of *Artemisia arbuscula* spatial distribution, which is recorded in all *I. webberi* sites, and e) inclusion of layers of spatial distribution of annual and perennial herbs in the Great Basin Desert (Wickham et al. 2014). However, all predictor variables representing biotic interactions, except the perennial herbaceous layer, were excluded following the three variable reduction processes. Additionally, preliminary modeling efforts (not reported here) that included layers of spatial distributions of *Artemisia arbuscula* and other native Great Basin Desert shrubs, suggesting facilitative biotic interaction, did not improve niche models. This is possibly due to the fact that *I. webberi* occupies a small portion of the distribution range of these native shrubs, which suggests a lack of true ecological fidelity between *I. webberi* and *A. arbuscula*. Previous studies have also included biotic interactions (competition, facilitation and resource availability) as spatial distribution layers of other species (e.g., Meier et al. 2010, Linder et al. 2012, Blois et al. 2013, Pellissier et al. 2013, de Araújo et al. 2014, Anderson 2016), co-occurrence matrix of vegetation types (e.g., Pellissier et al. 2010, Kissling et al. 2012, González-Salazar et al. 2013, Pollock et al. 2014), species' functional traits (e.g., Kraft et al. 2008, Morales-Castilla et al. 2015), remotely sensed vegetation indices and image texture which are proxies for species richness, plant productivity and habitat physiognomy (Oindo and Skidmore 2002, Zimmerman et al. 2007, Buermann et al. 2008), and mechanistic niche models (Peterson and Soberón 2005).

Experts have discouraged the use of topographical variables for niche modeling because such variables have no direct relationship with species, but are proxies of climatic variables and hydrological processes (Austin 2007). However, the Great Basin Desert and the adjacent Sierra Nevada Range are home to relatively young neo-endemic plant taxa, and the origin and spatial endemism of these young endemics is attributed to several environmental factors including topographic heterogeneity (Kraft et al. 2010, Thornhill et al. 2017). Furthermore, topographic variables have been reported as an important driver of species diversity in plants, amphibians, birds and mammals (O'Brien et al. 2000, Hortal et al. 2009, Albuquerque et al. 2019). Therefore, topographic variables are more than just micro-climate proxies for *I. webberi*, but they may also delineate the slope positions of the sites where the species may occur. Furthermore, species were reported to change their topographic positions due to variations in climatic conditions at fine scales (Guisan and Zimmerman 2000), which may explain why *I. webberi* was fitted in niche models to occur in either gentle lateral slopes or ridges. This validates field reports that have observed that known populations of *I. webberi* occur in mid-elevation, gentle slope areas (Witham 2000, USFWS 2014). Previous studies have also acknowledged the contribution of topographic variables in species distribution modeling for plant species (e.g., Guisan et al. 1999, Chardon et al. 2014, Kübler et al. 2016).

In this study, summer precipitation was the third important predictor for the 2020 niche modeling iteration, replacing AET and cosine aspect in the previous modeling iterations. *I. webberi* seeds are not adapted for long distance dispersal common with wind-dispersed seeds, but gravity-assisted seed dispersal by surface run-off resulting in the colonization of empty microsites and decolonized roads and trails have been observed. Therefore, summer precipitation may play an important role in the *I. webberi* climatic niche and seed dispersal. Moreover, summer precipitation represents rainfall seasonality that has been linked with annual grass dominance in the Mediterranean region (Clary 2008). Chen et al. (2019) also reported that increase in summer precipitation facilitates seed dormancy for spring germinating annual plant species in a cold desert. Water, sourced mainly from precipitation, is the principal limiting factor for plant physiology and growth in the Great Basin Desert (Donovan and Ehleringer 1994). Not only do climatic variables have an impact on species distributions (Bellard et al. 2012, Araújo and Rozenfeld 2014), they also influence the phenology of winter and spring annuals and perennials in the Great Basin Desert. For example, the timing of *I. webberi* regeneration from seeds and vegetative caudices is determined by increase in soil temperature which results in gradual snow melt and availability of soil moisture (Witham 2000, USFWS 2014).

Comparison of modeling algorithms

Rare species are given conservation priority and ecological niche models can inform the management of such imperiled species. Therefore, it is important to use modeling algorithms that are less sensitive to prevalence and biased occurrence records, while also having high predictive ability. In this study, machine learning algorithms, especially random forests and boosted regression trees, performed excellently in fitting the niche model of *Ivesia webberi*. This result is congruent with literature and demonstrates the statistical ability of machine learning algorithms in fitting niche models of species with fewer occurrences (Marmion et al. 2009, Mi et al. 2017). Moreover, the spatial distribution of co-occurring species, that is, biotic variables, is often influenced by abiotic variables (Anderson 2013); therefore, abiotic variables used for niche modeling may have incorporated the non-independent effects of biotic interactors (Costa et al. 2010, Brewer and Gaston 2003, Soberón and Nakamura 2009). Machine learning algorithms were reported to be ideal for modeling the non-independent effects of biotic interactors (Elith and Graham 2009, Latimer et al. 2009, Anderson 2016).

Conversely, GLM had the poorest model performance based on TSS and AUC, while ANN and MAXENT models performed poorly under the continuous Boyce index and specificity respectively. This is congruent with previous studies where RF was reported to have had the highest model performance while GLMs had the lowest, especially when an independent test data was not used (Oppel et al. 2012, Guo, Lek, Ye, Li, Liu, & Li 2015). Previous studies have shown that GLMs are sensitive to prevalence in the dataset (Fielding and Bell 1997), but produce a more reliable spatial interpolation (Iturbide, Bedia, & Gutiérrez 2018).

However, fitting weighted mean ensemble models using the models with high predictive performance from the six single algorithms produced the best overall model performance. The superior model performance of ensemble models over single algorithms is well established in literature, and demonstrates their ability to reduce prediction errors (Marmion et al. 2009, Li and Wang 2013, Guo et al. 2015).

Conclusion and future directions

Occurrence points for rare species are often incomplete and variable selection for niche modeling is commonly based on expert knowledge rather than from empirical studies; therefore, predictions and transferability of such niche models can be suspect. In this study, we explored the efficacy of two main approaches for addressing the challenges associated with niche modeling for rare species: ensemble modeling, and iterative niche modeling. Ensemble modeling helps to reduce prediction error that can arise from using incomplete and biased occurrence data for rare species, while iterative niche modeling and model-guided field surveys can reduce the spatial bias, allow for model fine tuning that can improve model performance, and increase the chances of detecting novel species locations that can either fill the realized niche space or expand species niche breadth and geographical distribution range. These two approaches increased the scientific understanding of species-environment relationships for *I. webberi*. Furthermore, our work also highlights the value of incorporating biotic predictor variables in ecological niche modeling. Future directions in this study should intensify model-guided surveys to unsampled areas, especially through collaborative efforts with citizen scientists, and the resulting spatial data should be used for further iterative niche modeling.

REFERENCES

Aguirre-Gutiérrez, J., Carvalheiro, L. G., Polce, C., van Loon, E. E., Raes, N., Reemer M., & Biesmeijer J. C. (2013). Fit-for-Purpose: Species distribution model performance depends on evaluation criteria – Dutch Hoverflies as a case study. *PLoS ONE,* 8(5), e63708. doi:10.1371/journal.pone.0063708

Aiello-Lammens, M. E., Boria, R. A., Radosavljevic, A., Vilela, B., & Anderson, R.P. (2015). spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. *Ecography*, 38(5), 541-545. https://doi.org/10.1111/ecog.01132

Aitken, M., Roberts, D. W., & Shultz, L. M. (2007). Modelling distributions of rare plants in the great basin, Western North America. *Western North American Naturalist*, 67, 26-38.

Albuquerque, F., Astudillo-Scalia, Y., Loyola, R., & Beier, P. (2019). Towards an understanding of the drivers of broad-scale patterns of rarity-weighted richness for vertebrates. *Biodiversity and Conservation* 28, 3733–3747. https://doi.org/10.1007/s10531-019-01847-z

Allouche, O., Tsoar, A., & Kadmon, R. (2006). Assessing the accuracy of species distribution models: Prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology*, 43, 1223–1232. https://doi.org/10.1111/j.1365-2664.2006.01214.x

Anderson, R. P. (2013). A framework for using niche models to estimate impacts of climate change on species distributions. *Annals of the New York Academy of Sciences* 1297 (1), 8– 28. https://doi.org/10.1111/nyas.12264

Anderson R. P. (2016). When and how should biotic interactions be considered in models of species niches and distributions? *Journal of Biogeography* 44(1), 8-17. https://doi.org/10.1111/jbi.12825

Aranda, S. C., & Lobo, J. M. (2011). How well does presence-only-based species distribution modelling predict assemblage diversity? A case study of the Tenerife flora. *Ecography*, 34, 31–38. https://doi.org/10.1111/j.1600-0587.2010.06134.x

Araújo, M. B., & New, M. (2007). Ensemble forecasting of species distributions. *Trends in Ecology & Evolution*, 22, 42-47. https://doi.org/10.1016/j.tree.2006.09.010

Araújo, M. B., & Peterson, A. T. (2012). Uses and misuses of bioclimatic envelope modeling. *Ecology*, 93, 1527-1539. https://doi.org/10.1890/11-1930.1

Araújo, M. B., & Rozenfeld, A. (2014). The geographic scaling of biotic interactions. *Ecography*, 37, 406-415. https://doi.org/10.1111/j.1600-0587.2013.00643.x

Araújo, M. B., Whittaker, R. J., Ladle, R. J., & Erhard, M. (2005). Reducing uncertainty in projections of extinction risk from climate change. *Global Ecology and Biogeography*, 14, 529–538. https://doi.org/10.1111/j.1466-822X.2005.00182.x

Araujo, M. B., & Guisan, A. (2006). Five (or so) challenges for species distribution modelling. *Journal of Biogeography*, 33, 1677–1688. https://doi.org/10.1111/j.1365-2699.2006.01584.x

Araujo, M. B., & Luoto, M. (2007). The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology and Biogeography*, 16, 743–753. https://doi.org/10.1111/j.1466-8238.2007.00359.x

Ashcroft, M. B., French, K. O., & Chisholm, L. A. (2011). An evaluation of environmental factors affecting species distributions. *Ecological Modelling*, 222, (3): 524– 531. https://doi.org/10.1016/j.ecolmodel.2010.10.003

Austin, M. (2007). Species distribution models and ecological theory: A critical assessment and some possible new approaches. *Ecological Modelling,* 200(1–2), 1-19. https://doi.org/10.1016/j.ecolmodel.2006.07.005

Austin, M. P. (2002). Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. *Ecological Modelling*, 157(2-3), 101-118. https://doi.org/10.1016/S0304-3800(02)00205-3

Banerjee, A. K., Mukherjee, A., Guo, W., Ng, W. L., & Huang, Y. (2019). Combining ecological niche modeling with genetic lineage information to predict potential distribution of Mikania micrantha Kunth in South and Southeast Asia under predicted climate change. *Global Ecology and Conservation*, 20, e00800. https://doi.org/10.1016/j.gecco.2019.e00800

Barbet-Massin M., Jiguet F., Albert C.H., & Thuiller W. (2012). Selecting pseudo-absences for species distribution models: how, where and how many? *Methods in Ecology and Evolution* 3(2), 327-338. https://doi.org/10.1111/j.2041-210X.2011.00172.x

Bateman, B. L., VanDerWal, J., Williams, S. E., & Johnson, C. N. (2012). Biotic interactions influence the projected distribution of a specialist mammal under climate change. *Diversity and Distributions*, 18, 861 – 872. https://doi.org/10.1111/j.1472-4642.2012.00922.x

Beaumont, L. J., Graham, E., Duursma, D. E., Wilson, P. D., Cabrelli, A., Baumgartner, J. B., Hallgren, W., Esperón-Rodríguez, M., Nipperess, D. A., Warren, D. L., Laffan, S. W., & VanDerWal, J. (2016). Which species distribution models are more (or less) likely to project broad-scale, climate-induced shifts in species ranges? *Ecological Modelling*, 342, 135-146. http://dx.doi.org/10.1016/j.ecolmodel.2016.10.004

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, 15(4), 365-377. https://doi.org/10.1111/j.1461-0248.2011.01736.x

Blois, J. L., Zarnetske, P. L., Fitzpatrick, M. C., & Finnegan, S. (2013). Climate change and the past, present, and future of biotic interactions. *Science,* 341(6145), 499–504. 10.1126/science.1237184

Booth, T. H., Nix, H. A., Busby, J. R., & Hutchinson, M. F. (2014). bioclim: the first species distribution modelling package, its early applications and relevance to most current MaxEnt studies. *Diversity and Distributions*, 20(1), 1-9. https://doi.org/10.1111/ddi.12144

Boyce, M. S., Vernier, P. R., Nielsen, S. E., & Schmiegelow, F. K. A. (2002). Evaluating resource selection functions. *Ecological Modelling*, 157(2-3), 281-300. https://doi.org/10.1016/S0304-3800(02)00200-4

Breiman L. (2001). Random Forests. *Machine Learning,* 45, 5–32. https://doi.org/10.1023/A:1010933404324

Breiner, F. T., Guisan, A., Bergamini, A., & Nobis, M. P. (2015). Overcoming limitations of modelling rare species by using ensembles of small models. *Methods in Ecology and Evolution*, 6, 1210–1218. https://doi.org/10.1111/2041-210X.12403

Brewer, A. M., & Gaston, K. J. (2003). The geographical range structure of the holly leaf-miner. II. Demographic rates. *Journal of Animal Ecology*, 72(1), 82– 93. https://doi.org/10.1046/j.1365-2656.2003.00682.x

Broennimann, O., Fitzpatrick, M. C., Pearman, P. B., Petitpierre, B., Pellissier, L., Yoccoz, N. G., Thuiller, W., Fortin, M.-J., Randin, C., Zimmermann, N. E., Graham, C.H., & Guisan, A. (2012). Measuring ecological niche overlap from occurrence and spatial environmental data. *Global Ecology and Biogeography*, 21, 481– 497. https://doi.org/10.1371/journal.pone.0049508

Broennimann, O., Thuiller, W., Hughes, G., Midgley, G. F., Alkemade, J. M. R., & Guisan, A. (2006). Do geographic distribution, niche property and life form explain plants' vulnerability to global change? *Global Change Biology*, 12, 1079-1093. https://doi.org/10.1111/j.1365-2486.2006.01157.x

Buermann, W., Saatchi, S., Smith, T. B., Zutta, B. R., Chaves, J. A., Milá, B., & Graham, C. H. (2008). Predicting species distributions across the Amazonian and Andean regions using remote sensing data. *Journal of Biogeography*, 35(7), 1160-1176. https://doi.org/10.1111/j.1365-2699.2007.01858.x

Burns, P. A., Clemann, N., & White, M. (2020). Testing the utility of species distribution modelling using Random Forests for a species in decline. *Austral Ecology*, 45(6), 706-716. https://doi.org/10.1111/aec.12884

Carvalho, S. B., Brito, J. C., Crespo, E. J., & Possingham, H. P. (2011). From climate change predictions to actions-conserving vulnerable animal groups in hotspots at a regional scale. *Global Change Biology*, 16, 3257–3270. https://doi.org/10.1111/j.1365-2486.2010.02212.x

Chardon, N. I., Cornwell, W. K., Flint, L. E., Flint, A. L., & Ackerly, D. D. (2014). Topographic, latitudinal and climatic distribution of *Pinus coulteri*: geographic range limits are not at the edge of the climate envelope. *Ecography*, 37, 001–012. https://doi.org/10.1111/ecog.00780

Chen, Y., Shi, X., Zhang, L., Baskin, J. M., Baskin, C. C., Liu, H., & Zhang, D. (2019). Effects of increased precipitation on the life history of spring- and autumn-germinated plants of the cold desert annual *Erodium oxyrhynchum* (Geraniaceae). *AoB PLANTS,* 11(2), plz004. https://doi.org/10.1093/aobpla/plz004

Clary, J. (2008). Rainfall seasonality determines annual/perennial grass balance in vegetation of Mediterranean Iberian. *Plant Ecology,* 195(1), 13-20. https://doi.org/10.1007/s11258-007-9294-9

Costa, G. C., Nogueira, C., Machado, R. B., & Colli, G. R. (2010). Sampling bias and the use of ecological niche modeling in conservation planning: a field evaluation in a biodiversity hotspot. *Biodiversity and Conservation*, 19, 883– 899. https://doi.org/10.1007/s10531-009-9746-8

Cutler, D. R., Edwards, T. C., Beard, K. H., Cutler, A., Hess, K. T., Gibson, J., & Lawler, J. J. (2007). Random forests for classification in ecology. *Ecology*, 88(11), 2783-2792. https://doi.org/10.1890/07-0539.1

Daly C., Halbleib M., Smith J. I., Gibson W. P., Doggett M. K., Taylor G. H., Curtis J., & Pasteris P. A. (2008). Physiographically-sensitive mapping of temperature and precipitation across the conterminous United States. *International Journal of Climatology*, 28, 2031-2064. https://doi.org/10.1002/joc.1688

de Araújo C. B., Marcondes-Machado L. O., & Costa G. C. (2014). The importance of biotic interactions in species distribution models: a test of the Eltonian noise hypothesis using parrots. *Journal of Biogeography*, 41, 513–523. https://doi.org/10.1111/jbi.12234

de Siqueira, M. F., Durigan, G., de Marco Jr., P., & Peterson, A. T. (2009). Something from nothing: Using landscape similarity and ecological niche modeling to find rare plant species. *Journal for Nature Conservation,* 17(1), 25-32. https://doi.org/10.1016/j.jnc.2008.11.001

De'ath G. (2007). Boosted Trees for ecological modeling and prediction. *Ecology*, 88(1), 243-251. https://doi.org/10.1890/0012-9658(2007)88[243:BTFEMA]2.0.CO;2

Deb, C. R., Jamir, N. S., & Kikon Z. P. (2017). Distribution prediction model of a rare Orchid species (Vanda bicolor Griff.) using small sample size. *American Journal of Plant Sciences,* 8, 1388-1398. https://doi.org/10.4236/ajps.2017.86094

Dilts, T. E., Weisberg, P. J., Dencker, C. M., & Chambers, J. C. (2015). Functionally relevant climate variables for arid lands: a climatic water deficit approach for modelling desert shrub distributions. *Journal of Biogeography,* 42(10), 1986-1997. https://doi.org/10.1111/jbi.12561

Dilts, T.E. (2014). Climatic Water Deficit Toolbox for ArcGIS 10.1. University of Nevada Reno. Available at: http://www.arcgis.com/home/item.html?id=de9ca57d43c041148b815da7ce4aa3a0

Donovan, L. A., & Ehleringer, J. R. (1994). Water-stress and use of summer precipitation in a Great Basin shrub community. *Functional Ecology* 8(3), 289– 297. https://doi.org/10.2307/2389821

Dormann, C. F., Calabrese, J. M., Guillera-Arroita, G., Matechou, E., Bahn, V., Bartoń, F., Beale, C. M., Ciuti, S., Elith, J., Gerstner, K., Guelat, J., Keil, P., Lahoz-Monfort, J. J., Pollock, L. J., Reineking, B., Roberts, D. R., Schröder, B., Thuiller, W., Warton, D. I., Wintle, B. A., Wood, S. N., Wüest, R. O., & Hartig, F. (2018). Model averaging in ecology: A review of Bayesian, information-theoretic and tactical approaches. *Ecological Monographs*, 88, 485– 504. https://doi.org/10.1002/ecm.1309

Drake, J. M. (2014). Ensemble algorithms for ecological niche modeling from presence-background and presence-only data. *Ecosphere*, 5(6), 1-16. https://doi.org/10.1890/ES13-00202.1

Elith, J., Graham, C., Anderson, R., Dudı´k, M., Ferrier, S., Guisan, A., Hijmans, R., Huettmann, F., Leathwick, J., Lehmann, A., Li, J., Lohmann, L., Loiselle, B., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J., Peterson, A. T., Phillips, S., Richardson, K., Scachetti-Pereira, R., Schapire, R., Soberon, J., Williams, S., Wisz, M., & Zimmermann, N. (2006). Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, 29, 129–151. https://doi.org/10.1111/j.2006.0906-7590.04596.x

Elith, J., & Graham, C. H. (2009). Do they? How do they? Why do they differ? -- on finding reasons for differing performances of species distribution models. *Ecography*, 32(1), 66-77. https://doi.org/10.1111/j.1600-0587.2008.05505.x

Elith, J., & Leathwick, J. R. (2009). Species Distribution Models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics*, 40, 677-697. https://doi.org/10.1146/annurev.ecolsys.110308.120159

Elith, J., Leathwick, J. R., & Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology*, 77, 802-813. https://doi.org/10.1111/j.1365-2656.2008.01390.x

Escober, L. E., Qiao, H., Cabello, J., & Peterson A. T. (2018). Ecological niche modeling re-examined: A case study with the Darwin's fox. *Ecology and Evolution*, 8, 4757–4770. https://doi.org/10.1002/ece3.4014

Fielding, A. H., & Bell, J. F. (1997). A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation*, 24(1), 38-49. https://doi.org/10.1017/S0376892997000088

Galante, P. J., Alade, B., Muscarella, R., Jansa, S. A., Goodman, S. M., & Anderson, R. P. (2018). The challenge of modeling niches and distributions for data-poor species: A comprehensive approach to model complexity. *Ecography*, 41(5), 726–736. https://doi.org/10.1111/ecog.02909

Garcia, R. A., Burgess, N. D., Cabeza, M., Rahbek, C., Araújo, M. B. (2012). Exploring consensus in 21st century projections of climatically suitable areas for African vertebrates. *Global Change Biology*, 18, 1253-1269. https://doi.org/10.1111/j.1365-2486.2011.02605.x

García-Callejas, D., & Araújo, M. B. (2016). The effects of model and data complexity on predictions from species distributions models. *Ecological Modelling*, 326, 4–12. https://doi.org/10.1016/j.ecolmodel.2015.06.002

González-Salazar, C., Stephens, C. R., & Marquet, P. A. (2013). Comparing the relative contributions of biotic and abiotic factors as mediators of species' distributions. *Ecological Modelling*, 248, 57– 70. https://doi.org/10.1016/j.ecolmodel.2012.10.007

Gorban, A., Pokidysheva, L., Smirnova, E., & Tyukina, T. (2011). Law of the minimum paradoxes. *Bulletin of Mathematical Biology*, 73, 2013–2044. 10.1007/s11538-010-9597-1

Grenouillet, G., Buisson, L., Casajus, N., & Lek, S. (2011). Ensemble modelling of species distribution: the effects of geographical and environmental ranges. *Ecography*, 34, 9-17. https://doi.org/10.1111/j.1600-0587.2010.06152.x

Groff, L. A., Marks, S. B., & Hayes, M. P. (2014). Using ecological niche models to predict rare amphibian surveys: A case study using the Oregon spotted frog (*Rana pretiosa*). *Herpetological Conservation and Biology*, 9(2), 354−368.

Gromping, U. (2009). Variable importance assessment in regression: linear regression versus random forest. *The American Statistician*, 63(4): 308-319. https://doi.org/10.1198/tast.2009.08199

Guisan, A, & Zimmermann, N. E. (2000). Predictive habitat distribution models in ecology. *Ecological Modelling*, 135, 147-186. https://doi.org/10.1016/S0304-3800(00)00354-9

Guisan, A., & Thuiller, W. (2005). Predicting species distributions: offering more than simple habitat models. *Ecology Letters*, 8, 993–1009. https://doi.org/10.1111/j.1461-0248.2005.00792.x

Guisan, A., Edwards, Jr T. C., & Hastie, T. (2002). Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling*, 157(2-3), 89-100. https://doi.org/10.1016/S0304-3800(02)00204-1

Guisan, A., Weiss, S. B., & Weiss, A. D. (1999). GLM versus CCA spatial modeling of plant species distribution. *Plant Ecology*, 143, 107–122. https://doi.org/10.1023/A:1009841519580

Guisan, A., Broennimann, O., Engler, R., Vust, M., Yoccoz, N. G., Lehmann, A., & Zimmermann, N. E. (2006). Using niche-based models to improve the sampling of rare species. *Conservation Biology*, 20(2), 501-511. https://doi.org/10.1111/j.1523-1739.2006.00354.x

Guo, C., Lek, S., Ye, S., Li, W., Liu, J., & Li, Z. (2015). Uncertainty in ensemble modelling of large-scale species distribution: Effects from species characteristics and model techniques. *Ecological Modelling*, 306, 67-75. http://dx.doi.org/10.1016/j.ecolmodel.2014.08.002

Gutiérrez, E. E., Boria, R. A., & Anderson, R. P. (2014). Can biotic interactions cause allopatry? Niche models, competition, and distributions of South American mouse opossums. *Ecography*, 37(8), 741-753. https://doi.org/10.1111/ecog.00620

Hanley, J. A., & McNeil, B. J. (1982). The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology*, 143, 29–36. https://doi.org/10.1148/radiology.143.1.7063747

Hao, T., Elith, J., Guillera-Arroita, G., & Lahoz-Monfort, J. J. (2019). A review of evidence about use and performance of species distribution modelling ensembles like BIOMOD. *Diversity and Distributions*, 25(5), 839-852. https://doi.org/10.1111/ddi.12892

Harrell, F. E., Lee, K. L., & Mark, D. B. (1996). Tutorial in biostatistics multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Statistics in medicine*, 15, 361–387. https://doi.org/10.1002/(SICI)1097-0258(19960229)15:4<361::AID-SIM168>3.0.CO;2-4

Hastie, T., & Tibshirani, R. (1990). *Generalized Additive Models*. Chapman & Hall, London.

Heikkinen, R. K., Luoto, M., Kuussaari, M., & Toivonen, T. (2007a). Modelling the spatial distribution of a threatened butterfly: Impacts of scale and statistical technique. *Landscape and Urban Planning*, 79(3-4), 347-357. https://doi.org/10.1016/j.landurbplan.2006.04.002

Heikkinen, R. K., Luoto, M., Virkkala, R., Pearson, R. G., & Körber, J. H. (2007b). Biotic interactions improve prediction of boreal bird distributions at macro-scales. *Global Ecology and Biogeography*, 16(6), 754–763. https://doi.org/10.1111/j.1466-8238.2007.00345.x

Hernandez, P. A., Graham, C. H., Master, L. L., & Albert, D. L. (2006). The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, 29, 773-785. https://doi.org/10.1111/j.0906-7590.2006.04700.x

Hijmans, R. J., Phillips, S., Leathwick, J., & Elith, J. (2017). dismo: Species Distribution Modeling. R Package Version 1.0-12. http://CRAN.R-project.org/package=dismo

Hirzel, A. H., & Le Lay, G. (2008). Habitat suitability modeling and niche theory. *Journal of Applied Ecology*, 45, 1372-1381. https://doi.org/10.1111/j.1365-2664.2008.01524.x

Hirzel, A. H., Le Lay, G., Helfer, V., Randin, C., & Guisan, A. (2006). Evaluating the ability of habitat suitability models to predict species presences. *Ecological Modelling*, 199(2), 142-152. https://doi.org/10.1016/j.ecolmodel.2006.05.017

Hortal, J., Triantis, K. A., Meiri, S., Thébault, E., & Sfenthourakis, S. (2009). Island species richness increases with habitat diversity. *The American Naturalist*, 174, 205–217. https://doi.org/10.1086/645085

Ikeda, D. H., Max, T. L., Allan, G. J., Lau, M. K., Shuster, S. M., & Whitham, T. G. (2016). Genetically informed ecological niche models improve climate change predictions. *Global Change Biology*, 23(1), 164-176. https://doi.org/10.1111/gcb.13470

Iturbide, M., Bedia, J., & Gutiérrez, J. M. (2018). Background sampling and transferability of species distribution model ensembles under climate change. *Global and Planetary Change*, 166, 19-29. https://doi.org/10.1016/j.gloplacha.2018.03.008

Jarnevich, C. S., Stohlgren, T. J., Kumar, S., Morisette, J. T., & Holcombe, T. R. (2015). Caveats for correlative species distribution modeling. *Ecological Informatics*, 29, 6-15. https://doi.org/10.1016/j.ecoinf.2015.06.007

Jiménez-Valverde, A., Lobo, J. M., & Hortal, J. (2008). Not as good as they seem: the importance of concepts in species distribution modelling. *Diversity and Distributions*, 14, 885–890. 10.1111/j.1472-4642.2008.00496.x

Jiménez-Valverde, A., Peterson, A. T., Soberón, J., Overton, J. M., Aragón, P. & Lobo, J. M. (2011). Use of niche models in invasive species risk assessments. *Biological Invasions*, 13, 2785-2797. https://doi.org/10.1007/s10530-011-9963-4

Keeley, A. T. H., Beier, P., Keeley, B. W., & Fagan, M. E. (2017). Habitat suitability is a poor proxy for landscape connectivity during dispersal and mating movements. *Landscape and Urban Planning*, 161, 90–102. https://doi.org/10.1016/j.landurbplan.2017.01.007

Kissling, W. D., Dormann, C. F., Groeneveld, J., Hickler, T., Kühn, I., McInerny, G. J., Montoya, J. M., Römermann, C., Schiffers, K., Schurr, F. M., Singer, A., Svenning, J. -C., Zimmermann, N. E., & O'Hara, R. B. (2012). Towards novel approaches to modelling biotic interactions in multispecies assemblages at large spatial extents. *Journal of Biogeography*, 39(12), 2163– 2178. https://doi.org/10.1111/j.1365-2699.2011.02663.x

Kraft, N. J., Baldwin, B. G., & Ackerly, D. D. (2010). Range size, taxon age and hotspots of neoendemism in the California flora. *Diversity and Distributions*, 16, 403–413. https://doi.org/10.1111/j.1472-4642.2010.00640.x

Kraft, N. J. B., Valencia, R., & Ackerly, D. D. (2008). Functional traits and niche-based tree community assembly in an Amazonian forest. *Science*, 322(5901), 580– 582. 10.1126/science.1160662

Kübler, D., Hildebrandt, P., Günter, S., Stimm, B., Weber, M., Mosandl, R., Muñoz, J., Cabrera, O., Aguirre, N., Zeilinger, J., & Silva, B. (2016). Assessing the importance of topographic variables for the spatial distribution of tree species in a tropical mountain forest. *Erdkunde,* 70(1), 19-47.

Kuhn, M. (2008). Building Predictive Models in R Using the caret Package. *Journal of Statistical Software*, 28(5), 1-26. 10.18637/jss.v028.i05

Kursa, M. B., & Rudnicki, W. R. (2010). Feature selection with the Boruta package. *Journal of Statistical Software,* 36(11), 1-13. 10.18637/jss.v036.i11

Latimer, A. M., Banerjee, S., Sang, H., Mosher, E. S., & Silander, J. A. (2009). Hierarchical models facilitate spatial analysis of large data sets: a case study on invasive plant species in the northeastern United States. *Ecology Letters*, 12(2), 144– 154. https://doi.org/10.1111/j.1461-0248.2008.01270.x

Lauzeral, C., Grenouillet, G., & Brosse, S. (2012). Dealing with noisy absences to optimize species distribution models: An iterative ensemble modelling approach. *PLoS ONE, 7,* e49508. https://doi.org/10.1371/journal.pone.0049508

Lavergne, S., Thuiller, W, Molina, J., & Debussche, M. (2005). Environmental and human factors influencing rare plant local occurrence, extinction and persistence: a 115-year study in the Mediterranean region. *Journal of Biogeography,* 32(5), 799-811. http://dx.doi.org/10.1111/j.1365-2699.2005.01207.x

Le Lay, G., Engler, R., Franc, E., & Guisan, A. (2010). Prospective sampling based on model ensembles improves the detection of rare species. *Ecography*, 33(6), 1015-1027. https://doi.org/10.1111/j.1600-0587.2010.06338.x

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: A package for multivariate analysis. *Journal of Statistical Software, 25*, 1–18. doi: 10.18637/jss.v025.i01.

Leathwick, J. R., Elith, J., Francis, M. P., Hastie, T., & Taylor, P. (2006). Variation in demersal fish species richness in the oceans surrounding New Zealand: an analysis using boosted regression trees. *Marine Ecology Progress Series*, 321, 267-281. doi:10.3354/meps321267

Lek, S., & Guegan, J. (1999). Artificial neural networks as a tool in ecological modelling, an introduction. *Ecological Modelling*, 120(2-3), 65-73. https://doi.org/10.1016/S0304-3800(99)00092-7

Li, X. H., & Wang, Y. (2013). Applying various algorithms for species distribution modelling. *Integrative Zoology,* 8, 124–135. https://doi.org/10.1111/1749-4877.12000

Linder, H. P., Bykova, O., Dyke, J., Etienne, R. S., Hickler, T., Kühn, I., Marion, G., Ohlemüller, R., Schymanski, S. J., & Singer, A. (2012). Biotic modifiers, environmental modulation and species distribution models. *Journal of Biogeography*, 39(12), 2179– 2190. https://doi.org/10.1111/j.1365-2699.2012.02705.x

Liu, C., White, M., & Newell, G. (2011). Measuring and comparing the accuracy of species distribution models with presence-absence data. *Ecography*, 34(2), 232–243. https://doi.org/10.1111/j.1600-0587.2010.06354.x

Lobo, J. M., Jiménez-Valverde, A., & Real, R. (2008). AUC: a misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography*, 17(2), 145– 151. https://doi.org/10.1111/j.1466-8238.2007.00358.x

Lobo, J. M. (2008). More complex distribution models or more representative data? *Biodiversity Informatics*, 5, 14–19. https://doi.org/10.17161/bi.v5i0.40

Lobo, J. M., & Tognelli, M. F. (2011). Exploring the effects of quantity and location of pseudo-absences and sampling biases on the performance of distribution models with limited point occurrence data. *Journal for Nature Conservation*, 19, 1– 7. https://doi.org/10.1016/j.jnc.2010.03.002

Lobo, J. M., Jiménez-Valverde, A., & Hortal, J. (2010). The uncertain nature of absences and their importance in species distribution modelling. *Ecography,* 33, 103–114. https://doi.org/10.1111/j.1600-0587.2009.06039.x

Lomba, A., Pellissier, L., Randin, C., Vicente, J., Moreira, F., Honrado, J., & Guisan, A. (2010). Overcoming the rare species modelling paradox: A novel hierarchical framework applied to an Iberian endemic plant. *Biological Conservation*, 143(11), 2647-2657. https://doi.org/10.1016/j.biocon.2010.07.007

Lomolino, M., Brown, J. H., & Riddle, B. (2005). *Biogeography*. Signaler Sunderland, MA, USA.

Marmion, M., Parviainen, M., Luoto, M., Heikkinen, R. K., & Thuiller, W. (2009). Evaluation of consensus methods in predictive species distribution modelling. *Diversity and Distributions*, 15, 59-69. https://doi.org/10.1111/j.1472-4642.2008.00491.x

McCullagh, P., & Nelder, J. A. (1989). *Generalized Linear Models*. Vol. 37, Monographs on Statistics and Applied Probability, 2 edn, Chapman and Hall, London.

McCune, J. L. (2016). Species distribution models predict rare species occurrences despite significant effects of landscape context. *Journal of Applied Ecology,* 53(6), 1871-1879. https://doi.org/10.1111/1365-2664.12702

McGill, B. J. (2010). *Matters of scale*. *Science*, 328, 575–576. 10.1126/science.1188528

McPherson, J. M., Jetz, W., & Rodgers, D. J. (2004). The effects of species' range sizes on the accuracy of distribution models: ecological phenomenon or statistical artefact? *Journal of Applied Ecology*, 41(5), 811-823. https://doi.org/10.1111/j.0021-8901.2004.00943.x

Meier, E. S., Kienast, F., Pearman, P. B., Svenning, J. -C., Thuiller, W., Araújo, M. B., Guisan, A., & Zimmermann, N. E. (2010). Biotic and abiotic variables show little redundancy in explaining tree species distributions. *Ecography*, 33, 1038–1048. https://doi.org/10.1111/j.1600-0587.2010.06229.x

Meller, L., Cabeza, M., Pironon, S., Barbet-Massin, M., Maiorano, L., Georges, D., & Thuiller, W. (2014). Ensemble distribution models in conservation prioritization: from consensus predictions to consensus reserve networks. *Diversity and Distributions*, 20, 309–321. https://doi.org/10.1111/ddi.12162

Menon, S., Choudhury, B. I., Khan, M. L., & Peterson, A. T. (2010). Ecological niche modeling and local knowledge predict new populations of *Gymnocladus assamicus* a critically endangered tree species. *Endangered Species Research*, 11, 175–181. https://doi.org/10.3354/esr00275

Merow, C., Smith, M. J., & Silander Jr., J. A. (2013). A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography*, 36(10), 1058-1069. https://doi.org/10.1111/j.1600-0587.2013.07872.x

Mi, C., Huettmann, F., Guo, Y., Han, X., & Wen, L. (2017). Why choose Random Forest to predict rare species distribution with few samples in large undersampled areas? Three Asian crane species models provide supporting evidence. *PeerJ*, 5, e2849. https://doi.org/10.7717/peerj.2849

Mishler, B. D., Guralnick, R., Soltis, P. S., Smith, S. A., Soltis, D. E., Barve, N., Allen, J. M., & Laffan, S. W. (2020). Spatial phylogenetics of the North American flora. *Journal of Systematics and Evolution*, 58(4), 393-405. https://doi.org/10.1111/jse.12590

Moisen, G. G., Freeman, E. A., Blackard, J. A., Frescino, T. S., Zimmermann, N. E., & Edwards, T. C. (2006). Predicting tree species presence and basal area in Utah: a comparison of stochastic gradient boosting, generalized additive models, and tree-based methods. *Ecological Modelling*, 199, 176– 187. https://doi.org/10.1016/j.ecolmodel.2006.05.021

Morales, N. S., Fernández, I. C., Carrasco, B., & Orchard, C. (2015). Combining niche modelling, land-use change, and genetic information to assess the conservation status of *Pouteria splendens* populations in Central Chile. *International Journal of Ecology*, Volume 2015, Article ID 612194, 12 pages. https://doi.org/10.1155/2015/612194

Morales-Castilla, I., Matias, M. G., Gravel, D., & Araújo, M. B. (2015). Inferring biotic interactions from proxies. *Trends in Ecology & Evolution*, 30(6), 347-356. http://dx.doi.org/10.1016/j.tree.2015.03.014

Moreno, R., Zamora, R., Molina, J. R., Vasquez, A., & Herrera, M. Á. (2011). Predictive modeling of microhabitats for endemic birds in South Chilean temperate forests using maximum entropy (maxent). *Ecological Informatics*, 6(6), 364-370. https://doi.org/10.1016/j.ecoinf.2011.07.003

Muñoz-Mas, R., Martínez-Capel, F., Alcaraz-Hernández J. D., Mouton A. M. (2017). On species distribution modelling, spatial scales and environmental flow assessment with Multi–Layer Perceptron Ensembles: A case study on the redfin barbel (*Barbus haasi*; Mertens, 1925). *Limnologica*, 62, 161-172. https://doi.org/10.1016/j.limno.2016.09.004

Newbold, T., Reader, T., El-Gabbas, A., Berg, W., Shohdi, W. M., Zalat, S., El Din, S. B., & Gilbert, F. (2010). Testing the accuracy of species distribution models using species records from a new field survey. *Oikos,* 119, 1326-1334. https://doi.org/10.1111/j.1600-0706.2009.18295.x

O'Brien, E. M., Field, R., & Whittaker, R. J. (2000). Climatic gradients in woody plant (tree and shrub) diversity: water–energy dynamics, residual variation, and topography. *Oikos*, 89, 588–600. https://doi.org/10.1034/j.1600-0706.2000.890319.x

Oindo, B. O., & Skidmore, A. K. (2002). Interannual variability of NDVI and species richness in Kenya. *International Journal of Remote Sensing*, 23(2), 285-298. https://doi.org/10.1080/01431160010014819

Oppel, S., Meirinho, A., Ramírez, I., Gardner, B., O'Connell, A. F., Miller, P. I., & Louzao, M. (2011). Comparison of five modelling techniques to predict the spatial distribution and abundance of seabirds. *Biological Conservation*, 156, 94–104. https://doi.org/10.1016/j.biocon.2011.11.013

Pearson, R. G., & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography*, 12(5), 361-371. https://doi.org/10.1046/j.1466-822X.2003.00042.x

Pearson, R. G., Thuiller, W., Araújo, M. B., Martinez-Meyer, E., Brotons, L., McClean, C., Miles, L., Segurado, P., Dawson, T. P., & Lees, D. C. (2006). Model-based uncertainty in species range prediction. *Journal of Biogeography*, 33(10), 1704-1711. https://doi.org/10.1111/j.1365-2699.2006.01460.x

Pearson, R. G., Raxworthy, C. J., Nakamura, M., & Townsend, P. A. (2007). Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, 34, 102–117.

Pellissier, L., Bråthen, K. A., Pottier, J., Randin, C. F., Vittoz, P., Dubuis, A., Yoccoz, N. G., Alm, T., Zimmermann, N. E., & Guisan, A. (2010). Species distribution models reveal apparent competitive and facilitative effects of a dominant species on the distribution of tundra plants. *Ecography*, 33(6), 1004-1014. https://doi.org/10.1111/j.1600-0587.2010.06386.x

Pellissier, L., Rohr, R. P., Ndiribe, C., Pradervand, J. -N., Salamin, N., Guisan, A., & Wisz, M. (2013). Combining food web and species distribution models for improved community projections. *Ecology and Evolution*, 3(13), 4572– 4583. https://doi.org/10.1002/ece3.843

Peterson, A. T., & Soberón, J. (2005). Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics*, 2, 1– 10. https://doi.org/10.17161/bi.v2i0.4

Petitpierre, B., Kueffer, C., Broennimann, O., Randin, C., Daehler, C., & Guisan. A. (2012). Climatic niche shifts are rare among terrestrial plant invaders. *Science*, 335, 1344–1348. 10.1126/science.1215933

Phillips, S. J., & Dudík, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, 31(2), 161-175. https://doi.org/10.1111/j.0906-7590.2008.5203.x

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, 190(3-4), 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Pollock, L. J., Tingley, R., Morris, W. K., Golding, N., O'Hara, R. B., Parris, K. M., Vesk, P. A., & Mccarthy, M. A. (2014). Understanding co-occurrence by modelling species simultaneously with a Joint Species Distribution Model (JSDM). *Methods in Ecology and Evolution*, 5(5), 397– 406. https://doi.org/10.1111/2041-210X.12180

Prasad, A. M., Iverson, L. R., & Liaw, A. (2006). Newer classification and regression tree techniques: bagging and random forests for ecological prediction. *Ecosystems*, 9, 181-199. https://doi.org/10.1007/s10021-005-0054-1

Qian, H., Zhang, Y., Zhang, J., & Wang, X. (2013). Latitudinal gradients in phylogenetic relatedness of angiosperm trees in North America. *Global Ecology and Biogeography*, 22(11), 1183–1191. https://doi.org/10.1111/geb.12069

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [online]. Available from URL: http://www.R-project.org/

Ramesh, V., Gopalakrishna, T., Barve, S., & Melnick, D. J. (2017). IUCN greatly underestimates threat levels of endemic birds in the Western Ghats. *Biological Conservation*, 210, 205– 221. https://doi.org/10.1016/j.biocon.2017.03.019

Rebelo, H., & Jones, G. (2010). Ground validation of presence-only modelling with rare species: a case study on barbastelles *Barbastella barbastellus* (Chiroptera: Vespertilionidae). *Journal of Applied Ecology*, 47, 410–420. https://doi.org/10.1111/j.1365-2664.2009.01765.x

Rinnhofer, L. J., Roura-Pascual, N., Arthofer, W., Dejaco, T., Thaler-Knoflach, B., Wachter, G. A., Christian, E., Steiner, F. M., & Schlick-Steiner, B. C. (2012).

Iterative species distribution modelling and ground validation in endemism research: An Alpine jumping bristletail example. *Biodiversity Conservation*, *21*, 2845–2863. https://doi.org/10.1007/s10531-012-0341-z

Ripley, B. D. (1996). *Pattern recognition and neural network*. Cambridge University Press, Cambridge New York Melbourne

Sánchez-Cordero, V., Stockwell, D., Sarkar, S., Liu, H., Stephens, C., & Gimenez, J. (2008). Competitive interactions between felid species may limit the southern distribution of bobcats *Lynx rufus*. *Ecography*, 31(6), 757–764. https://doi.org/10.1111/j.1600-0587.2008.05327.x

Särkinen, T., Gonzáles, P., & Knapp, S. (2013). Distribution models and species discovery: the story of a new Solanum species from the Peruvian Andes. *PhytoKeys*, 31, 1–20. https://doi.org/10.3897/phytokeys.31.6312

Scherson, R. A., Thornhill, A. H., Urbina-Casanova, R., Freyman, W. A., Pliscoff, P. A., & Mishler, B. D. (2017). Spatial phylogenetics of the vascular flora of Chile. *Molecular Phylogenetics and Evolution*, 112, 88– 95. https://doi.org/10.1016/j.ympev.2017.04.021

Segurado, P., & Araújo, M. B. (2004). An evaluation of methods for modelling species distributions. *Journal of Biogeography*, 31(10), 1555–1568. https://doi.org/10.1111/j.1365-2699.2004.01076.x

Shabani, F., Kumar, L., & Ahmadi, M. (2016). A comparison of absolute performance of different correlative and mechanistic species distribution models in an independent area. *Ecology and Evolution*, 6(16), 5973–5986. https://doi.org/10.1002/ece3.2332

Singh, N. J., Yoccoz, N. G., Bhatnagar, Y. V., & Fox, J. L. (2009). Using habitat suitability models to sample rare species in high-altitude ecosystems: a case study with Tibetan argali. *Biodiversity Conservation, 18*, 2893–2908. https://doi.org/10.1007/s10531-009-9615-5

Soberón, J., & Nakamura, M. (2009). Niches and distributional areas: concepts, methods, and assumptions. *Proceedings of the National Academy of Sciences of the United State of America*, 106(Supplement 2), 19644–19650. https://doi.org/10.1073/pnas.0901637106

Sousa-Silva, R., Alves, P., Honrado, J., & Lomba, A. (2014). Improving the assessment and reporting on rare and endangered species through species distribution models. *Global Ecology and Conservation*, 2, 226-237. http://dx.doi.org/10.1016/j.gecco.2014.09.011

Stockwell, D. R. B., & Peterson, A. T. (2002). Effects of sample size on accuracy of species distribution models. *Ecological Modelling*, 148, 1-13. https://doi.org/10.1016/S0304-3800(01)00388-X

Taylor S.D., and White E.P. (2020). Automated data-intensive forecasting of plant phenology throughout the United States. *Ecological Applications*, 30(1), e02025. https://doi.org/10.1002/eap.2025

Thornhill, A. H., Baldwin, B. G., Freyman, W. A., Nosratinia, S, Kling, M. M., Morueta-Holme, N., Madsen, T. P., Ackerly, D. D., & Mishler, B. D. (2017). Spatial phylogenetics of the native California flora. *BMC Biology*, 15, 96. https://doi.org/10.1186/s12915-017-0435-x

Thuiller, W. (2004). Patterns and uncertainties of species' range shifts under climate change. *Global Change Biology*, 10(12), 2020–2027. https://doi.org/10.1111/j.1365-2486.2004.00859.x

Thuiller, W., Lafourcade, B., Engler, R., & Araújo, M. B. (2009). BIOMOD – a platform for ensemble forecasting of species distributions. *Ecography*, 32, 1-5. https://doi.org/10.1111/j.1600-0587.2008.05742.x

Töpel, M., Antonelli, A., Yesson, C., & Eriksen, B. (2012). Past climate change and plant evolution in Western North America: a case study in Rosaceae. *PLoS ONE*, *7*, e50358. https://doi.org/10.1371/journal.pone.0050358

U.S. Geological Survey (2017). 1 Arc-second Digital Elevation Models (DEMs) - USGS National Map 3DEP Downloadable Data Collection: U.S. Geological Survey. https://www.usgs.gov/core-science-systems/ngp/tnm-delivery/

USFWS (2014). Species Report for *Ivesia webberi* (Webber's ivesia). Nevada Fish and Wildlife Office, Reno. United States Fish and Wildlife Services, 49 pp.

Wall, M. E., Rechtsteiner, A., & Rocha, L. M. (2003). Singular value decomposition and principal component analysis. In D. P. Berrar, W. Dubitzky, & M. Granzow (Eds.), *A Practical approach to microarray data analysis*, pp. 91-109. Norwell, MA: Kluwer Academic Publishers.

Warren, D. L., & Seifert, S. N. (2011). Ecological niche modeling in Maxent: The importance of model complexity and the performance of model selection criteria. *Ecological Applications*, 21(2), 335– 342. https://doi.org/10.1890/10-1171.1

Warren, D. L., Glor, R. E., & Turelli, M. (2008). Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution*, 62(11), 2868-2883. https://doi.org/10.1111/j.1558-5646.2008.00482.x

Weiss, A. (2001). Topographic position and landform analysis. Poster presentation, ESRI User Conference, San Diego, California, USA.

White, E. P., Yenni, G. M., Taylor, S. D., Christensen, E. M., Bledsoe, E. K., Simonis, J. L., & Ernest, S. K. M. (2018). Developing an automated iterative near-term forecasting system for an ecological study. *Methods in Ecology and Evolution*, 10, 332–344. https://doi.org/10.1111/2041-210X.13104

Whittaker, R. J., Willis, K. J., & Field, R. (2001). Scale and species richness: towards a general, hierarchical theory of species diversity. *Journal of Biogeography*, 28(4), 453–470. https://doi.org/10.1046/j.1365-2699.2001.00563.x

Wickham, J., Homer, C., Vogelmann, J., McKerrow, A., Mueller, R., Herold, N., & Coulston, J. (2014). The Multi-Resolution Land Characteristics (MRLC) Consortium — 20 Years of Development and Integration of USA National Land Cover Data. *Remote Sensing*, 6, 7424-7441. https://doi.org/10.3390/rs6087424

Wiesmeier, M., Barthold, F., Blank, B., & Kögel-Knabner, I. (2011). Digital mapping of soil organic matter stocks using random forest modeling in a semi-arid steppe ecosystem. *Plant Soil*, 340, 7-24. https://doi.org/10.1007/s11104-010-0425-z

Williams, J. N. Seo, C., Thorne, J., Nelson, J. K., Erwin, S., O’Brien, J., & Schwartz, M. W. (2009). Using species distribution models to predict new occurrences for rare plants. *Diversity and Distributions*, 15(4), 565-576. https://doi.org/10.1111/j.1472-4642.2009.00567.x

Wisz, M. S., Hijmans, R. J., Li, J., Peterson, A. T., Graham, C. H., Guisan, A. & NCEAS Predicting Species Distributions Working Group (2008). Effects of sample size on the performance of species distribution models. *Diversity and Distributions*, 14, 763-773. https://doi.org/10.1111/j.1472-4642.2008.00482.x

Witham, C. W. (2000). Current Knowledge and Conservation Status of *Ivesia webberi* A. Gray (Rosaceae), the Webber ivesia, in Nevada. Status Report prepared for the Nevada Natural Heritage Program, Dept. of Conservation and Natural Resources, Carson City, NV. 52pp.

Xian, G., Homer, C., Meyer, D., & Granneman, B. (2013). An approach for characterizing the distribution of shrubland ecosystem components as continuous fields as part of NLCD. *ISPRS Journal of Photogrammetry and Remote Sensing, 86*, 136-149. https://doi.org/10.1016/j.isprsjprs.2013.09.009

Young, N. E., Fairchild, M., Belcher, T., Evangelista, P., Verdone, C. J., & Stohlgren, T. J. (2019). Finding the needle in the haystack: iterative sampling and modeling for rare taxa. *Journal of Insect Conservation, 23,* 589–595. https://doi.org/10.1007/s10841-019-00151-z

Zimmermann, N. E., Edwards Jr., T. C., Moisen, G. G., Frescino, T. S., & Blackard, J. A. (2007). Remote sensing-based predictors improve distribution models of rare, early successional and broadleaf tree species in Utah. *Journal of Applied Ecology*, 44(5), 1057-1067. https://doi.org/10.1111/j.1365-2664.2007.01348.x

TABLES

Table 1. Iterative niche modeling across five years with increasing number of presence and absence points for *Ivesia webberi*

Year	**Presence points**	**Raw absence points**	**Thinned absence**	**Predictor variables used for final modeling**
2015	21	758	53	Perennial herbaceous, topographic position index (TPI), and annual evapotranspiration
2018	26	1652	90	Perennial herbaceous, TPI, and cosine aspect
2019	27	1881	75	Layers of perennial herbaceous, TPI, and cosine aspect
2020	31	2289	102	Perennial herbaceous, TPI, and annual precipitation

Table 2. The six uncorrelated predictor variables used to fit preliminary niche models for *Ivesia webberi*. The three predictor variables used for the iterative niche models were selected from this pool. All predictors were downscaled from 4 km to 30 m resolution

Predictor variable	**Relationship with species**
Actual evapotranspiration (AET)	An estimate of the amount of water removed from an area by both evaporation and transpiration. AET, a direct predictor, is a proxy estimate of plant productivity.
Cosine aspect	Higher values indicate more north-facing slope aspects, which receive less sunlight.
Perennial herbaceous vegetative cover	A spatial vegetative cover delineation representing native grasses, perennial forbs, and cacti, which includes areas of *I. webberi* distribution. We considered this as a representation of biotic interactions covering the coexistence of all native forbs in *I. webberi* communities.
Minimum annual temperature	A direct predictor that potentially influences plant distribution (Araújo and Rozenfeld 2014). Vegetative and seed regeneration of *I. webberi* are dependent on cold stratification that characterizes late winter and early spring seasons.
Summer precipitation	A direct predictor that potentially influences plant distribution. Summer precipitation causes surface runoffs which facilitate localized gravity-enhanced seed dispersal and colonization of empty niches.

Topographic position index (TPI)	A scale-dependent variable describing the elevation of a cell in relation to the mean elevation of the neighboring cells. At the scale of 333 m, TPI distinguishes between mountains and valleys in the study area. The study area is characterised by topographic heterogeneity which can limit dispersal and distribution, and also act as a proxy for climatic variables.

Table 3. The relationship between the environmental variables and the first two axes of the principal component analysis based on *Ivesia webberi* presence and true absence points. Values represent correlation between the environmental variables and PC axes

Environmental variables	**PC1 (28.7%)**	**PC2 (20.3%)**
Actual evapotranspiration	-0.154	-0.607
Cosine aspect	0.159	0.791
Perennial herbaceous layer	0.015	0.436
Mean summer precipitation	0.868	-0.122
Minimum annual temperature	-0.742	0.121
Topographic position index	0.605	-0.051

Table 4. Similarity measure among the ensemble-projected habitat suitability from iterative niche modeling. Similarity was assessed using the niche overlap function in *dismo* R package

Iterative models	**2015**	**2018**	**2019**	**2020**
2015	1			
2018	0.946	1		
2019	0.927	0.989	1	
2020	0.894	0.882	0.845	1

Table 5. Adjusted *p* values from the pairwise Tukey post-hoc test on the multivariate multiple linear regression on four performance metrics of the niche model iterations from 2015 to 2020 for *Ivesia webberi*. *P* values < 0.05 are in bold text

Pairwise comparisons	**TSS**	**AUC**	**Boyce Index**	**Specificity**
2015: 2018	0.19	0.25	0.12	0.90
2015: 2019	**0.01**	**0.01**	0.53	0.99
2015: 2020	**0.01**	**0.02**	0.66	0.88
2018: 2019	**0.01**	**0.01**	0.82	0.74
2018: 2020	0.13	0.47	0.70	0.99
2019: 2020	0.67	0.25	0.99	0.72

Table 6. AUC values for the iterative niche models from a separate niche modeling using blocking to partition spatial data into training and test data. Block AUC and test AUC represent average values of the model replicates from random forest, artificial neural networks and maximum entropy

Iterative model	Block AUC	Test AUC	Difference AUC
2015	0.81	0.47	0.34
2018	0.63	0.59	0.04
2019	0.66	0.46	0.20
2020	0.47	0.52	-0.05

FIGURE LEGENDS

Figure 1. A diagram illustrating the iterative niche modeling framework for fitting *Ivesia webberi* ecological niche

Figure 2. PCA biplot of the environmental predictors that influence the *Ivesia webberi* niche in the western Great Basin Desert. 2015 represents the initial spatial data used for the first niche model iteration, while 2020 represents all new spatial data added to the iterative niche models from 2016-2020. Both “a” and “p” represent absence and presence points respectively

Figure 3a-f. Box plots representing environmental conditions in the original occurrence points compared to those in the novel locations. The environmental conditions represent the six uncorrelated predictor variables, including (a) actual evapotranspiration, (b) cosine aspect, (c) perennial herbaceous cover, (d) summer mean precipitation, (e) topographic position index, and (f) minimum monthly temperature

Figure 4. Variable contributions to the iterative niche modeling for *Ivesia webberi* from a) 2015 to d) 2020. The three predictors used for each year of iterative modeling were selected from the preliminary modeling. Herb represents the perennial herb layer, TPI stands for topographic position index at 333 m, AET stands for cumulative actual evapotranspiration, aspect represents cosine aspect, while precip stands for summer mean precipitation

Figure 5. Response plots showing the predicted probability of *Ivesia webberi* occurrence in a) topographic position index, b) annual precipitation, and c) perennial herb spatial distribution layer. The response plots were generated using the Random forest models

Figure 6a-d. Maps of predicted suitable areas and geographical distribution of *Ivesia webberi* in the western Great Basin Desert, with occurrence points overlay. Red colored pixels represent areas of predicted high probability of *I. webberi* occurrence, yellow pixels represent intermediate probability of species occurrence, while blue pixels are predicted areas of zero to low probability of occurrence. The occurrence points in green are the original *I. webberi* occurrence points used for niche modeling, while yellow colored occurrence points represent the novel population

Figure 7. Values for the a) area under curve (AUC) of the receiver operating characteristic (ROC) plot, b) continuous Boyce index (CBI), c) specificity, and d) true statistic skill (TSS) metrics across the years of iterative niche modeling (shown in x axes). Niche models for 2015 and 2018 were the highest and lowest respectively

Figure 8. Model performance of the six algorithms based on the a) area under curve (AUC) of the receiver operating characteristic (ROC) plot, b) continuous Boyce index (CBI), c) specificity, and d) true statistic skill (TSS), after accounting for the effect of years of iterative modeling

Figure 9. Comparative model performance between ensemble and single algorithm-based niche models for *Ivesia webberi*, after accounting for the effect of years of iterative modeling in a) TSS, and b) AUC metrics

FIGURES

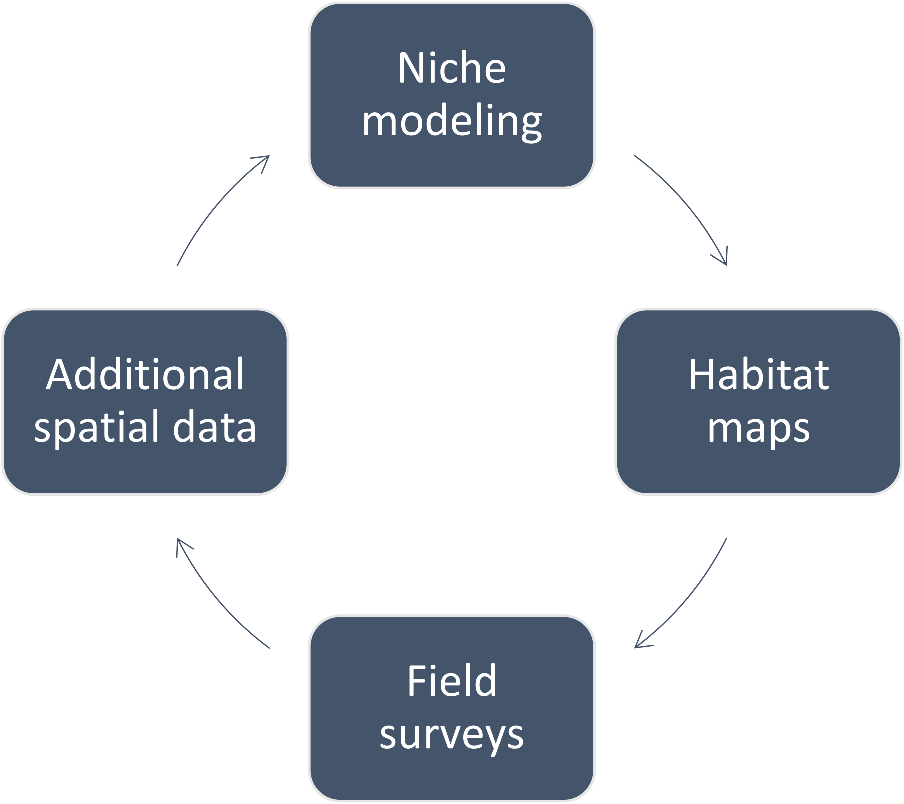


Figure 1.

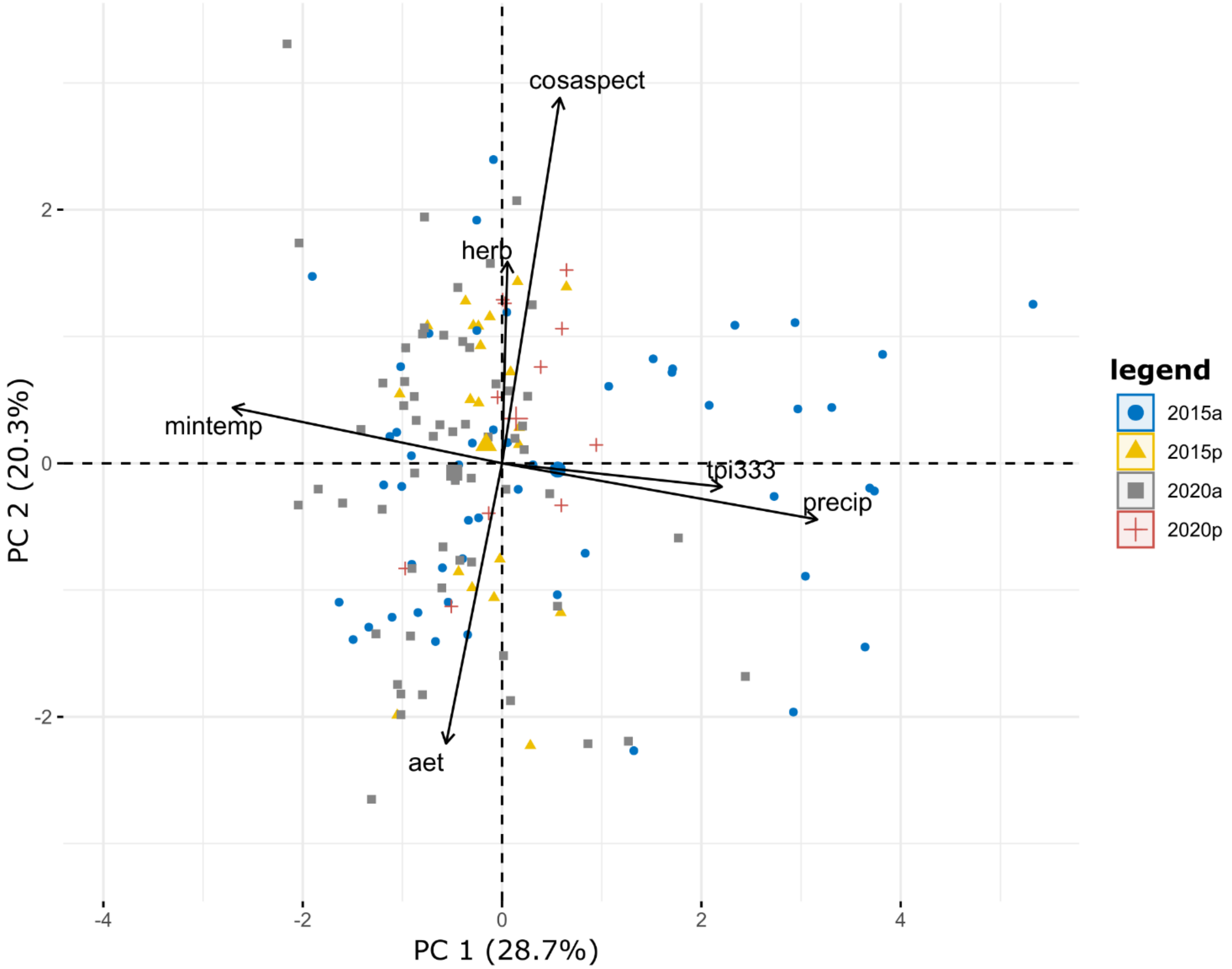

cosaspect
herb
mintemp
tpi333
precip
aet
PC 2 (20.3%)
PC 1 (28.7%)
2
0
-2
-4
-2
0
2
4
legend
2015a
2015p
2020a
2020p

Figure 2.

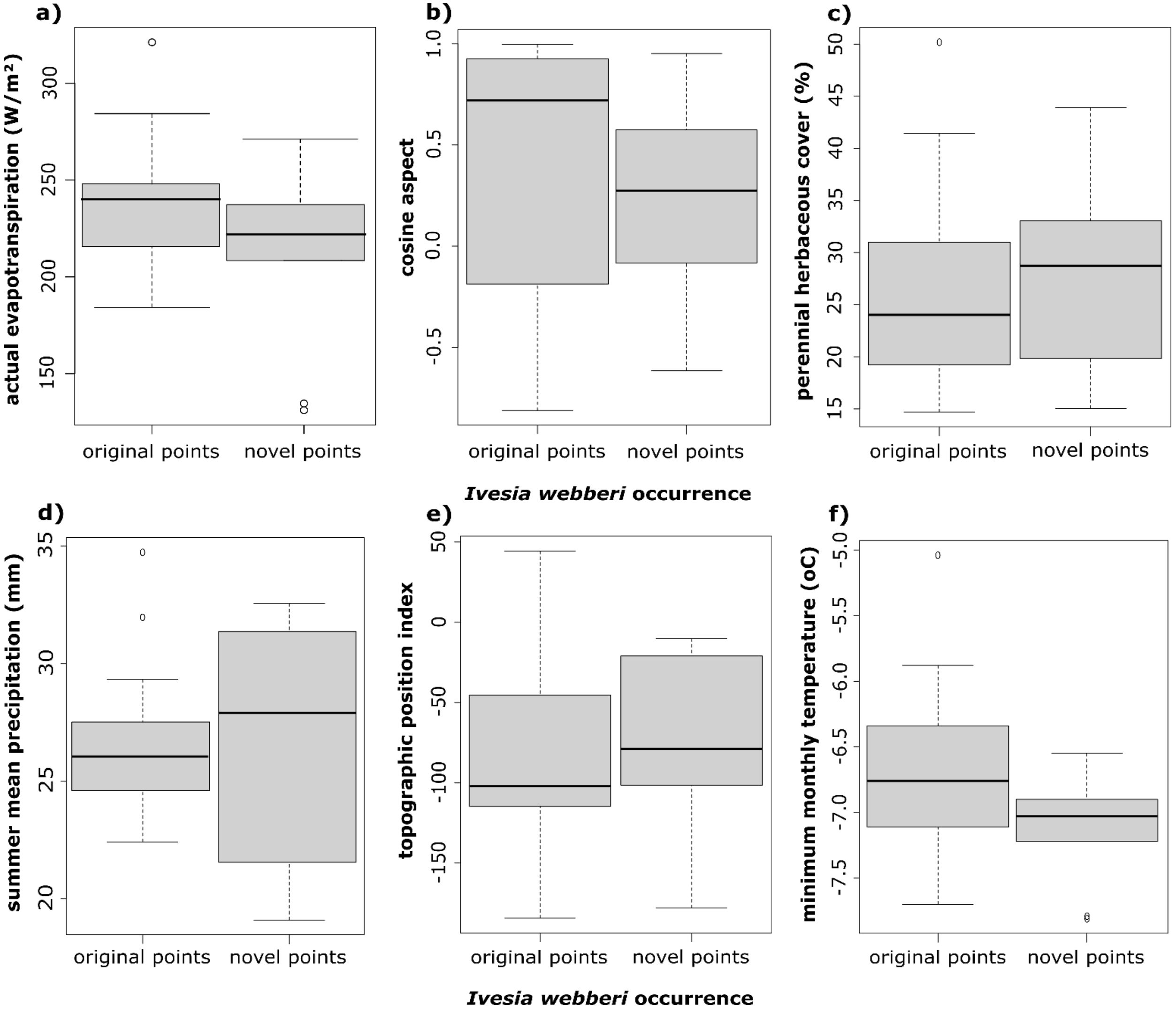

a)
actual evapotranspiration (W/m²)
150
200
250
300
original points
novel points
b)
cosine aspect
-0.5
0.0
0.5
1.0
original points
novel points
c)
perennial herbaceous cover (%)
15
20
25
30
35
40
45
50
original points
novel points
Ivesia webberi occurrence
d)
summer mean precipitation (mm)
20
25
30
35
original points
novel points
e)
topographic position index
-150
-100
-50
0
50
original points
novel points
f)
minimum monthly temperature (oC)
-7.5
-7.0
-6.5
-6.0
-5.5
-5.0
original points
novel points
Ivesia webberi occurrence

Figure 3.

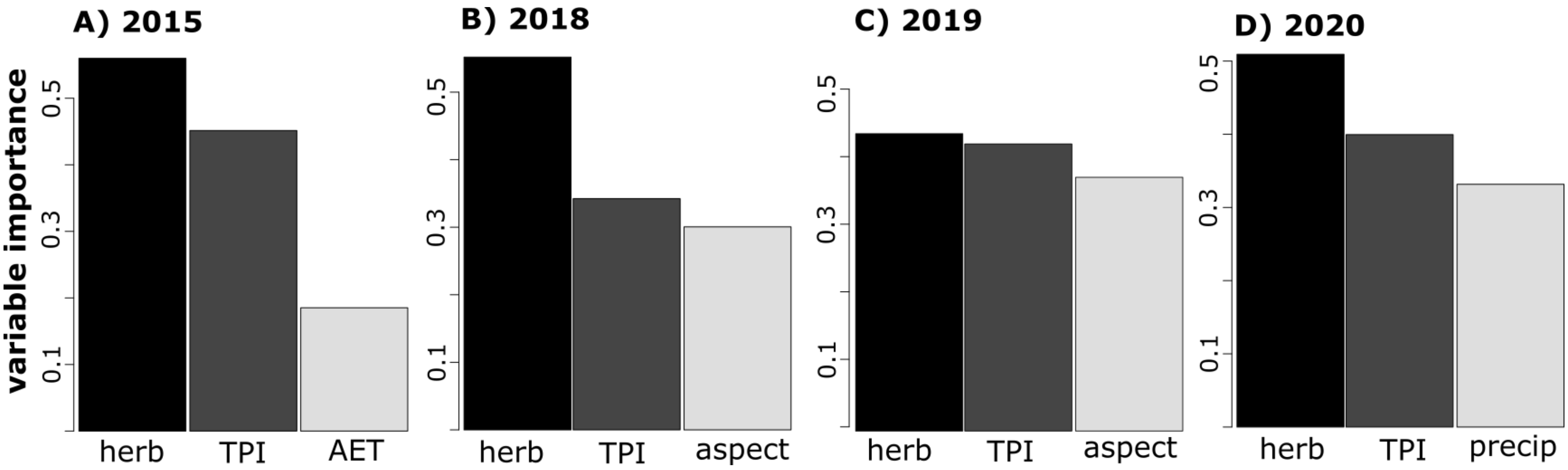

A) 2015
B) 2018
C) 2019
D) 2020
variable importance
0.1
0.3
0.5
herb
TPI
AET
aspect
precip

Figure 4.

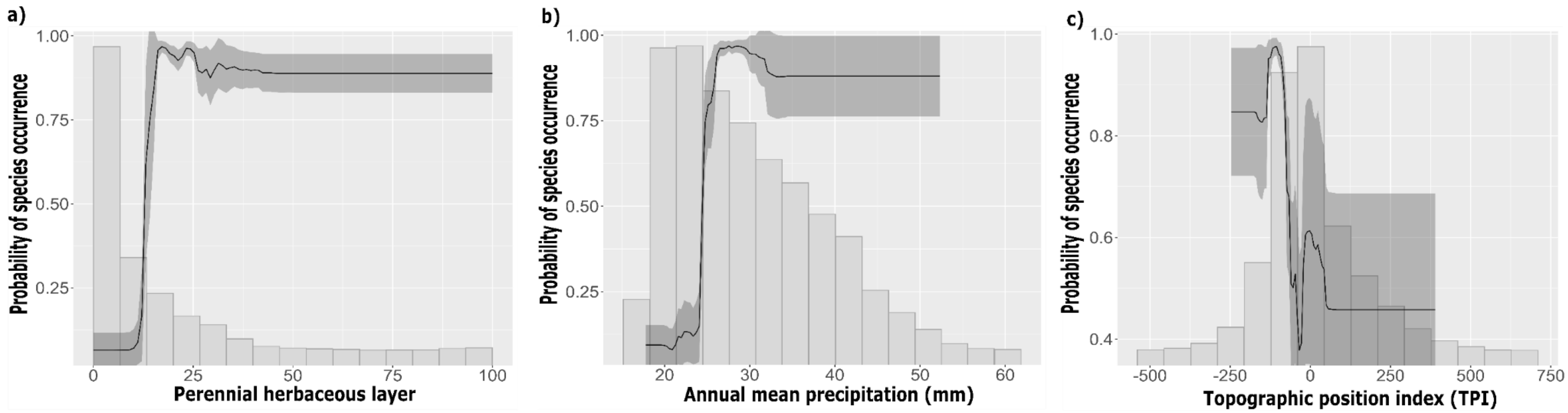

a)
Probability of species occurrence
1.00
0.75
0.50
0.25
0
25
50
75
100
Perennial herbaceous layer
b)
Probability of species occurrence
1.00
0.75
0.50
0.25
20
30
40
50
60
Annual mean precipitation (mm)
c)
Probability of species occurrence
1.0
0.8
0.6
0.4
-500
-250
0
250
500
750
Topographic position index (TPI)

Figure 5.

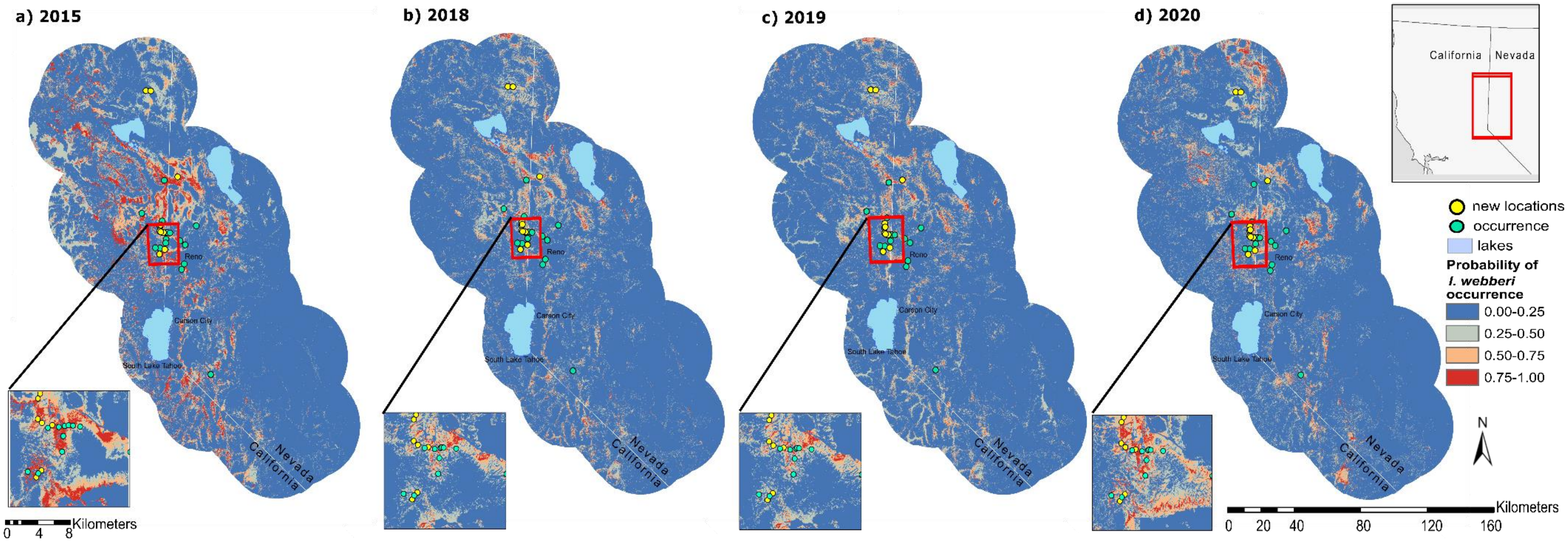

a) 2015
b) 2018
c) 2019
d) 2020
California
Nevada
Reno
Carson City
South Lake Tahoe
Nevada
California
new locations
occurrence
lakes
Probability of *I. webberi* occurrence
0.00-0.25
0.25-0.50
0.50-0.75
0.75-1.00
N
Kilometers
0 4 8
Kilometers
0 20 40 80 120 160

Figure 6a-d.

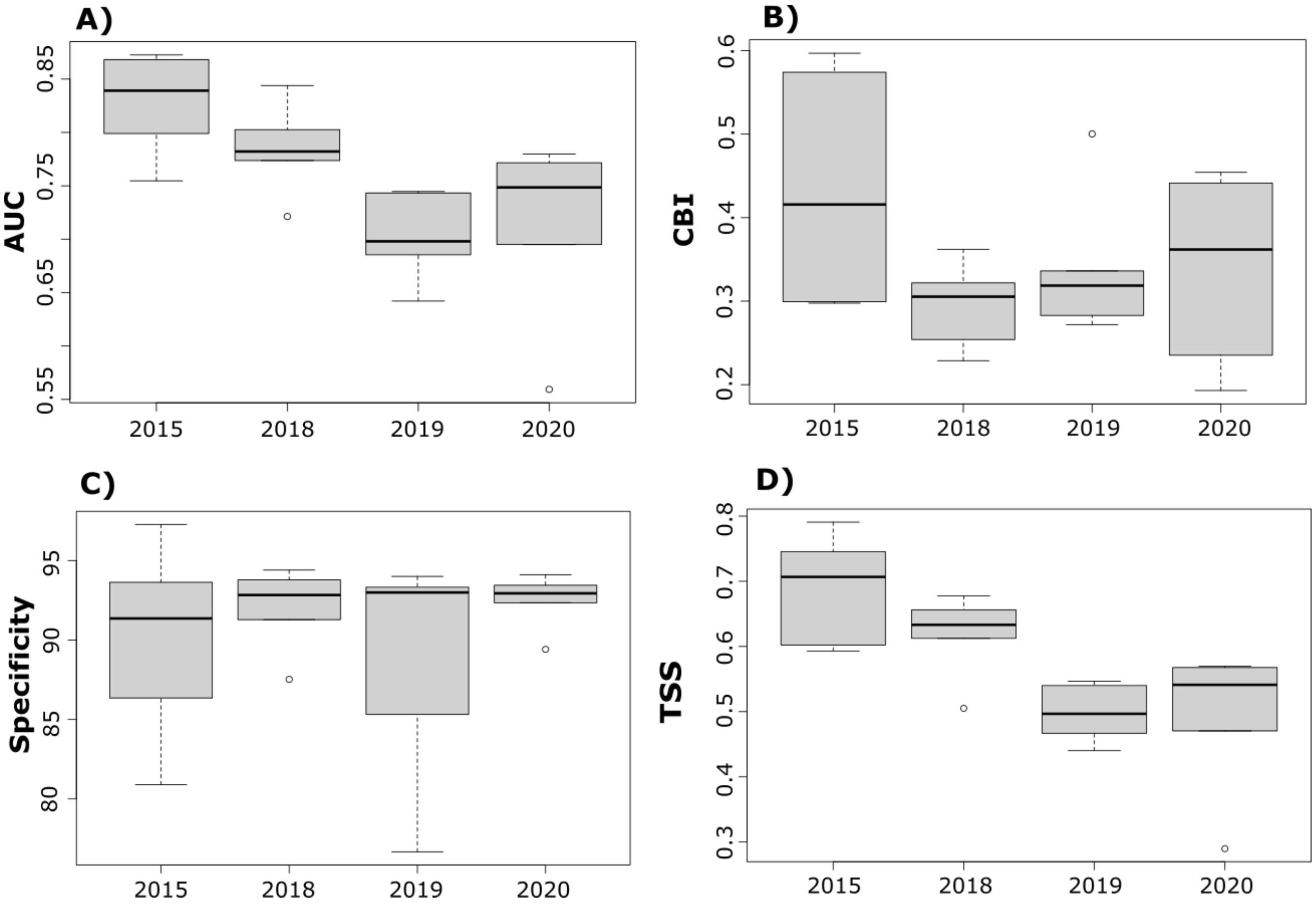

A)
AUC
0.55
0.65
0.75
0.85
2015
2018
2019
2020
B)
CBI
0.2
0.3
0.4
0.5
0.6
2015
2018
2019
2020
C)
Specificity
80
85
90
95
2015
2018
2019
2020
D)
TSS
0.3
0.4
0.5
0.6
0.7
0.8
2015
2018
2019
2020

Figure 7.

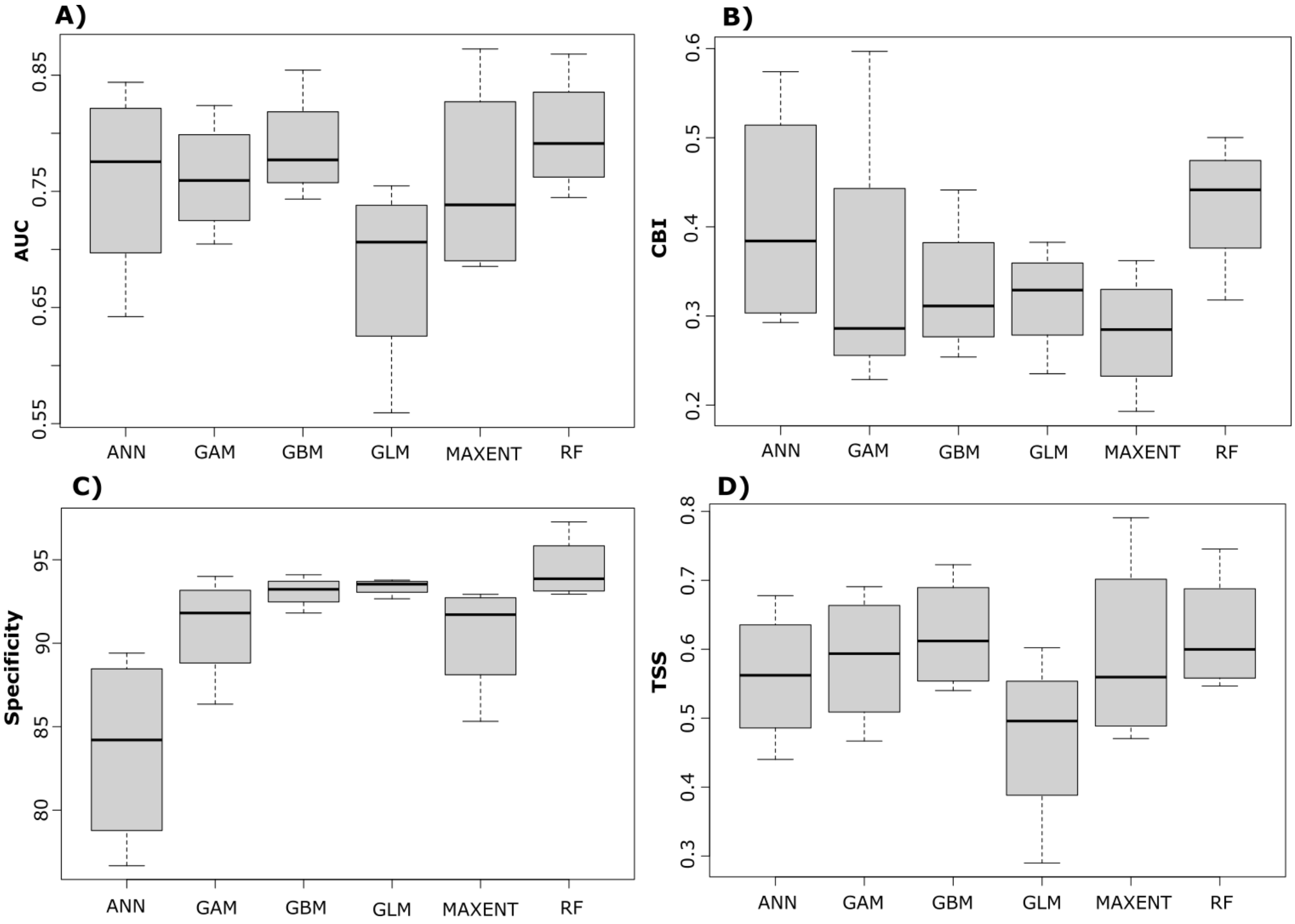

A)
AUC
0.55
0.65
0.75
0.85
ANN
GAM
GBM
GLM
MAXENT
RF
B)
CBI
0.2
0.3
0.4
0.5
0.6
C)
Specificity
80
85
90
95
D)
TSS
0.3
0.4
0.5
0.6
0.7
0.8

Figure 8.

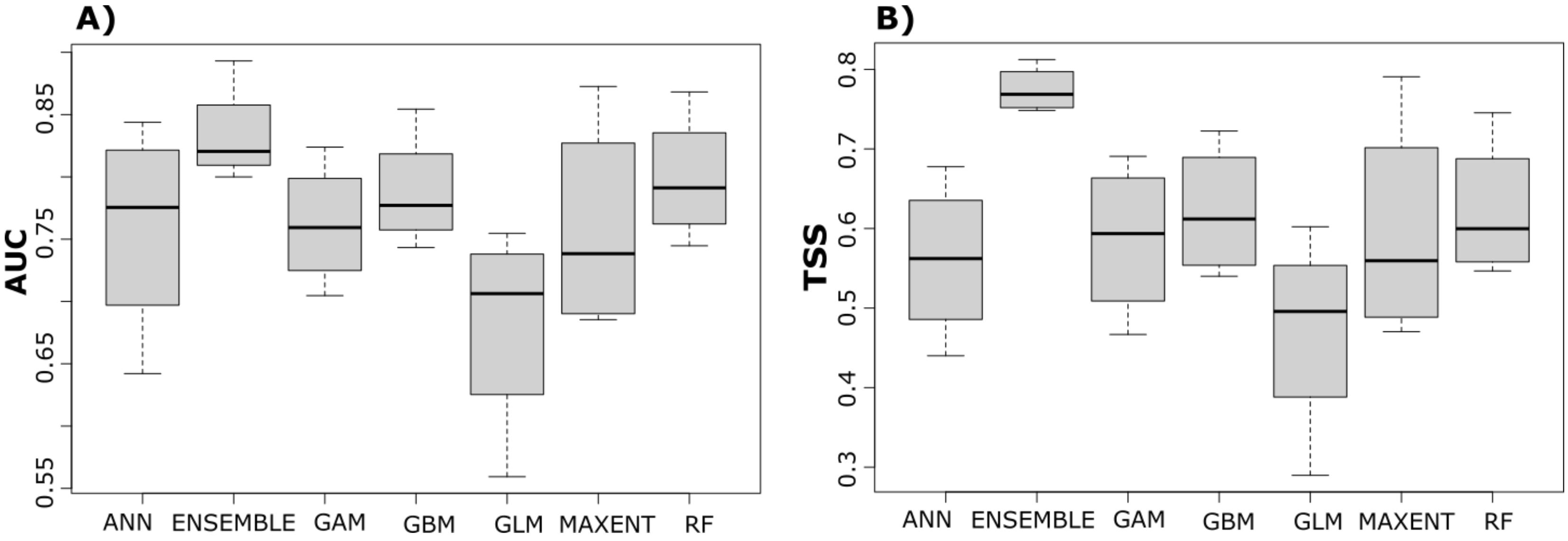

A)
AUC
0.55
0.65
0.75
0.85
ANN
ENSEMBLE
GAM
GBM
GLM
MAXENT
RF
B)
TSS
0.3
0.4
0.5
0.6
0.7
0.8
ANN
ENSEMBLE
GAM
GBM
GLM
MAXENT
RF

Figure 9.

ACKNOWLEDGEMENTS: This research was funded through a grant from the U.S. Fish and Wildlife Service CFDA program (award number F15AC00784). The authors are grateful to the Nevada Natural Heritage Program for supplying the species occurrence data and absence points for the preliminary modeling. Additional data from Ms. Sarah Kulpa of the U.S. Fish and Wildlife Service, Reno Field Office, Ms. Valda Lockie of the Bureau of Land Management, Susanville CA Office, and the California Native Plant Society is also appreciated. The authors are also grateful to David Board of the Rocky Mountain Research Station of the U.S. Department of Agriculture (USDA) Forest Service.

SUPPLEMENTAL INFORMATION

Table S1. List of 72 predictor variables assembled for fitting the ecological niche models of *Ivesia webberi*. They were reduced to six uncorrelated (r<0.59) predictors using Kendall r correlation, and feature selection runs in *Boruta* R package and RFE function in *caret* R package

Predictor name	**Description and source**
Elevation	A 30 m (1 arc second) digital elevation model (DEM) from National elevation dataset (USGS, 2017)
Slope	Slope layer was calculated from the USGS (2017) DEM layer, using the slope tool in ArcMap version 10.7
Curvature	Curvature layer was calculated from the USGS (2017) DEM layer, using the slope tool in ArcMap version 10.7
Land cover	Sourced from the 30 m U.S. National Land Cover Dataset (NLCD) 2011 product (Homer, Dewitz, Yang, Jin, Danielson, Xian, … Megown, 2015)
Sine aspect	Sine aspect was calculated in ArcMap version 10.6.1 using USGS (2017) DEM and the slope layers
Cosine aspect	Cosine aspect was calculated in ArcMap version 10.6.1 using USGS (2017) DEM and the slope layers, the formula: $= \theta \times \cos(\alpha)$, where $= \theta$ is slope (in percentage), and α is aspect (in radians)
Cosine aspect at 45°	Cosine aspect was calculated in ArcMap version 10.6.1 using USGS (2017) DEM and the slope layers, the formula: $= \theta \times \cos(\alpha)$, where $= \theta$ is slope (in percentage), and α is aspect (in radians). Here, aspect is tilted at 45°

Hillshade	A topographic layer calculated from the USGS (2017) DEM layer, using the hillshade tool in ArcMap version 10.6.1
Topographic position index (TPI33)	Calculated from USGS (2017) DEM in ArcMap version 10.6.1, using formula introduced by Weiss (2001), and a 33 m neighborhood
Topographic position index (TPI333)	Calculated from USGS (2017) DEM in ArcMap version 10.6.1, using formula introduced by Weiss (2001), and a 333 m neighborhood. At 333 m scale, the entire landscape is classified into either a valley or a mountain range
Topographic wetness index	Calculated from USGS (2017) DEM following the formula of Beven & Kirkby (1979) and is defined using the following formula: ln(upslope area / tan(slope)).
Annual herb cover	Data was sourced from the Multi-Resolution Land Characteristics (MRLC) development of the U.S. National Land Cover Database (NLCD) 2016 Shrub component products (Xian, Homer, Meyer, & Granneman, 2013)
Bare ground	
Artemisia tridentata cover	
Perennial herbaceous cover	
Soil litter	
Artemisia spp. cover	
Shrub layer	
Shrub height	
Sagebrush height	
Forest cover	A 30 m raster layer developed by the United States Forest Service (USFS) from multispectral LANDSAT imagery (Coulston, Moisen, Wilson, Finco, Cohen, & Brewer, 2012)

Modified soil-adjusted vegetation index (MSAVI2)	Vegetation indices were created in Google Earth Engine, using remotely sensed data between January 1985 and December 2010, from LANDSAT 5, 7 and 8 images, using code developed by Brehm & Matos (2019)
Normalized difference vegetation index (NDVI)	
Solar radiation	Solar radiation was calculated using a hemispherical viewshed algorithm (Fu & Rich, 2002) on the USGS (2017) DEM layer, in ArcMap version 10.6.1. Solar radiation was calculated for one day each in the four seasons: Julian days 1, 90, 225 and 287 for winter, spring, summer and fall respectively (except for leap years where Julian days 91, 226 and 288 represent spring, summer and fall respectively). Five-year interval area solar radiation was calculated between 1975 and 2018.
Available water content (AWC)	Edaphic layers sourced from POLARIS, a 30 m probabilistic soil series map of the United States (Chaney, Wood, McBratney, Hempel, Nauman, Brungard, & Odgers, 2016)
Soil mean $CaCO_3$	
Soil mean clay	
Soil mean silt	
Soil mean sand	
Soil mean pH	
Soil mean bulk density	
Soil organic matter	
Depth to restrictive layer (bedrock)	

Soil class	A gridded soil survey dataset (gSSURGO) developed by the USDA Natural Resources Conservation Service (Soil Survey Staff, 2017)
Geology	Layer obtained from the Geologic maps of U.S. states (Horton, San Juan, & Stoeser, 2017) at https://mrdata.usgs.gov/geology/state
Cumulative annual actual evapo-transpiration (AET)	Water balance variable calculated from 800 m 1971-2000 PRISM climate normals (Daly et al., 2008), available water capacity (Chaney et al., 2016), and the 1-arcsecond DEM (USGS, 2017) combined in the Climatic Water Deficit Toolbox for ArcGIS (Dilts, 2014). Methods are described in Dilts, Weisberg, Dencker, & Chambers (2015). AET represents the simultaneous availability of water and energy to support plant productivity.
Cumulative annual climatic water deficit (CWD)	Water balance variable calculated from 800 m 1971-2000 PRISM climate normals (Daly et al., 2008), available water capacity (Chaney et al., 2016), and the 1-arcsecond DEM (USGS, 2017) combined in the Climatic Water Deficit Toolbox for ArcGIS (Dilts, 2014). Methods are described in Dilts et al. (2015). CWD is a measure of aridity and is difference between evaporative demand and supply
Cumulative annual potential evapo-transpiration (PET)	Water balance variable calculated from 800 m 1971-2000 PRISM climate normals (Daly et al., 2008), available water capacity (Chaney et al., 2016), and the 1-arcsecond DEM

	(USGS, 2017) combined in the Climatic Water Deficit Toolbox for ArcGIS (Dilts, 2014). Methods are described in Dilts et al. (2015). PET was calculated using the Thornthwaite approach outlined in Lutz et al. (2010).
Cumulative annual soil water balance (SWB)	Water balance variable calculated from 800 m 1971-2000 PRISM climate normals (Daly et al., 2008), available water capacity (Chaney et al., 2016), and the 1-arcsecond DEM (USGS, 2017) combined in the Climatic Water Deficit Toolbox for ArcGIS (Dilts, 2014). Methods are described in Dilts et al. (2015). SWB represents the amount of soil water storage summed across all months of the year.
Absolute cumulative soil water balance	Absolute value of the SWB. Calculated using 800 m resolution PRISM climatic data 1970-2001 normals (Daly et al., 2008), in BIOLCLIM (Booth, Nix, Busby, & Hutchinson, 2014)
Cumulative annual water supply (WS)	Water balance variable calculated from 4 km 1971-2000 PRISM climate normals (Daly et al., 2008), available water capacity (Chaney et al., 2016), and the 1-arcsecond DEM (USGS, 2017) combined in the Climatic Water Deficit Toolbox for ArcGIS (Dilts, 2014). Methods are described in Dilts et al. (2015). WS is the sum of rainfall and snowmelt in all months in the calendar year
Monsoonality	Proportion of annual precipitation falling between July and September (Romme, Allen, Bailey, Baker, Bestelmeyer, Brown, ... & Miller, 2009). Original data source from 4 km

	resolution PRISM climatic data normals 1971-2000 (Daly et al., 2008)
Heatload	Static measure of solar radiation exposure based on slope, aspect, and latitude, based on methods published in McCune and Keon (2002). DEM derivatives were based on the 1-arcsecond National Elevation Dataset (USGS, 2017).
Minimum monthly temperature	Based on the 4 km PRISM climatic data normals 1971-2000 (Daly et al., 2008)
Maximum monthly temperature 1970-2001	
Minimum annual temperature	
Minimum spring temperature	
Minimum fall temperature	
Minimum summer temperature	
Minimum winter temperature	
Maximum spring temperature	
Maximum summer temperature	
Maximum fall temperature	

Maximum winter temperature	
Annual monthly precipitation	
Fall seasonal precipitation	
Spring seasonal precipitation	
Summer seasonal precipitation	
Winter seasonal precipitation	
Temperature range	
Ratio of AET and CWD	Water balance variables calculated from 4 km 1950-2015 PRISM climate monthly data (Daly et al., 2008), available water capacity (Chaney et al., 2016), and the 1-arcsecond DEM (USGS, 2017) combined in the Climatic Water Deficit Toolbox for ArcGIS (Dilts, 2014). PRISM climate data were downscaled from 4 km using the delta method and the PRISM 1971-2000 normals. Variables are described in Barga, Dilts, & Leger (2018).
Ratio of AET and PET	
Ratio of SWB and AET	
Ratio of WS and AET	
Positive difference between AET and SWB	
Absolute ratio of SWB and AET	
Spring ratio of WS and the greater of AET or SWB	
Precipitation seasonality	Based on PRISM climatic data normals 1971-2000 (Daly et al., 2008), in BIOCLIM (Booth et al., 2014).

April snowpack (1951 – 1980)	Water balance variables calculated from 4 km 1950-2015 PRISM climate monthly data (Daly et al., 2008), available water capacity (Chaney et al., 2016), and the 1-arcsecond DEM (USGS, 2017) combined in the Climatic Water Deficit Toolbox for ArcGIS (Dilts, 2014). PRISM climate data were downscaled to 800 m using the delta method and the PRISM 1971-2000 normals. Variables are described in Barga et al. (2018).
April snowpack (1981 – 2010)	
Recharge 1951-1980	
Recharge 1981-2010	

References

Barga, S. C., Dilts, T. E., & Leger, E. A. (2018). Contrasting climate niches among co-occurring subdominant forbs of the sagebrush steppe. *Diversity and Distributions*, 24(9), 1291-1307. https://doi.org/10.1111/ddi.12764

Beven, K. J., & Kirkby, M. J. (1979). A physically based, variable contributing area model of basin hydrology. *Hydrological Sciences Journal*, *24*(1), 43-69. https://doi.org/10.1080/02626667909491834

Booth, T. H., Nix, H. A., Busby, J. R., & Hutchinson, M. F. (2014). BIOCLIM: the first species distribution modelling package, its early applications and relevance to most current MaxEnt studies. *Diversity and Distributions*, 20(1), 1-9. https://doi.org/10.1111/ddi.12144

Brehm, J., & Matos, A. (2019) Google Earth Engine Landsat Compositor Javascript code for Google Earth Engine. University of Nevada Reno. Available at: https://code.earthengine.google.com/a86bca4669160b79b3bb60eec08556cc

Chaney, N. W., Wood, E. F., McBratney, A. B., Hempel, J. W., Nauman, T. W., Brungard, C. W., & Odgers, N. P. (2016). POLARIS: A 30-meter probabilistic soil series map of the contiguous United States. *Geoderma*, 274, 54–67. https://doi.org/10.1029/2018WR022797

Coulston, J. W., Moisen, G. G., Wilson, B. T., Finco, M. V., Cohen, W. B., & Brewer, C. K. (2012). Modeling percent tree canopy cover: a pilot study. *Photogrammetric Engineering & Remote Sensing*, 78(7), 715-727. doi: 10.14358/PERS.78.7.715

Daly, C., Halbleib, M., Smith, J. I., Gibson, W. P., Doggett, M. K., Taylor, G. H., Curtis, J., & Pasteris, P. A. (2008). Physiographically-sensitive mapping of temperature and precipitation across the conterminous United States. *International Journal of Climatology*, 28, 2031-2064. https://doi.org/10.1002/joc.1688

Dilts, T. E., Weisberg, P. J., Dencker, C. M., & Chambers, J. C. (2015). Functionally relevant climate variables for arid lands: a climatic water deficit approach for modelling desert shrub distributions. *Journal of Biogeography*, 42(10), 1986-1997. https://doi.org/10.1111/jbi.12561

Dilts, T.E. (2014). Climatic Water Deficit Toolbox for ArcGIS 10.1. University of Nevada Reno. Available at: http://www.arcgis.com/home/item.html?id=de9ca57d43c041148b815da7ce4aa3a0

Fu, P., & Rich, P. M. (2002). A geometric solar radiation model with applications in agriculture and forestry. *Computers and Electronics in Agriculture*, 37, 25–35. https://doi.org/10.1016/S0168-1699(02)00115-1

Homer, C. G., Dewitz, J. A., Yang, L., Jin, S., Danielson, P., Xian, G., Coulston, J., Herold, N. D., Wickham, J. D., & Megown, K. (2015). Completion of the 2011 National Land Cover Database for the conterminous United States-Representing a decade of land cover change information. *Photogrammetric Engineering & Remote Sensing*, 81(5), 345-354. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=309950

Horton, J. D., San Juan, C. A., & Stoeser, D. B. (2017). The State Geologic Map Compilation (SGMC) geodatabase of the conterminous United States (ver. 1.1, August 2017). U.S. Geological Survey Data Series 1052. Accessed at https://doi.org/10.3133/ds1052.

Lutz, J. A., Van Wagtendonk, J. W., & Franklin, J. F. (2010). Climatic water deficit, tree species ranges, and climate change in Yosemite National Park. *Journal of Biogeography*, 37(5), 936-950. https://doi.org/10.1111/j.1365-2699.2009.02268.x

McCune, B., & Keon, D. (2002). Equations for potential annual direct incident radiation and heat load. *Journal of Vegetation Science*, 13, 603-606. https://doi.org/10.1111/j.1654-1103.2002.tb02087.x

Romme, W. H., Allen, C. D., Bailey, J. D., Baker, W. L., Bestelmeyer, B. T., Brown, P. M., Eisenhart, K. S., Floyd, M. L., Huffman, D. W., Jacobs, B. F., Miller, R. F., Muldavin, E. H., Swetnam, T. W., Tausch, R. J., & Weisberg, P. J. (2009). Historical and modern disturbance regimes, stand structures, and landscape dynamics in pinon–juniper vegetation of the western United States. *Rangeland Ecology & Management*, 62(3), 203-222. https://doi.org/10.2111/08-188R1.1

Soil Survey Staff (2017). Gridded Soil Survey Geographic (gSSURGO) Database for the United States of America and the Territories, Commonwealths, and Island Nations served by the USDA-NRCS. United States Department of Agriculture, Natural Resources Conservation Service. Available online at http://datagateway.nrcs.usda.gov/. January 2017.

U.S. Geological Survey [USGS] (2017). 1 Arc-second Digital Elevation Models (DEMs) - USGS National Map 3DEP Downloadable Data Collection. U.S. Geological Survey. Accessed at https://www.usgs.gov/core-science-systems/ngp/tnm-delivery/

Weiss, A. (2001). Topographic position and landform analysis. Poster presentation, ESRI User Conference, San Diego, California, USA.

Xian, G., Homer, C., Meyer, D., & Granneman, B. (2013). An approach for characterizing the distribution of shrubland ecosystem components as continuous fields as part of NLCD. *ISPRS Journal of Photogrammetry and Remote Sensing*, *86*, 136-149. https://doi.org/10.1016/j.isprsjprs.2013.09.009

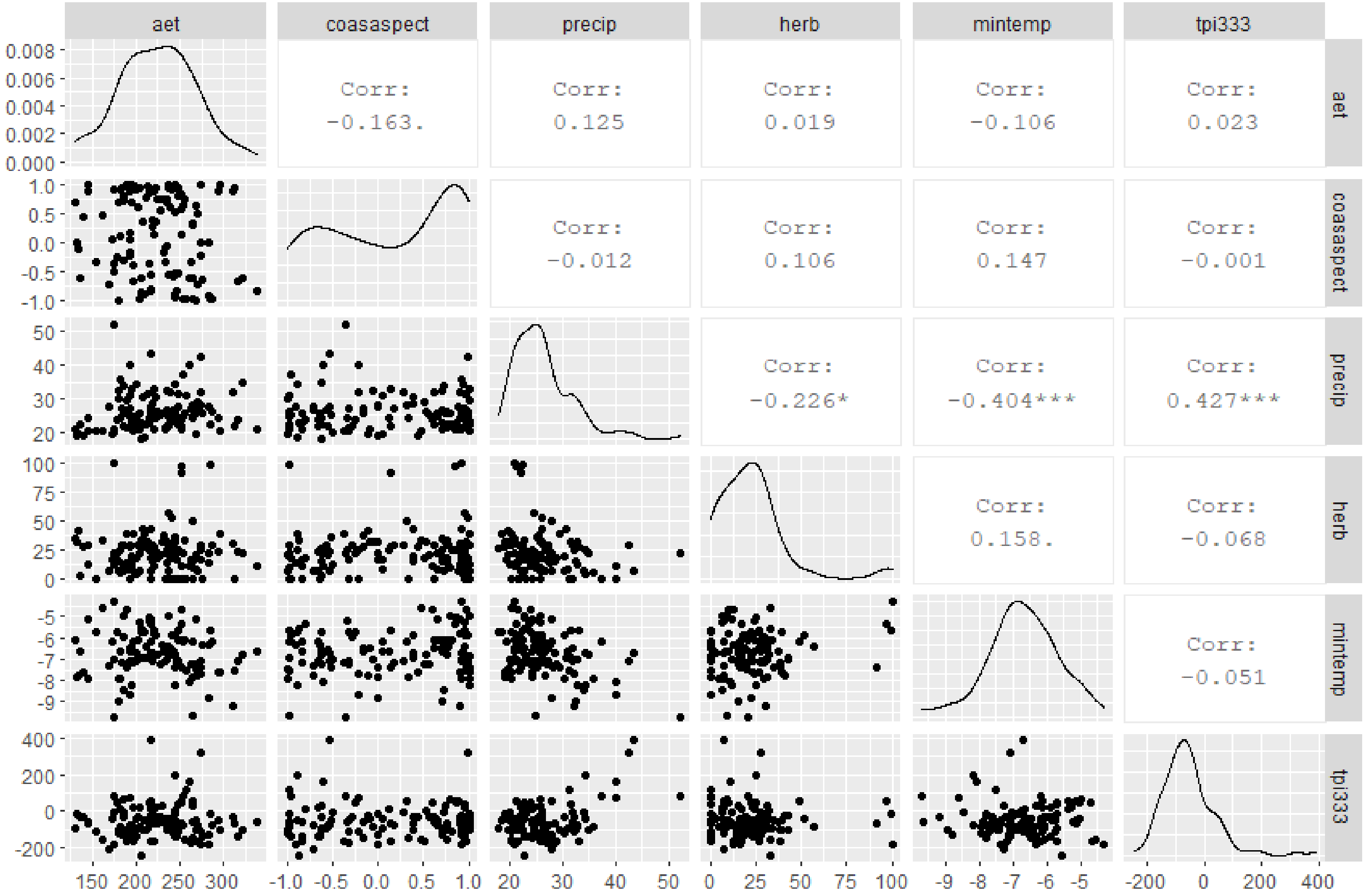


Figure S1. Correlation plot of the six uncorrelated predictor variables used for the iterative niche modeling of *Ivesia webberi* A. Gray. AET = actual evapotranspiration, cosaspect = cosine aspect, precip = summer mean precipitation, herb = perennial herb vegetative cover, mintemp = minimum annual temperature, tpi333 = topographic position index calculated at 333 m

Figure S2. Photo illustrating the vegetative cover in *Ivesia webberi* site at the Hallelujah Junction Wildlife Area (USFWS-designated unit 4). Photo taken by Israel Borokini in June 2020.

Figure S3a. Photo illustrating the vegetative cover in *Ivesia webberi* site at the Dog Valley Meadows, near Verdi NV (USFWS-designated unit 5a). Photo taken by Israel Borokini in June 2018.

Figure S3b. Photo illustrating the vegetative cover in *Ivesia webberi* site at the Dog Valley Meadows, near Verdi NV (USFWS-designated unit 5a). Photo taken near the historical California trail marker by Israel Borokini in June 2017.

Figure S4. Photo illustrating the vegetative cover in *Ivesia webberi* site at the Dog Valley Meadows, near Verdi NV (USFWS-designated unit 5b). Photo taken by Israel Borokini in May 2017.

Figure S5. Photo illustrating the vegetative cover in *Ivesia webberi* site at the *Ivesia* flat on Peavine Mountain Range (USFWS-designated unit 8). Photo taken by Israel Borokini in June 2018.

Figure S6. Photo illustrating the vegetative cover in *Ivesia webberi* site in the Humboldt-Toiyabe National Forest, near the Nevada-California Stateline (USFWS-designated unit 9a). Photo taken by Israel Borokini in June 2018.

Figure S7. Photo illustrating the vegetative cover in *Ivesia webberi* site in the Humboldt-Toiyabe National Forest, near the Nevada-California Stateline (USFWS-designated unit 9b). This site is characterized by mosaics of *I. webberi-Balsamorhiza hookeri* and the invasive *Taeniatherum caput-medusae* (green patches). Photo taken by Israel Borokini in June 2018.

Figure S8. Photo illustrating the vegetative cover in *Ivesia webberi* site in the Humboldt-Toiyabe National Forest, near the Nevada-California Stateline (USFWS-designated unit 10). Photo taken by Israel Borokini in June 2018.

Figure S9. Photo illustrating the vegetative cover in *Ivesia webberi* site near Dutch Louie Flat on the Carson Range (USFWS-designated unit 14). Photo taken by Israel Borokini in June 2018.

Figure S10. Photo illustrating the vegetative cover in *Ivesia webberi* site at the base of the Carson Range near Caughlin Ranch neighborhood (USFWS-designated unit 15). *I. webberi* grows under the dense tufts of *Bromus tectorum* (senesced plants) and *Taeniatherum caput-medusae* which invaded the site. Photo taken by Israel Borokini in June 2018.

Figure S11. Photo illustrating the vegetative cover in one of the newly discovered *Ivesia webberi* sites on a private property adjacent to the Humboldt-Toiyabe National Forest. Photo taken by Israel Borokini in May 2020.

Figure S12. Photo illustrating the vegetative cover in one of the newly discovered *Ivesia webberi* sites, the second patch on the same private property adjacent to the Humboldt-Toiyabe National Forest. Photo taken by Israel Borokini in May 2020.

Figure S13. Photo illustrating the vegetative cover in one of three newly discovered *Ivesia webberi* sites in the Hallelujah Junction Wildlife Area. This site is located in the southern end of the western portion of the Wildlife Area. Photo taken by Israel Borokini in May 2020.

Figure S14. Photo illustrating the vegetative cover in one of the newly discovered *Ivesia webberi* sites on public land managed by the Bureau of Land Management Eagle Lake District, along the Smoke Creek Road, northeast of Honey Lake, off U.S. 395 highway in California. This site extends the northern distribution range of *I. webberi* by 65 km. *I. webberi* is found along a long but narrow stretch of ephemeral dry wash surrounded by *Bromus tectorum* and *Taeniatherum caput-medusae* monocultures. Photo taken by Israel Borokini in May 2020.

CHAPTER 2 Quantifying the relationship between soil seed bank and plant community assemblage in sites harboring the threatened *Ivesia webberi* in the western Great Basin Desert*

Israel T Borokini[1], Peter J. Weisberg[2], Mary M Peacock[3]

[1]Program in Ecology, Evolution and Conservation Biology, Department of Biology, University of Nevada, Reno, Reno NV 89557

[2]Department of Natural Resources and Environmental Science, University of Nevada, Reno, Reno NV 89557

[3]Department of Biology, University of Nevada, Reno, Reno NV 89557

*Published in *Applied Vegetation Science*

ABSTRACT

Questions: The soil seed bank is an important ecosystem component that can be pivotal for long-term persistence of many plant species, especially after disturbances followed by the invasion of alien weeds. However, many Great Basin Desert perennials produce fewer viable seeds, while large areas of the Great Basin are currently invaded by alien weeds. This could result in dissimilarity in floristic composition between the aboveground vegetative community and the soil seed bank, causing abrupt plant community shifts following disturbance. Therefore, we asked what is the relationship in the floristic composition between the aboveground communities and the soil seed bank in sites where the threatened *Ivesia webberi* occurs?

Location: The Great Basin Desert, United States.

Methods: We used Dice-Sorensen's similarity index to estimate similarity between the standing vegetation and the soil seed bank. Redundancy analysis and variation partitioning were used to quantify the relationship between the total abundance of the sampled aboveground flora and the soil seed bank, accounting for effects of spatial processes and environmental variables describing climate, soils, and vegetation in the 10 sites.

Results: Findings reveal high dissimilarity in species assemblage and abundance between the aboveground plant communities and the soil seed bank. Most of the dominant native plant species sampled in the standing vegetation were absent in the soil seed bank, and the soil seed bank was dominated by invasive alien weeds.

Conclusions: Divergence in the floristic composition between the aboveground communities and the soil seed bank in *I. webberi* habitat indicates low resilience and high risk of native species loss following perturbation. Post-disturbance succession in these plant communities may be largely dominated by invasive annual species; therefore, reduction of invasive species and native plant seeding may be necessary to sustain the ecological legacies of the desert ecosystem.

Keywords: *Ivesia webberi*, invasive species, community assemblage, redundancy analysis, soil seed bank, variation partitioning, ecological resilience, ecological restoration, native plant seeding, Great Basin Desert

Data accessibility statement: Data used in this study are published in Knowledge Network for Biocomplexity: doi:10.5063/F1FN14J7.

INTRODUCTION

An assessment of the relationship between the aboveground plant community and the soil seed bank is critical to understanding the ecological resistance and resilience of an ecosystem and post-disturbance successional pattern (Korb, Springer, Powers, & Moore, 2005; Ma et al., 2019; Mndela, Madakadze, Nherera-Chokuda, & Dube, 2020). Such an understanding is needed to support successful restoration efforts, and reverse biodiversity loss (Ma, Zhou, & Du, 2010; Haapalehto et al., 2017). However, the majority of studies that investigated the association between the aboveground plant communities and the soil seed bank reported low floristic similarity, and many soil seed banks are dominated by native and exotic annual ruderal and early-successional species (Peco, Ortega, & Levassor, 1998; Decocq et al., 2004; Hopfensperger, 2007; Bossuyt & Honnay, 2008). This raises questions concerning the role of the soil seed bank in fostering plant community resilience to disturbance (Kalamees & Zobel, 2002; Gioria & Pyšek, 2016).

The Great Basin Desert plant communities are dominated by perennial species (Kemp, 1989; Allen & Nowak, 2008). Many of these species produce fewer healthy seeds which have limited dispersal capacity, and form a transient seed bank (Lee, 2004; Goodwillie, Kalisz, & Eckert, 2005; Pekas & Schupp, 2013). Most studies in the Great Basin Desert have reported dissimilarities in the aboveground vs. belowground floristic composition (Pekas & Schupp, 2013; Martyn, Bradford, Schlaepfer, Burke, & Lauenroth, 2016), suggesting a limited role of the soil seed bank in the regeneration of desert perennial species (Bossuyt & Honnay, 2008; Allen & Nowak, 2008; Gomaa, 2012).

Moreover, vegetative recovery is not dependent on the seed bank alone, as regrowth from surviving vegetative structures of perennial plants can be quite important (Milberg, 1993; Bullock, Hill, Silvertown, & Sutton, 1994). For arid ecosystems in particular, the soil seed bank can be vulnerable to surface disturbance as the seed density is highest within the top 5 cm of the soil (Walters, 2004; DeFalco, Esque, Kane, & Nicklas, 2009).

Plant community structure in the Great Basin Desert has been significantly altered as a result of the spread of invasive species, intense grazing, climatic stress, increased frequency and intensity of wildfires, and heavy recreational land use (Cox & Allen, 2008; Balch, Bradley, D'Antonio, & Gómez-Dans, 2013; Coates et al., 2016; Pilliod, Welty, & Arkle, 2017). These disturbances deplete the soil seed bank for most native plant species, and reduce the ecological resistance and resilience to invasive alien weeds and disturbance (Chambers et al., 2007; Brooks & Chambers, 2011). Consequently, the Great Basin Desert is listed as the third most endangered ecosystem in the United States (Stein, Kutner, & Adams, 2000; Nachlinger, Sochi, Comer, Kittel, & Dorfman, 2001). Experts have therefore cautioned that the remaining native vegetation in the Great Basin Desert could be lost to invasive alien weeds (Wisdom & Chambers, 2009).

In this study, we investigated the relationship in species composition between the soil seed bank and the aboveground plant communities for 10 sites occupied by the threatened *I. webberi*, listed under the United States Endangered Species Act of 1973 (ESA; 16 U.S.C. § 1531 et seq.). These sites were selected with a focus on understanding the regeneration dynamics of *I. webberi* and the role of the soil seed bank in maintaining these plant communities that are currently threatened by the invasive, nonnative weeds.

Therefore, we asked the following questions: 1) What is the degree of similarity between the species assemblage in the aboveground vegetation and the soil seed bank of the sites where *I. webberi* occur? 2) Is there a significant relationship between the soil seed bank and the aboveground plant communities in these sites? 3) If so, what proportion of the aboveground plant community structure is explained by the soil seed bank in comparison to other environmental variables? Given a high density of nonnative and invasive annual plant species in the study sites, and based on existing literature (e.g., Humphrey & Schupp, 2001; Hopfensperger, 2007; Vanstockem, Ceusters, Van Dyck, Somers, & Hermy 2018; Barga & Leger, 2018), we predict high dissimilarity between the aboveground vegetation and the soil seed bank in these relatively undisturbed sites. We defined the soil seed bank as a collection of viable but ungerminated seeds, originating from standing, but also locally extirpated plants and vegetation from neighboring sites (Baskin & Baskin, 1998; Boussyt & Honnay, 2008; Solomon, 2011).

METHODS

Study sites

I. webberi (Rosaceae; Flora of North America) is a federally-listed threatened perennial forb, narrowly distributed along the eastern foothills of the northern Sierra Nevada and western margin of the Great Basin Desert (Figure 1). There are 16 known populations, most of which are located in small and isolated habitat patches that are dominated by nonnative and invasive annual grasses including *Bromus tectorum* and *Taeniatherum caput-medusae. I. webberi* regenerates mainly from the dormant root caudex in late winter or early spring (Witham, 2000), but age-class difference, colonization of bare-soil microsites created by the decommissioned roads, and field detection of seedlings in many of the sites indicate additional recruitment from seeds. This necessitates the importance of characterizing the soil seed bank in the sites harboring the species to assess the potential implications for persistence of this threatened forb under current disturbance levels.

Only 10 of the 16 known sites with *I. webberi* populations were accessible for this study, but were representative of the spatial distribution of the species (Figure 1). Climatic conditions at these sites are typical of the Great Basin Desert, which is influenced by rain shadow effects from the Sierra Nevada and Cascade Mountain ranges, and is therefore characterized by relatively mild winters and hot summers (Svejcar, Boyd, Davies, Hamerlynck, & Svejcar, 2017). Across the sites, temperature ranged from an average of -6 °C in the winter to an average of 28 °C in the summer, while annual precipitation varies between 25 and 33 cm. The areal extent of the selected sites for this study varies widely (Table 1) based on the patch occupancy of *I. webberi* estimated by the U.S. Fish and Wildlife Service (USFWS), which may indicate the relative suitability of ecological conditions at these sites for this species. The soil across these 10 study sites is characterized by argillic (clayey) subsurface horizon of volcanic origin overlaid by rocky pavement soil surface (USFWS, 2014). Over the past 30 years, all 10 sites have experienced varying degrees of grazing, off-highway vehicle use, wildfires and extensive colonization by nonnative and invasive plants (Witham, 2000; USFWS, 2014).

Soil seed bank sampling and estimation

To characterize species composition of the soil seed bank, 20 soil samples were collected in November 2015 from each site (n = 200), after seed set and before the soil was covered in snow (Allen & Nowak, 2008). At each site, collection points were randomly selected within *I. webberi* patches (Adams, Marsh, & Knox, 2005). Within a 1 m^2 quadrat placed at each of the collection points, three soil samples were collected from the top 3 cm, and combined as one sample. Due to a rocky surface pavement above a thick and hard argillic subsurface horizon in all sites (USFWS, 2014), the chosen soil depth is expected to capture optimal seed density in the soil seed bank.

The seedling emergence method was used to quantify species composition of the soil samples (Thompson & Grime, 1979). This method is preferable for plant communities in desert and grassland ecosystems (ter Heerdt, Verweij, Bekker, & Bakker, 1996), which often produce small seeds that could be lost using seed extraction methods (Gonzalez & Ghermandi, 2012). Importantly, the emergence method estimates the abundance of viable, non-dormant seeds (Tessema, de Boer, Baars, & Prins, 2012).

Germination trials of the collected soil seed bank were implemented in the Greenhouse complex at the University of Nevada, Reno. Soil samples were left to dry for one month, and the soil was then sieved with 50 mm mesh to remove rocks. The soil samples were spread evenly over 1 m thick vermiculite on 25 × 25 cm, 5 cm deep nursery trays (n = 200), which were divided into four blocks (Espeland, Perkins, & Leger, 2010). The greenhouse was maintained under an ambient temperature of 21 ˚C, during the day and 16 ˚C at night, to enhance optimal germination in a stratified environment

(Humphrey & Schupp, 2001). To ensure equal exposure to light, the positions of the trays in the four blocks were changed every month (Sileshi & Abraha, 2014). Randomized sampling and cold-moist stratification conditions in the greenhouse were used to mitigate potential limitations of the seedling emergence method, which may not account for dormant and ungerminated seeds (Allen & Nowak, 2008; Gonzalez & Ghermandi, 2012).

The soil samples were subjected to sequential treatments to enhance the probability of all seeds germinating. Transitioning each tray to the next treatment was done when no additional germination was observed in the trays after one week. The first treatment was a 2-minute daily watering which continued for five months until all germination ceased. This was followed by a dry phase for four weeks, to mimic natural fluctuations in soil moisture which is required for germination of some desert seeds (Baskin & Baskin, 1998; Meyer, Quinney, Nelson, & Weaver, 2007). The dry phase was followed by another daily watering phase for four weeks, the application of gibberellic acid to break seed dormancy of some species, and finally, the application of vinegar and sugar solution, which has been reported to stimulate seed germination through mycorrhizal association (Mu, Uehara, & Furuno, 2003). Also, the soil was stirred once every two months to allow for repeat germination of new seedlings (Ma et al., 2010). The experiment was ended when no additional seedling emerged for two consecutive weeks.

Germinated seedlings were identified and removed, or potted for later identification, to reduce seedling competition and facilitate optimal germination of seeds in the soil samples (Ma et al., 2010; Aponte, Kazakis, Ghosn, & Papanastasis, 2010; Gomaa, 2012). The number of species (species richness) and number of individuals for each species (species abundance) per site was recorded. Five morpho-species were also included, as they could not be identified to species. In addition, some seedlings did not survive beyond the seed-leaf stage and could not be identified. This is a common issue with employing the seedling emergence method, which has been reported in previous studies in the Great Basin Desert (see Allen & Nowak, 2008; Martyn et al., 2016).

Vegetative community sampling

In May 2016, the 10 study sites were revisited for plant community sampling. Twenty locations were randomly selected at each site using a stratified random sampling strategy focusing on *I. webberi* occupied patches. For each sampling point, we used a 1 m^2 quadrat to record total plant cover, plant richness, abundance, elevation and GPS coordinates. For each site, we also recorded soil type, dominant vegetation, distance (m) to major/minor road, disturbance intensity and physiognomic description. As in the soil seed bank estimate (section 2.2 above), species richness was taken as the number of species per site, while the total number of individuals per species was summed and used as species abundance per site. Disturbance intensity was described using an ordinal ranking of five classes as described in Appendix S1. Cover was measured as the total land area covered by plants and their shadows, estimated by eye as percentage of the quadrat. Percentage total vegetation cover for each site was obtained from the mean percent cover for the 20 quadrats sampled.

Environmental predictor variables

In addition to the field data, we also included 10 geospatial variables describing climate (seasonal mean temperature and precipitation for winter, spring, fall and summer, and solar radiation), and soil properties (soil mean clay, silt and sand). Selected predictor variables represented different components of water availability for plants, as is expected to be limiting for vegetation in arid ecosystems. However, the set of 17 predictor variables was reduced to six uncorrelated variables ($r < 0.59$) using an informed interpretation of Kendall correlation coefficients. The five selected abiotic variables, including solar radiation, soil mean clay, vegetation cover, and mean temperature for spring and winter seasons, together with the soil seed bank richness and diversity, were used as predictor variables (Table 2). Soil seed bank diversity was taken as the effective number of species, which are natural numbers produced from converting the logarithmic values of the Shannon-Wiener H' index for each site (Jost, 2006). Spatial variables were computed as the principal coordinates of the neighbor matrix (PCNM, Borcard & Legendre, 2002; Dray, Legendre, & Peres-Neto, 2006), obtained from the truncated pairwise geographical distance matrix among sampling sites. The resulting eight independent and orthogonal PCNM eigenvalues were further reduced to one spatial variable (PCNM dimension 8) following forward variable selection runs, which was used for variation partitioning.

Statistical analysis

Dice-Sorensen's quantitative similarity index was used to estimate similarities in species composition between the aboveground plant communities and the soil seed bank for each site (Osem, Perevolotsky, & Kigel, 2006). We also used transformation-based redundancy analysis (tb-RDA) to assess variation in the Hellinger-transformed species abundance across all sites, as explained by linear gradients in the five abiotic variables, and soil seed bank richness and diversity. The choice of RDA over canonical correspondence analysis (CCA) was supported by a linear relationship of species data to gradients of environmental predictors in a detrended correspondence analysis (DCA; Hill, 1979), following the recommendation of Lepš & Šmilauer (2003). Permutation tests (10,000 Monte-Carlo permutations) were used to evaluate the overall RDA model fitness relative to a permuted data, the significance of the constrained axes, and the partial effects of the variables used.

Variation partitioning was used to quantify the proportion of variation in the plant communities explained by the soil seed bank, abiotic and spatial variables (Borcard, Legendre, & Drapeau, 1992). The variables were clustered into climatic (seasonal mean temperatures for winter and spring, and solar radiation), site conditions (soil mean clay content and vegetation cover), soil seed bank (richness and diversity), and a spatial variable. Data analyses were done in R statistical software version 3.5.3 (R Core Team, 2019), using functions in various packages including *vegan* (Oksanen et al., 2019), *phytools* (Revell, 2012), and *ecodist* (Goslee & Urban, 2007). The raw data used for this study were published in Knowledge Network for Biocomplexity (Borokini, Weisberg & Peacock, 2020).

RESULTS

Floristic structure and association of the aboveground plant community with the soil seed bank

There was a 37% overall similarity in the floristic composition between the aboveground vegetation (hereafter, AGV) and the soil seed bank (hereafter, SSB) community (Table 3), with 21 species in common out of 82 total species. Species richness and diversity were higher in the AGV but total species abundance was higher in the SSB (Table 3). The AGV had higher richness of native perennial flora, in contrast to higher species richness of annuals in the SSB, but the dominance of invasive species in both the AGV and the SSB was apparent (see Appendix S2). Only one site had a disproportionately low abundance of invasive plants, which also had the highest species diversity in both the AGV and the SSB, and a significantly low similarity (Sorensen's index $w = 1441$, $p < 0.001$) between the AGV and the SSB (Table 3). *Ivesia webberi* was recorded in the AGV but not in the SSB of all studied sites.

Effect of environmental variables on the structure of the aboveground plant community

The transformation-based redundancy analysis illustrated key relationships among the environmental variables and the aboveground plant community structure in the sampled sites. The tb-RDA model was significantly different than a random model of permuted data ($F = 2.24$, $p < 0.004$), produced seven constrained axes capturing 88.67% of the variance in the AGV data, 50.15% of which was captured in the first RDA axis ($F = 5.24$, $p < 0.006$) and the second RDA axis ($F = 3.62$, $p < 0.04$). The first ordination axis describes a gradient from sites with warmer winter temperatures, higher solar radiation and greater overall vegetation cover, all of which were associated with greater levels of invasive *B. tectorum* dominance, to sites with colder winters, lower solar radiation and lower cover, associated with dominance of invasive *Draba verna* (Figure 2). Along axis

2, soil mean clay content was positively correlated with seed bank diversity, both of which were negatively correlated with seed bank richness and abundance of invasive plants (Figure 2). This suggests that sites with higher abundance of invasive *B. tectorum* and *D. verna* have lower seed bank diversity. Correlation among the environmental variables (Table 2) is congruent with their contribution to the structure of the sampled aboveground plant communities in ordination space, however these correlations were not statistically significant. The ordination biplot also showed that some sites have higher abundance of invasive *B. tectorum*, and *D. verna* (Figure 2). Species diversity in the SSB was the only variable with significant partial effects on the RDA model (Table 4).

Quantifying the relationship of the environmental variables and aboveground plant community composition

Climatic variables explained most of the variance in the sampled plant communities, followed by the soil seed bank, site properties and lastly the spatial variable. The variance explained by the synergistic effect of the environmental variables was much lower, except for the variance shared among the grouped climate, site and seed bank variables (Figure 3). Overall, 61% of the variation in the aboveground plant communities was explained by the environmental variables.

DISCUSSION

An assessment of soil seed banks and their floristic similarities with the aboveground vegetation could be used to infer the ability of a vegetative community to maintain its ecological integrity post-disturbance. In this study, we observed high dissimilarity in the floristic composition of the aboveground communities and the soil seed bank in the 10 sites occupied by *Ivesia webberi*. This dissimilarity is due primarily to the absence in the seed bank of the majority of the native plant species recorded in the standing vegetation. The majority of native plants in the Great Basin Desert produce fewer seeds (Guo, Rundel, & Goodall, 1998; Lucero & Callaway, 2018) compared to the prolific seed production in the invasive alien grasses (Gioria & Osborne, 2010; Gremer & Venable, 2014; Martyn et al., 2016), which could result in greater sampling of the soil seed bank of invasive plant species than the native plants. Furthermore, native plants may be represented in the pool of seedlings that died before they could be identified during the study, or could have dormancy requirements that were not met in our experimental conditions. However, the seedling mortality observed in this study is similar to previous studies that reported high seedling mortality of native plants in the Great Basin Desert (James, Svejcar, & Rinella, 2011; Boyd & Lemos, 2015). Species compositional dissimilarity between AGV and SSB has been previously reported in many ecosystems including grasslands (Peco et al., 1998; Valkó et al., 2014), heathland (Valbuena & Trabaud, 2001), salt marshes (Egan & Unger, 2000), alpine sites (Ma et al. 2010), wetlands (Jutila, 2003; Aponte et al., 2010), forest-grassland mosaic (Díaz-Villa, Marañón, Arroyo, & Garrido, 2003), natural and planted forests (Lemenih & Teketay, 2006), and particularly for desert ecosystems (Guo, Rundel, & Goodall, 1999; Gomaa, 2012), including the Great Basin desert (Martyn et al., 2016; Barga & Leger 2018).

The redundancy analysis showed an association of greater vegetation cover, especially of the invasive *B. tectorum*, with warmer winter temperatures and higher solar radiation. Similar relationships with incident solar radiation have been observed from studies elsewhere in the Great Basin (Condon, Weisberg, & Chambers, 2011; Williamson et al., 2020). Warmer winter temperatures result in rapid snowmelt and increasing soil moisture which supports regeneration of winter annuals, such as the invasive *B. tectorum*. Furthermore, reduced snowpack decimates the population of the snow molds which damage *B. tectorum* seedlings (Smull, Pendleton, Kleinhesselink, & Adler, 2019).

Alien plant invasions are well known to lead to plant community homogenization through the suppression of native plant species (Richardson, Macdonald, & Forsythe, 1989; Hejda, Pyšek, & Jarošik, 2009). Consistent with this, we observed an inverse relationship between the abundance of invasive plants and species diversity. For example, the only site with low abundance of invasive plants also had the highest native plant species richness and diversity in both the aboveground vegetation and the soil seed bank. Additionally, high abundance of invasive plants in the standing vegetation corresponded with high abundance in the soil seed bank, which was also associated with reduced diversity and richness of native plant species in the soil seed banks. These findings show that the plant community structure in the sampled sites is driven largely by the abundance of invasive species. This pattern is supported by earlier work that show that a large proportion of the standing vegetation and the soil seed bank in the Great Basin Desert has been invaded by nonnative plant species (Humphrey & Schupp, 2001; 2004; Cox & Allen, 2008; Aponte et al., 2010; Bradley et al., 2018). The disproportionately higher abundance of alien plant species in the sampled soil seed bank could have had negative effects on the emergence of native perennials and resulted in competitive exclusion of native plant seedlings (Brooks, 2000; Humphrey & Schupp, 2004; Chambers, Roundy, Blank, Meyer, & Whittaker, 2007).

Results of the variation partitioning analysis showed varying contributions of the environmental, spatial variables and the soil seed bank on the floristic assemblage and structure of the aboveground communities across the 10 studied sites. It is not surprising that climatic variables contributed the most to the plant community structure, because the regeneration of many Great Basin Desert flora, including *I. webberi*, is influenced by climatic factors, especially by winter and spring temperature (USFWS, 2014; Chick, Nitschke, Cohn, Penman, & Yoek, 2018). The contribution of the soil seed bank was also relatively high in the variation partitioning, but given a high floristic dissimilarity in the AGV and the SSB, the proportion explained by the soil seed bank is primarily due to the abundance of the invasive species. Furthermore, it is particularly interesting that the synergistic effects of climatic, site, and seedbank variables explained 11.5% of the aboveground plant community structure. This may highlight the relationship among surface and soil temperatures in winter and spring and the soil water retention capacity, which provide the natural stratification needed for seed germination and vegetative regeneration of perennials, as well as winter and spring annuals (Humphrey & Schupp, 2001).

The observed disparity between species composition of the aboveground plant community and the soil seed bank suggests that these study sites have low resistance and resilience to invasion by nonnative weeds following disturbance, and are in high risk of losing their ecological legacies. The selected sites in this study are of the mid-elevation *I. webberi-Artemisia arbuscula* vegetative association (USFWS, 2014), which experience frequent inter-annual soil moisture fluctuations (Chambers et al., 2007), and which may be vulnerable to shifts in species assemblages due to climate change (Kiss, Deák, Török, Tóthmérész, & Valkó, 2018). Post-fire recovery of the dominant native perennials in these sites is weak and slow (Miller, Chambers, Pyke, Pierson, & Williams, 2013). This implies that post-disturbance plant early succession may be driven by invasive alien weeds (DiVittorio, Corbin, & D'Antonio, 2007; Gioria & Osborne, 2010; Morris, Monaco, & Sheley, 2011). However, given the natural history of these sites, and depending on the severity of the disturbance, some native perennials may regenerate from surviving vegetative structures. Overall, these findings caution against reliance on the soil seed bank for restoration of desert perennials (Laughlin, 2003; Maccherini & De Dominicis, 2003; Handlová & Münzbergová, 2006; Allen & Nowak, 2008; Gomaa, 2012), underscore the importance of seeding with native plants as a key restoration program (Knutson et al., 2014; Svejcar, Boyd, Davies, Hamerlynck, & Svejcar, 2017), and highlight the importance of research and management practices aimed at controlling invasive plants (Elseroad & Rudd, 2011).

REFERENCES

Adams, V. M., Marsh, D. M., & Knox, J. S. (2005). Importance of the seed bank for population viability and population monitoring in a threatened wetland herb. *Biological Conservation, 124*, 425–436. https://doi.org/10.1016/j.biocon.2005.02.001

Allen, E. A., & Nowak, R. S. (2008). Effect of pinyon-juniper tree cover on the soil seed bank. *Rangeland Ecology and Management, 61,* 63–73. https://doi.org/10.2111/07-007R2.1

Aponte, C., Kazakis, G., Ghosn, D., & Papanastasis, V. P. (2010). Characteristics of the soil seed bank in Mediterranean temporary ponds and its role in ecosystem dynamics. *Wetlands Ecology and Management, 18,* 243-253. https://doi.org/10.1007/s11273-009-9163-5

Balch, J. K., Bradley, B. A., D'Antonio, C. M., & Gómez-Dans, J. (2013). Introduced annual grass increases regional fire activity across the arid western USA (1980-2009). *Global Change Biology, 19,* 173–183. https://doi.org/10.1111/gcb.12046

Barga, S., & Leger, E. A. (2018). Shrub cover and fire history predict seed bank composition in Great Basin shrublands. *Journal of Arid Environments, 154,* 40-50. https://doi.org/10.1016/j.jaridenv.2018.03.004

Baskin C.C., and Baskin J.M. (1998). *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination* (1st ed.). New York: Academic Press.

Borcard, D., & Legendre, P. (2002). All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling, 153,* 51–68. https://doi.org/10.1016/S0304-3800(01)00501-4

Borcard, D., Legendre, P., & Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology, 73,* 1045–1055. https://doi.org/10.2307/1940179

Borokini, T., Weisberg, P., & Peacock, M. (2020). Quantifying the relationship between soil seed bank and plant community assemblage in *Ivesia webberi* A. Gray populations in the western Great Basin Desert. Knowledge Network for Biocomplexity. doi:10.5063/F1FN14J7.

Bossuyt, B., & Honnay, O. (2008). Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. *Journal of Vegetation Science, 19*, 875-884. https://doi.org/10.3170/2008-8-18462

Boyd, C. S., & Lemos, J. A. (2015). Evaluating Winter/Spring Seeding of a Native Perennial Bunchgrass in the Sagebrush Steppe. *Rangeland Ecology & Management, 68,* 494-500. https://doi.org/10.1016/j.rama.2015.07.009

Bradley, B. A., Curtis, C. A., Fusco, E. J., Abatzoglou, J. T., Balch, J. K., Dadashi, S., & Tuanmu, M. N. (2018) Cheatgrass (*Bromus tectorum*) distribution in the Intermountain Western United States and its relationship to fire frequency, seasonality, and ignitions. *Biological Invasions, 20*, 1493–1506. https://doi.org/10.1007/s10530-017-1641-8

Brooks, M. L. (2000). Competition between Alien Annual Grasses and Native Annual Plants in the Mojave Desert. *The American Midland Naturalist, 144*, 92-108. https://doi.org/10.1674/0003-0031(2000)144[0092:CBAAGA]2.0.CO;2

Bullock, J. M., Hill, B. C., Silvertown, J., & Sutton, M. (1995). Gap colonization as a source of grassland community change: effects of gap size and grazing on the rate and mode of colonization by different species. *Oikos*, *72*, 273– 282. https://doi.org/10.2307/3546229

Chambers, J. C., Roundy, B. A., Blank, R. R., Meyer, S. E., and Whittaker, A. (2007). What makes Great Basin sagebrush ecosystems invasible by *Bromus tectorum*? *Ecological Monographs, 77*, 117-145. https://doi.org/10.1890/05-1991

Chaney, N. W., Wood, E. F., McBratney, A. B., Hempel, J. W., Nauman, T. W., Brungard, C. W., & Odgers, N. P. (2016). POLARIS: A 30-meter probabilistic soil series map of the contiguous United States. *Geoderma, 274,* 54–67. https://doi.org/10.1029/2018WR022797

Chick, M. P., Nitschke, C. R., Cohn, J. S., Penman, T. D., & Yoek, A. (2018). Factors influencing above-ground and soil seed bank vegetation diversity at different scales in a quasi-Mediterranean ecosystem. *Journal of Vegetation Science, 29*, 684-694. https://doi.org/10.1111/jvs.12649

Coates, P. S., Ricca, M. A., Prochazka, B. G., Brooks, M. L., Doherty, K. E., Kroger T., …, Casazza M.L. (2016). Wildfire, climate, and invasive grass interactions negatively impact an indicator species by reshaping sagebrush ecosystems. *Proceedings of the National Academy of Science, 113,* 12745-12750. https://doi.org/10.1073/pnas.1606898113

Condon, L., Weisberg, P. J., & Chambers, J. C. (2011). Abiotic and biotic influences on *Bromus tectorum* invasion and *Artemisia tridentata* recovery after fire. *International Journal of Wildland Fire*, *20,* 597-604. https://doi.org/10.1071/WF09082

Cox, R. D., & Allen, E. B. (2008). Composition of Soil Seed Banks in Southern California Coastal Sage Scrub and Adjacent Exotic Grassland. *Plant Ecology, 198*, 37-46. https://doi.org/10.1007/s11258-007-9383-9

Decocq, G., Valentin, B., Toussaint, B., Hendoux, F., Saguez, R., & Bardat, J. (2004). Soil seed bank composition and diversity in a managed temperate deciduous forest. *Biodiversity and Conservation, 13*, 2485–2509. https://doi.org/10.1023/B:BIOC.0000048454.08438.c6

DeFalco, L. A., Esque, T. C., Kane, J. M., & Nicklas, M. B. (2009). Seed banks in a degraded desert shrubland: Influence of soil surface condition and harvester ant activity on seed abundance. *Journal of Arid Environments, 73*, 885-893. https://doi.org/10.1016/j.jaridenv.2009.04.017

Díaz-Villa, M. D., Marañón, T., Arroyo, J., & Garrido, B. (2003). Soil seed bank and floristic diversity in a forest-grassland mosaic in southern Spain. *Journal of Vegetation Science, 14*, 701-709. https://doi.org/10.1111/j.1654-1103.2003.tb02202.x

DiVittorio, C. T., Corbin, J. D., & D'Antonio, C. M. (2007). Spatial and temporal patterns of seed dispersal: an important determinant of grassland invasion. *Ecological Applications, 17*, 311– 316. https://doi.org/10.1890/06-0610

Dray, S., Legendre, P., & Peres-Neto, P. R. (2006). Spatial modeling: a comprehensive framework for principal coordinate analysis of neighbor matrices (PCNM). *Ecological Modelling, 196*, 483–493. https://doi.org/10.1016/j.ecolmodel.2006.02.015

Egan, T. P., & Unger, I. A. (2000). Similarity between seed bank and aboveground vegetation along salinity gradient. *Journal of Vegetation Science, 11*, 189-194. https://doi.org/10.2307/3236798

Elseroad, A. C., & Rudd, N. T. (2011). Can imazapic increase native species abundance in cheatgrass (*Bromus tectorum*) invaded native plant communities? *Rangeland Ecology and Management, 64*, 641-648. https://doi.org/10.2111/REM-D-10-00163.1

Espeland, E. K., Perkins, L. B., & Leger, E. A. (2010). Comparison of seed bank estimation techniques using six weed species in two soil types. *Rangeland Ecological and Management, 63*, 243-247. https://doi.org/10.2111/REM-D-09-00109.1

Franckowiak, R. P., Panasci, M., Jarvis, K. J., Acuña-Rodriguez, I. S., Landguth, E. L., Fortin, M.-J., & Wagner, H. H. (2017). Model selection with multiple regression on distance matrices leads to incorrect inferences. *PLoS ONE, 12*, e0175194. https://doi.org/10.1371/journal.pone.0175194

Fu, P., & Rich, P. M. (2002). A geometric solar radiation model with applications in agriculture and forestry. *Computers and Electronics in Agriculture, 37*, 25–35. https://doi.org/10.1016/S0168-1699(02)00115-1

Gioria, M., & Osborne, B. (2010). Similarities in the impact of three large invasive plant species on soil seed bank communities. *Biological Invasions, 12*, 1671-1683. https://doi.org/10.1007/s10530-009-9580-7

Gioria, M., & Pyšek, P. (2016). The legacy of plant invasions: changes in the soil seed bank of invaded plant communities. *BioScience, 66*, 40-53. https://doi.org/10.1093/biosci/biv165

Gomaa, N. H. (2012). Soil seed bank in different habitats of the Eastern Desert of Egypt. *Saudi Journal of Biological Science, 19*, 211-220. https://doi.org/10.1016/j.sjbs.2012.01.002

Gonzalez, S. L, & Ghermandi, L. (2012). Comparison of methods to estimate soil seed banks: the role of seed size and mass. *Community Ecology, 13*, 238-242. https://doi.org/10.1556/ComEc.13.2012.2.14

Goodwillie, C., Kalisz, S., & Eckert, C. G. (2005). The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Reviews on Ecology, Evolution and Systematics, 36*, 47-79. https://doi.org/10.1146/annurev.ecolsys.36.091704.175539

Goslee, S. C., & Urban, D. L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software, 22*, 1-19. doi: 10.18637/jss.v022.i07

Gremer, J. R., & Venable, D. L. (2014). Bet hedging in desert winter annual plants: optimal germination strategies in a variable environment. *Ecology Letters, 17*, 380–387. https://doi.org/10.1111/ele.12241

Guo, Q., Rundel, P. W., & Goodall, D. W. (1998). Horizontal and vertical distribution of desert seed banks: patterns, causes, and implications. *Journal of Arid Environments, 38*, 465-478. https://doi.org/10.1006/jare.1997.0353

Guo, Q., Rundel, P. W., & Goodall, D. W. (1999). Structure of desert seed banks: comparisons across four North American deserts. *Journal of Arid Environments, 42*, 1-14. https://doi.org/10.1006/jare.1999.0502

Haapalehto, T., Juutinen, R., Kareksela, S., Kuitunen, M., Tahvanainen, T., Vuori, H., & Kotiaho, J. S. (2017). Recovery of plant communities after ecological restoration of forestry-drained peatlands. *Ecology and Evolution, 7*, 7848–7858. https://doi.org/10.1002/ece3.3243

Handlová, V., & Munzbergová, Z. (2006). Seed banks of managed and degraded grasslands in the Krkonose Mts., Czech Republic. *Folia Geobotanica, 41*, 275-288. https://doi.org/10.1007/BF02904942

Hejda, M., Pyšek, P. & Jarošík, V. (2009). Impact of invasive plants on the species richness, diversity and composition of invaded communities. *Journal of Ecology, 97*, 393-403. https://doi.org/10.1111/j.1365-2745.2009.01480.x

Hill, M. O. (1979). *DECORANA – a FORTRAN Program of Detrended Correspondence Analysis and Reciprocal Averaging. Ecology and Systemics*. Ithaca, New York: Cornell University.

Hopfensperger, K. N. (2007). A review of similarity between seed bank and standing vegetation across ecosystems. *Oikos, 116*, 1438–1448. https://doi.org/10.1111/j.0030-1299.2007.15818.x

Humphrey, L. D., & Schupp, E. W. (2001). Seed banks of *Bromus tectorum*-dominated communities in the Great Basin. *Western North American Naturalist, 61*, 85–92.

Humphrey, L. D., & Schupp, E. W. (2004). Competition as a barrier to establishment of a native perennial grass (*Elymus elymoides*) in alien annual grass (*Bromus* *tectorum*) communities. *Journal of Arid Environments, 58*, 405-422. https://doi.org/10.1016/j.jaridenv.2003.11.008

James, J. J., Svejcar, T. J., & Rinella, M. J. (2011). Demographic processes limiting seedling recruitment in arid grassland restoration. *Journal of Applied Ecology,* 48, 961–969. https://doi.org/10.1111/j.1365-2664.2011.02009.x

Jost L. (2006). Entropy and diversity. *Oikos*, *113,* 363–375. https://doi.org/10.1111/j.2006.0030-1299.14714.x

Jutila, H. M. (2003). Germination in Baltic coastal wetland meadows: similarities and differences between vegetation and seed bank. *Plant Ecology, 166,* 275-293. https://doi.org/10.1023/A:1023278328077

Kalamees, R., & Zobel, M. (2002). The role of the seed bank in gap regeneration in a calcareous grassland community. *Ecology, 83,* 1017-1025. https://doi.org/10.1890/0012-9658(2002)083[1017:TROTSB]2.0.CO;2

Kemp, P. R. (1989). Seed banks and vegetation processes in deserts. In M. A. Leck, V. T. Parker, & R. L. Simpson (Eds.), *The ecology of soil seed banks* (1st ed., pp. 257-281). New York, NY: Academic Press.

Kiss, R., Deák, B., Török, P., Tóthmérész, B., & Valkó, O. (2018). Grassland seed bank and community resilience in a changing climate. *Restoration Ecology, 26,* S141-S150. https://doi.org/10.1111/rec.12694

Knutson, K. C., Pyke, D. A., Wirth, T. A., Arkle, R. S., Pilliod, D. S., Brooks, M. L., …, Grace, J. B. (2014). Long-term effects of seeding after wildfire on vegetation in Great Basin shrubland ecosystems. *Journal of Applied Ecology, 51,* 1414-1424. https://doi.org/10.1111/1365-2664.12309

Korb, J. E., Springer, J. D., Powers, S. R., & Moore, M. M. (2005). Soil seed banks in *Pinus ponderosa* forests in Arizona, clues to site history and restoration potential. *Applied Vegetation Science, 8,* 103–112. https://doi.org/10.1111/j.1654-109X.2005.tb00634.x

Laughlin, D. C. (2003). Lack of native propagules in a Pennsylvania, USA, limestone prairie seed bank: futile hopes for a role in ecological restoration. *Natural Areas Journal*, *23,* 158-164.

Lee, P. (2004). The impact of burn intensity from wildfires on seed and vegetative banks, and emergent understorey in aspen-dominated boreal forests. *Canadian Journal of Botany, 82,* 1468-1480. https://doi.org/10.1139/b04-108

Legendre, P., & Fortin, M.-J. (2010). Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. *Molecular Ecology Resources, 10,* 831-844. https://doi.org/10.1111/j.1755-0998.2010.02866.x

Lemenih, M., & Teketay, D. (2006). Changes in soil seed bank composition and density following deforestation and subsequent cultivation of a tropical dry Afromontane forest in Ethiopia. *Tropical Ecology, 47*, 1–12.

Lepš, J., & Šmilauer, P. (2003). *Multivariate analysis of ecological data using CANOCO*. Cambridge, UK: Cambridge University Press.

Lucero, J. E., & Callaway, R. M. (2018). Native granivores reduce the establishment of native grasses but not invasive *Bromus tectorum*. *Biological Invasions, 20*, 3491–3497. https://doi.org/10.1007/s10530-018-1789-x

Ma, M., Baskin, C. C., Li, W., Zhao, Y., Zhao, Y., Zhao, L., …, Du, G. (2019). Seed banks trigger ecological resilience in subalpine meadows abandoned after arable farming on the Tibetan Plateau. *Ecological Applications, 29*, e01959. https://doi.org/10.1002/eap.1959

Ma, M., Zhou, X., & Du, G. (2010). Role of soil seed bank along a disturbance gradient in an alpine meadow on the Tibet plateau. *Flora, 205*, 128-134. https://doi.org/10.1016/j.flora.2009.02.006

Maccherini, S., & De Dominicis, V. (2003). Germinable soil seed-bank of former grassland converted to coniferous plantation. *Ecological Research, 18*, 739-751. https://doi.org/10.1111/j.1440-1703.2003.00592.x

Martyn, T. E., Bradford, J. B., Schlaepfer, D. R., Burke, I. C., & Lauenroth, W. K. (2016). Seed bank and big sagebrush plant community composition in a range margin for big sagebrush. *Ecosphere, 7*, e01453. https://doi.org/10.1002/ecs2.1453

Meyer, S. E., Quinney, D., Nelson, D. L., & Weaver, J. (2007). Impact of the pathogen *Pyrenophora semeniperda* on *Bromus tectorum* seedbank dynamics in North American cold deserts. *Weed Research, 47*, 54-62. https://doi.org/10.1111/j.1365-3180.2007.00537.x

Milberg, P. (1993). Seed bank and seedling emerging after soil disturbance in a wet semi-natural grassland in Sweden. *Annales Botanici Fennici, 30*, 9– 13.

Miller, R., Chambers, J. C., Pyke, D. A., Pierson, F. B., & Williams, C. J. (2013). *A review of fire effects on vegetation and soils in the Great Basin Region: response and ecological site characteristics*. Gen. Tech. Rep. RMRS-GTR-308. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 126 p.

Mndela, M., Madakadze, C. I., Nherera-Chokuda, F., & Dube, S. (2020). Is the soil seed bank a reliable source for passive restoration of bush-cleared semi-arid rangelands of South Africa? *Ecological Processes, 9*, 1. https://doi.org/10.1186/s13717-019-0204-6

Morris, L.R., Monaco, T.A., & Sheley, R.L. (2011). Land-use legacies and vegetation recovery 90 Years after cultivation in Great Basin Sagebrush ecosystems. *Rangeland Ecology & Management, 64*, 488-497. https://doi.org/10.2111/REM-D-10-00147.1

Mu, J., Uehara, T., & Furuno, T. (2003). Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants. *Journal of Wood Science, 49*, 262-270. https://doi.org/10.1007/s10086-002-0472-z

Nachlinger, J., Sochi, K., Comer, P., Kittel, G., & Dorfman, D. (2001). *Great Basin: an ecoregion-based conservation blueprint*. Reno, NV: The Nature Conservancy.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., …, Wagner, H. (2019). *vegan*: Community Ecology Package. R package version 2.5-4. https://CRAN.R-project.org/package=vegan

Osem, Y., Perevolotsky, A., & Kigel, J. (2006). Similarity between seed bank and vegetation in a semi-arid annual plant community: The role of productivity and grazing. *Journal of Vegetation Science, 17*, 29-36. https://doi.org/10.1111/j.1654-1103.2006.tb02420.x

Peco, B., Ortega, M., & Levassor, C. (1998). Similarity between seed bank and vegetation in Mediterranean grassland: a predictive model. *Journal of Vegetation Science, 9*, 815-828. https://doi.org/10.2307/3237047

Pekas, K. M., & Schupp, E. W. (2013). Influence of aboveground vegetation on seed bank composition and distribution in a Great Basin Desert sagebrush community. *Journal of Arid Environments, 88*, 113–120. https://doi.org/10.1016/j.jaridenv.2012.08.013

Pilliod, D. S., Welty, J. L., & Arkle, R. S. (2017). Refining the cheatgrass–fire cycle in the Great Basin: precipitation timing and fine fuel composition predict wildfire trends. *Ecology and Evolution, 7*, 8126-8151. https://doi.org/10.1002/ece3.3414

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Revell, L. J. (2012) *phytools*: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution, 3*, 217-223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Richardson, D. M., Macdonald, I. A., & Forsyth, G. C. (1989). Reduction in plant species richness under stands of alien trees and shrubs in fynbos biome. *South African Forestry Journal*, *149*, 1–8. https://doi.org/10.1080/00382167.1989.9628986

Sileshi, D., & Abraha, B. (2014). Assessment of soil seedbank composition of woody species in Hgumbirda National Forest Priority Area, Northeastern Ethiopia. *Momona Ethiopian Journal of Science, 6*, 25-44. doi: 10.4314/mejs.v6i1.102413

Smull, D. M., Pendleton, N., Kleinhesselink, A. R., & Adler, P. B. (2019). Climate change, snow mold and the *Bromus tectorum* invasion: mixed evidence for release from cold weather pathogens. *AoB PLANTS, 11*, plz043. https://doi.org/10.1093/aobpla/plz043

Solomon, T. B. (2011). Soil seed bank dynamics in relation to land management and soil types in the semi-arid savannas of Swaziland. *African Journal of Agricultural Research*, *6*, 2494–2505. https://doi.org/10.5897/AJAR10.1054

Stein, B., Kutner, L. S., & Adams, J. S. (2000). *Precious Heritage: The Status of Biodiversity in the United States*. New York: Oxford University Press.

Svejcar, T., Boyd, C., Davies, K., Hamerlynck, E., & Svejcar, L. (2017). Challenges and limitations to native species restoration in the Great Basin, USA. *Plant Ecology, 218*, 81-94. https://doi.org/10.1007/s11258-016-0648-z

Ter Heerdt, G. N. J., Verweij, G. L., Bekker, R. M., & Bakker, J. P. (1996). An improved method for seed-bank analysis: seedling emergence after removing the soil by sieving. *Functional Ecology, 10*, 144-151. doi: 10.2307/2390273

Tessema, Z. K., de Boer, W. F., Baars, R. M. T., & Prins, H. H. T. (2012). Influence of Grazing on soil seed banks determines the restoration potential of aboveground vegetation in a semi-arid savanna of Ethiopia. *Biotropica, 44*, 211–219. https://doi.org/10.1111/j.1744-7429.2011.00780.x

Thompson, K., & Grime, J. P. (1979). Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology*, 67, 893–921. https://doi.org/10.2307/2259220

U.S. Geological Survey [USGS] (2016). USGS National Elevation Dataset (NED) 1 arc-second Downloadable Data Collection from The National Map 3D Elevation Program (3DEP) - National Geospatial Data Asset (NGDA) National Elevation Data Set (NED): U.S. Geological Survey.

United States Fish and Wildlife Services [USFWS] (2014). *Species Report for Ivesia webberi (Webber's ivesia)*. Nevada Fish and Wildlife Office, Reno, 49 pp.

Valbuena, L., & Trabaud, L. (2001). Contribution of the soil seed bank to post-fire recovery of a heathland. *Plant Ecology, 152*, 175-183. https://doi.org/10.1023/A:1011419721307

Valkó, O., Tóthmérész, B., Kelemen, A., Simon, E., Miglécz, T., Lukács, B. A., & Török, P. (2014). Environmental factors driving seed bank diversity in alkali grasslands. *Agriculture, Ecosystems and Environment, 182*, 80–87. https://doi.org/10.1016/j.agee.2013.06.012

Vanstockem, J., Ceusters, C., Van Dyck, K., Somers, B., & Hermy, M. (2018). Is there more than meets the eye? Seed bank analysis of a typical novel ecosystem, the extensive green roof. *Applied Vegetation Science, 21*, 419-430. https://doi.org/10.1111/avsc.12383

Walters, G. M. (2004). Perennial plants and ephemeral seed banks in Papago Park, Phoenix, Arizona. *Natural Areas Journal, 24*, 36-43

Williamson, M. A., Fleishman, E., Mac Nally, R. C., Chambers, J. C., Bradley, B. A., Dobkin, D. S., Board, D. I., Fogarty, F. A., Horning, N., Leu, M., & Zillig, M. W. (2020). Fire, livestock grazing, topography, and precipitation affect occurrence and prevalence of cheatgrass (*Bromus tectorum*) in the central Great Basin, USA. *Biological Invasions*, *22*, 663-680. https://doi.org/10.1007/s10530-019-02120-8

Wisdom, M. J., & Chambers, J. C. (2009). A landscape approach for ecologically based management of Great Basin shrublands. *Restoration Ecology*, *17*, 740-749. https://doi.org/10.1111/j.1526-100X.2009.00591.x

Witham, C. W. (2000). *Current Knowledge and Conservation Status of Ivesia webberi A. Gray (Rosaceae), the Webber ivesia, in Nevada*. Status Report prepared for the Nevada Natural Heritage Program, Department of Conservation and Natural Resources, Carson City, NV. 52pp.

TABLES

Table 1. Characteristics of the 10 *Ivesia webberi* study sites in the western Great Basin Desert, United States

Unit[a]	**Site location**	**County and State**	**Site area (m^2)**[b]	**Solar radiation (WH/ha)**[c]	**Soil clay (%)**[d]	**Elevation (m)**[e]	**Disturbance rank**[f]
2	Near Constantia	Lassen CA	7,700	1.42	8	1435.97	2
3	East of Hallelujah Junction	Lassen CA	1,400	1.47	8	1561.68	1
5	Dog Valley Meadows	Sierra CA	289,700	1.51	16	1834.70	1
6	White Lake Overlook	Sierra CA	54,900	1.47	11	1715.15	1
7	Mules Ear flat	Sierra CA	1,400	1.46	12	1680.01	1
8	Ivesia flat	Washoe NV	3,000	1.43	10	1775.63	1
11	Hungry Valley	Washoe NV	600	1.47	9	1599.01	3
12	Black Springs	Washoe NV	25,500	1.46	14	1730.17	2
13	Raleigh Heights	Washoe NV	38,600	1.48	14	1602.60	3
16	Dante Mine Road	Douglas NV	2,300	1.54	12	1894.81	1

[a]USFWS unit assignment for the *I. webberi* populations (see USFWS, 2014); [b]Site area was calculated from USFWS (2014); [c]Solar radiation was calculated in ArcMap using hemispherical viewshed algorithm (Fu & Rich, 2002) in ArcMap version 10.6.1; [d]Soil mean clay content was extracted from soil series probabilistic map (Chaney et al., 2016) in ArcMap; [e]Elevation was extracted from 30 m digital elevation model of (United States Geological Survey [USGS], 2016) in ArcMap; [f]Disturbance ranking was used (see Appendix S1).

Table 2. Correlation matrix of the predictor variables used for the redundancy analysis. The upper matrix contains the Kendall correlation coefficient, while the lower matrix contains the corresponding *p* values

Predictor variable	**Solar radiation**	**Winter temperature**	**Spring temperature**	**Soil clay content**	**Vegetative cover**	**Seedbank diversity**	**Seedbank richness**
Solar radiation	1	0.43	0.54	0.52	0.01	0.41	-0.23
Winter temperature	0.2194	1	0.51	-0.09	0.20	-0.41	0.22
Spring temperature	0.1048	0.13	1	-0.24	-0.21	-0.28	0.60
Soil clay content	0.1217	0.79	0.51	1	0.37	0.53	-0.10
Vegetative cover	0.9945	0.58	0.57	0.29	1	0.21	0.43
Seedbank diversity	0.2434	0.24	0.44	0.12	0.55	1	-0.42
Seedbank richness	0.5178	0.55	0.07	0.79	0.21	0.23	1

Table 3. Species richness, abundance, diversity indices, effective number of species and similarity indices in the aboveground vegetation (AGV) and the soil seed bank (SSB) in 10 sampled *Ivesia webberi* sites

***Ivesia webberi* sites**	**Species richness**		**Sorensen's similarity index**	**Species abundance**		**H' index (alpha diversity)**		**Effective number of species**	
	AGV	**SSB**		**AGV**	**SSB**	**AGV**	**SSB**	**AGV**	**SSB**
Unit 2	18	18	0.28	3667	21439	1.23	0.32	3.41	1.38
Unit 3	20	14	0.29	2323	6453	1.26	0.59	3.53	1.80
Unit 5	34	14	0.21	1021	86	2.51	2.17	12.35	8.72
Unit 6	26	16	0.33	2405	2031	2.33	0.54	10.32	1.72
Unit 7a	26	16	0.33	9197	10472	1.40	0.85	4.07	2.33
Unit 8	22	16	0.26	9091	2419	1.84	1.12	6.28	3.05
Unit 11	24	21	0.31	3321	1304	0.64	1.11	1.89	3.04
Unit 12	25	20	0.36	3300	7911	1.28	0.29	3.60	1.33
Unit 13	20	18	0.26	5494	4371	1.84	0.58	6.26	1.79
Unit 16	21	16	0.43	1211	1285	2.18	0.97	8.81	2.65
Total	82**	32**	0.37**	41030	57771				

**Morphospecies were not included the soil seed bank data; **Species richness and similarity index in the total row for both the aboveground community and the soil seed bank is the overall species richness or similarity index, not a sum of all sampled sites in the rows above it.*

Table 4. Permutation test for the redundancy analysis of the effects of the predictor variables on the plant community structure in the 10 *Ivesia webberi* sites. *F* and *P* distributions were generated using 10,000 permutations

Variable	***df***	**Variance**	***F***	***P***
Vegetative cover	1	0.036	1.521	0.169
Solar radiation	1	0.044	1.873	0.074
Mean winter temperature	1	0.040	1.693	0.115
Mean spring temperature	1	0.037	1.569	0.148
Soil mean clay content	1	0.040	1.692	0.111
Seed bank species richness	1	0.035	1.475	0.184
Seed bank species diversity	1	0.055	2.334	0.026
Residual	2	0.047		

FIGURE LEGENDS

Figure 1. Global distribution of *Ivesia webberi* populations. *Unit numbers follow the USFWS designations, circles represent the geographic center of extant, mapped occurrences, and circles of the same color indicate USFWS-designated subpopulations of the same population. Asterisk on unit 17 indicates it is a new proposed unit, as it was recently discovered.*

Figure 2. Redundancy analysis biplot of the predictor variables on the Hellinger-transformed species abundance in the aboveground plant community. The biplot was scaled symmetrically by eigenvalues of both the species and site scores. Arrow length represents the strength of the variables in the ordination space, and the arrow direction illustrates the gradients. For clarity, species reported in at least seven sites were displayed. See Appendix S3 for a full list of all species and their associated acronyms used in this Figure.

Figure 3. Venn diagram showing variation in the aboveground plant communities partitioned to variables associated individually with climate, site, soil seed bank and spatial properties across all 10 sampled *Ivesia webberi* sites. The values represent the adjusted R squared values of each fraction in the partitioning.


120°0'0"W
40°0'0"N
39°0'0"N
Unit 17*
Unit 2
Unit 1
Unit 3
Unit 4
Unit 7
Unit 11
Unit 6
Unit 8
Unit 13
Unit 9
Unit 10
Unit 12
Unit 5
Reno
Unit 14
Unit 15
Carson City
Lake Tahoe
Nevada
California
Unit 16
CALIFORNIA
NEVADA
WASATCH RANG
an Francisco
SIERRA NEVADA
ARI
0 5 10 20 30 40 Kilometers
1 cm = 7 km
N

Figure 1.

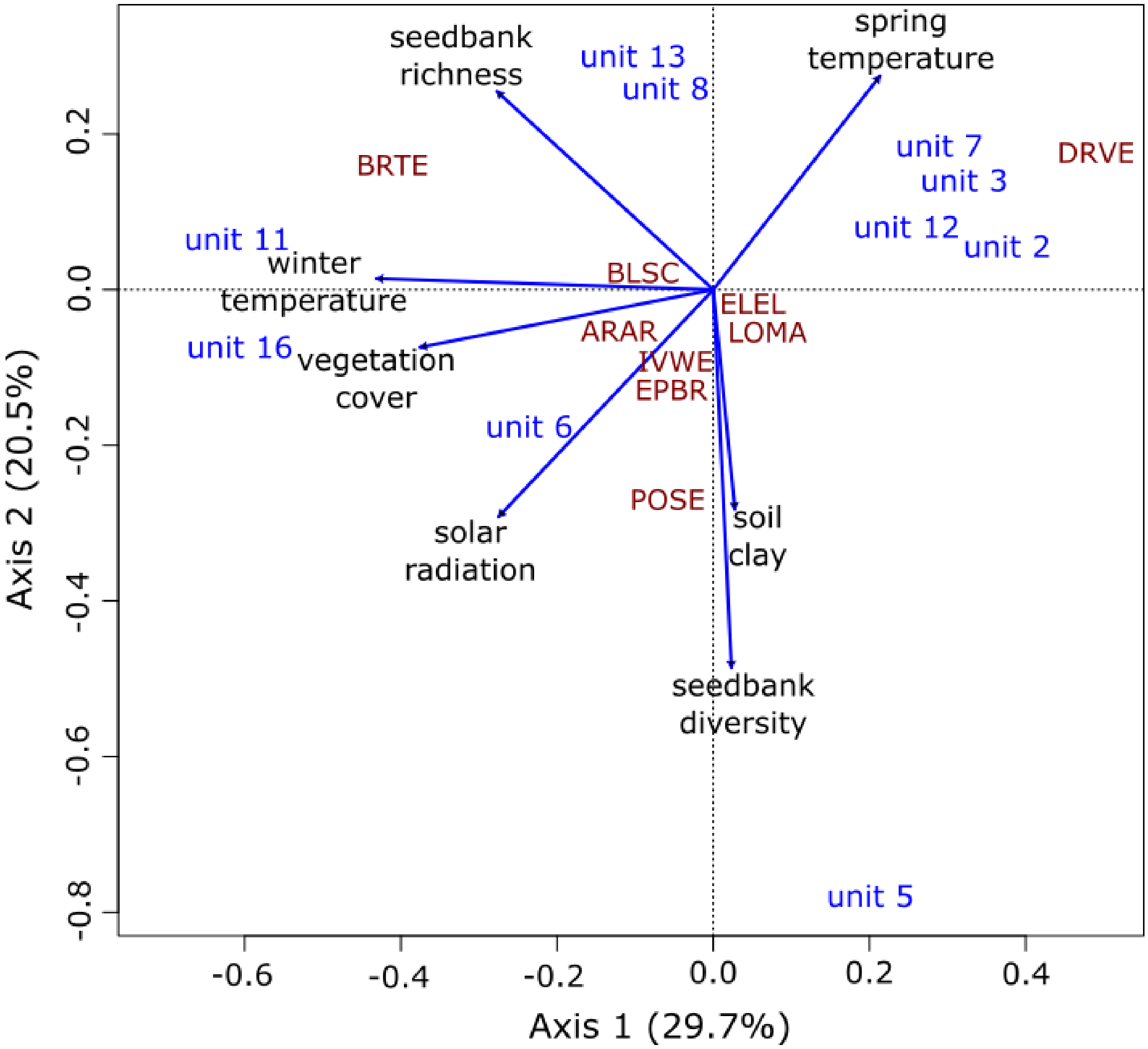

seedbank
richness
unit 13
unit 8
spring
temperature
BRTE
unit 7
unit 3
DRVE
unit 11
unit 12
unit 2
winter
temperature
BLSC
ELEL
ARAR
LOMA
unit 16
vegetation
cover
IVWE
EPBR
unit 6
POSE
soil
clay
solar
radiation
seedbank
diversity
unit 5
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
Axis 2 (20.5%)
Axis 1 (29.7%)

Figure 2.

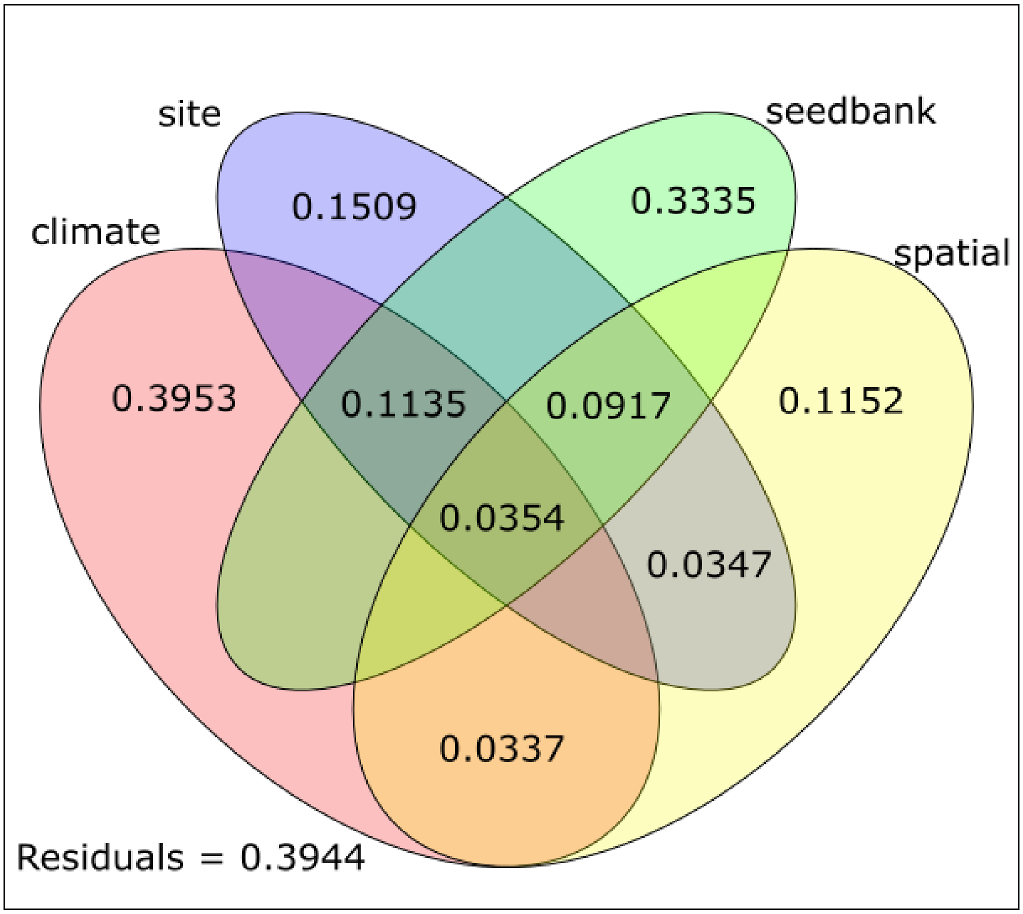

site
seedbank
climate
spatial
0.1509
0.3335
0.3953
0.1135
0.0917
0.1152
0.0354
0.0347
0.0337
Residuals = 0.3944

Figure 3.

Acknowledgements: The authors are grateful to Mr. Arnold Tiehm of the Museum of Natural History, University of Nevada, Reno, for his help with plant species identification, Dr. Sarah Barga and Dr. Elizabeth Leger for their advice on the greenhouse experimental set up, and Dr. Pierre Legendre for his advice on statistical analysis.

Appendix S1. Ranking of disturbance in *Ivesia webberi* sites

Rank value	**Description**
1	No visible or current anthropogenic disturbance across the entire site
2	Historical disturbance still visible, but discontinuous. For example, decommissioned off-highway roads where *Ivesia* is observed recovering. Disturbance affects less than 10% of site
3	Minor anthropogenic activity, such as active minor road, with *Ivesia* occurring on both sides, infrequent cattle grazing, hiking trails, etc. Disturbance affects between 10 and 25% of site
4	Moderate anthropogenic activity, including illegal dumping, and heavy grazing. Disturbance affects at least 50% of total site
5	Heavy anthropogenic activity such as housing and urban development, recent wildfires (≤2 years ago), or other activities that can potentially extirpate the population. Disturbance affects between 50 and 100% of total site

Appendix S2. Species richness and abundance for the 10 sites harboring *Ivesia webberi* decomposed into their component annual and perennial, and native and alien species. The first values under each heading representing the species richness, while the values in parenthesis represent the total abundance count per site

Site Name	Richness and counts of annuals		Richness and counts of perennials		Richness and counts of natives		Richness and counts of alien species	
	AGV	SSB*	AGV	SSB*	AGV	SSB*	AGV	SSB*
Unit 2	4 (3138)	15 (21280)	14 (529)	3 (159)	15 (839)	12 (473)	3 (2828)	6 (20966)
Unit 3	11 (2240)	12 (6451)	9 (83)	2 (2)	18 (487)	9 (331)	2 (1836)	5 (6122)
Unit 5	8 (369)	9 (71)	26 (652)	3 (12)	30 (773)	7 (64)	4 (248)	5 (19)
Unit 6	10 (1615)	11 (1989)	16 (790)	4 (37)	21 (1080)	13 (143)	5 (1325)	2 (1883)
Unit 7a	11 (8809)	11 (10215)	15 (388)	3 (255)	23 (3393)	10 (731)	3 (5804)	4 (9739)
Unit 8	8 (7542)	11 (2363)	14 (1549)	3 (49)	19 (2104)	10 (251)	3 (6987)	4 (2161)
Unit 11	8 (3169)	18 (1281)	16 (152)	3 (23)	20 (228)	14 (80)	4 (3093)	7 (1224)
Unit 12	10 (2736)	17 (7865)	15 (564)	3 (46)	20 (668)	14 (234)	5 (2632)	6 (7677)
Unit 13	10 (4913)	14 (4022)	10 (581)	3 (348)	15 (857)	12 (514)	5 (4637)	5 (3856)
Unit 16	9 (903)	13 (1246)	12 (308)	2 (38)	16 (554)	11 (201)	5 (657)	4 (1083)
Total	31 (35434	23 (56783)	51 (5596)	4 (969)	70 (10983)	20 (3022)	12 (30047)	7 (54730)

Appendix S3. List of all species recorded in the aboveground plant communities and the soil seed bank in the 10 sampled sites where *Ivesia webberi* occurs

#	**Species name***	**Code**	**Species abundance**		**Life form**	**Distribution**
			Aboveground	**Soil seed bank**		
1	*Acmispon americanus*	ACAM	3	0	annual	native
2	*AG*	AG	0	4	morphospecies	N/A
3	*Agoseris heterophylla*	AGHE	4	0	annual	native
4	*Agropyron cristatum*	AGCR	2	0	perennial	alien
5	*Aliciella leptomeria*	ALLE	0	3	annual	native
6	*Allium lemmonii*	ALLE	7	0	perennial	native
7	*Alopecurus pratensis*	ALPR	18	0	perennial	alien
8	*Alyssum desertorum*	ALDE	40	40	annual	native
9	*Antennaria dimorpha*	ANDI	695	0	perennial	native
10	*Antennaria luzuloides*	ANLU	231	0	perennial	native
11	*Arenaria kingii* var *glabrescens*	ARKG	290	0	perennial	native
12	*Artemisia arbuscula*	ARAR	324	44	perennial	native
13	*Artemisia tridentata*	ARTR	1	0	perennial	native
14	*Astragalus andersonii*	ASAN	217	0	perennial	native
15	*Balsamorrhiza hookeri*	BAHO	25	0	perennial	native
16	*Blepharipappus scaber*	BLSC	210	39	annual	native
17	*Bromus japonicus*	BRJA	211	0	annual	alien
18	*Bromus tectorum*	BRTE	9557	446	annual	alien
19	*Camissonia pusilla*	CAPU	255	450	annual	native
20	*Castilleja chromosa*	CACH	5	0	perennial	native
21	*Collinsia parviflora*	COPA	38	35	annual	native
22	*Crepis acuminata*	CRAC	4	0	perennial	native
23	*Crepis occidentalis*	CROC	269	0	perennial	native
24	*Cryptantha pterycarya*	CRPT	2	299	annual	native
25	*Cusickiella douglasii*	CUDO	63	3	perennial	native
26	*Danthonia unispicata*	DAUN	31	0	perennial	native
27	*Descurainia sophia*	DESO	0	7	annual	alien

28	*Draba verna*	DRVE	15805	50908	annual	alien
29	*Elymus elymoides*	ELEL	594	0	perennial	native
30	*Ephedra sp*	EPSP	7	0	perennial	native
31	*Epilobium brachycarpum*	EPBR	230	0	annual	native
32	*Ericameria nauseosa*	ERNA	3	0	perennial	native
33	*Ericameria parryi*	ERPA	7	0	perennial	native
34	*Erigeron bloomeri*	ERBL	37	0	perennial	native
35	*Eriogonum caespitosum*	ERCA	69	0	perennial	native
36	*Eriogonum douglasii*	ERDO	62	0	perennial	native
37	*Eriogonum microthecum*	ERMI	10	0	perennial	native
38	*Eriogonum nudum*	ERNU	6	0	perennial	native
39	*Erodium cicutarium*	ERCI	3499	0	annual	alien
40	*Gutierrezia sarothrae*	GUSA	14	0	perennial	native
41	*Holosteum umbellatum*	HOUM	2598	2681	annual	native
42	*Chorizanthe watsonii*	CHWA	7	0	annual	native
43	*Chrysothamnus viscidiflorus*	CHVI	2	0	perennial	native
44	*Chrysothamnus viscidiflorus* var *puberulus*	CHVP	3	0	perennial	native
45	*Idahoa scapigera*	IDSC	0	301	annual	native
46	*Ivesia aperta var canina*	IVAC	2	0	perennial	native
47	*Ivesia webberi*	IVWE	1253	0	perennial	native
48	*Juncus bufonius*	JUBU	62	11	annual	native
49	*Lactuca serriola*	LASE	1	0	annual	alien
50	*Lagophylla ramosissima*	LARA	404	9	annual	native
51	*Lepidium perfoliatum*	LEPE	1	0	annual	alien
52	*Lewisia rediviva*	LERE	108	0	perennial	native
53	*Leymus cinereus*	LECI	1	0	perennial	native
54	*Lithophragma glabrum*	LIGL	0	852	perennial	native
55	*Lomatium macrocarpum*	LOMA	204	0	perennial	native
56	*Lomatium nudicaule*	LONU	3	0	perennial	native
57	*Lotus sp.*	LOSP	3	0	annual	native
58	*Lupinus argenteus*	LUAR	1	0	perennial	native

59	*Lupinus lepidus* var *sellulus*	LULS	30	0	perennial	native
60	*Lupinus malacophyllus*	LUMA	40	70	perennial	native
61	*Macrosteris gracilis*	MAGR	139	16	annual	native
62	*Madia exigua*	MAEX	10	16	annual	native
63	*Mimulus susksdorfii*	MISU	0	38	annual	native
64	*Muilla transmontana*	MUTR	8	0	perennial	native
65	*N*	N	0	1	morphospecies	N/A
66	*Navarretia breweri*	NABR	15	0	annual	native
67	*Nestotus stenophyllus*	NEST	33	0	perennial	native
68	*Orobanche corymbosa*	ORCO	1	0	annual	native
69	*Pectocarya setosa*	PESE	2	8	annual	native
70	*Penstemon roezlii*	PERO	30	0	perennial	native
71	*Perideridia bolanderi*	PEBO	1	0	perennial	native
72	*Phlox longifolia*	PHLO	51	0	perennial	native
73	*Plagiobothrys tenellus*	PLTE	28	0	annual	native
74	*Plectritis macrocera*	PLMA	38	1	annual	native
75	*Pleicanthus spinosus*	PLSP	5	0	perennial	native
76	*Poa bulbosa*	POBU	297	0	perennial	alien
77	*Poa secunda*	POSE	443	0	perennial	native
78	*Prunus andersonii*	PRAN	2	0	perennial	native
79	*Purshia tridentata*	PUTR	3	0	perennial	native
80	*Ranunculus testiculatus*	RATE	0	538	annual	alien
81	*Rigiopappus leptocladus*	RILE	498	229	annual	native
82	*Salsola tragus*	SATR	609	76	annual	alien
83	*Sidalcea*	Code	2	0	perennial	native
84	*Sisymbrium altissimum*	SIAL	44	74	annual	alien
85	*Stipa sp*	STSP	32	0	perennial	native
86	*T*	T	0	1	morphospecies	N/A
87	*Tetradymia canescens*	TECA	10	0	perennial	native
88	*Tragopogon dubius*	TRDU	3	0	annual	alien
89	*Trifolium lemmonii*	TRLE	38	0	perennial	native
90	*U*	U	0	1	morphospecies	N/A

91	*Vulpia microstachys*	VUMI	1117	558	annual	native
92	*Wyethia mollis*	WYMO	3	0	perennial	native
93	*Y*	Y	0	12	morphospecies	N/A

*Species names follow the scientific nomenclature in the Flora of North America Flora

CHAPTER 3 Life in the desert: habitat spatial complexity, gene flow and functional connectivity in *Ivesia webberi**

Israel T Borokini[1], Kelly B. Klingler[2], & Mary M Peacock[2]

[1]Program in Ecology, Evolution and Conservation Biology, Department of Biology,

University of Nevada, Reno, Reno NV 89557

[2]Department of Biology, University of Nevada, Reno, Reno NV 89557

*Prepared for submission to *Ecology and Evolution*

ABSTRACT

Habitat protection is not sufficient to conserve range-restricted species with disjunct populations. It is also critical to characterize gene flow among the populations and factors that influence functional connectivity in order to design effective conservation programs. In this study, we genotyped 314 individuals of *Ivesia webberi*, a United States federally threatened Great Basin Desert perennial forb using six microsatellite loci, to estimate genetic diversity and population genetic structure, as well as rates and direction of gene flow among 16 extant *I. webberi* populations. We also assessed the effects of Euclidean distance, landscape features, and ecological dissimilarity on the genetic structure in the sampled populations, as well as the relationship between *I. webberi* genetic diversity and floristic diversity in the vegetative communities. The results show low levels of genetic diversity ($H_e = 0.200–0.441$; $H_o = 0.192–0.605$) and high genetic differentiation among the populations. Genetic diversity was structured along geographic and latitudinal gradients, indicating isolation by distance and central-marginal patterns. Genotype cluster membership supports west to east gene flow for populations near the species' range center. Populations at the range margins have significantly higher genetic distances, higher relatedness, and evidence of genetic bottlenecks. Pairwise genetic distance strongly correlates with actual evapotranspiration and precipitation, indicating a pattern of isolation by environment. An inverse of habitat suitability, but not land cover, contributes to an isolation by landscape resistance and highlights the importance of maintaining suitable habitats that act as corridors to facilitate functional connectivity. The significant correlation between pairwise genetic distance and dissimilarity in the soil seed bank suggest that annual regeneration of the floristic communities contributes to the genetic diversity in *I. webberi*.

Keywords: gene flow, *Ivesia webberi*, central marginal hypothesis, isolation by distance, species-genetic diversity, isolation by environment

DATA ACCESSIBILITY STATEMENT: Data generated or analyzed during this study are included in this article and the supplementary information files. The microsatellite loci and primers used in this study were developed and described in the Dobeš and Scheffknecht (2012) paper.

INTRODUCTION

Effective species conservation involves not only protecting habitat, but also maintaining genetic diversity within and among populations in order to ensure evolutionary potential on the rapidly changing landscapes of the Anthropocene (Murphy et al., 2010; Spear et al., 2010; Auffret et al., 2017). Gene flow among populations is critical to the maintenance of genetic variation (Cruzan, 2001; Hughes et al., 2008; Ellstrand, 2014), especially among spatially discrete populations of organisms (Peacock and Smith, 1997; Consuegra et al., 2005; Shirk et al., 2010; Neville et al., 2016). For species found in naturally fragmented habitats gene flow among populations may be constrained by both distance and movement across unsuitable habitat (Templeton et al., 1990; Peacock and Smith, 1997; Murphy et al., 2010). Such varied landscapes may then produce a pattern of isolation by resistance, which could impede successful recolonization of locally extirpated habitat patches (McRae, 2006; McRae and Beier, 2007; Zeller et al., 2012). In addition, local adaptation to divergent micro-ecological conditions, or isolation by environment, can also contribute to differentiation among populations and limit successful recolonization potential (Wang and Bradburd, 2014; Sexton et al., 2014).

For narrowly distributed endemic species found on spatially complex landscapes, habitat fragmentation, degradation and climate change are likely to elevate the risk of both population isolation and extinction probability (Zwick, 1992; Dirnböck et al., 2011; Canales-Delgadillo et al., 2012). Therefore, characterizing patterns of gene flow, levels of genetic diversity and population genetic structure among populations of rare, endemic and threatened species is critical for informing conservation actions that facilitate functional connectivity (Nevill et al., 2017) and the identification of evolutionarily significant units (Peacock and Dochterman, 2012, Brown et al., 2016).

Here we characterize genetic diversity and population genetic structure in *Ivesia webberi* A. Gray, in the Rosaceae family, a federally listed (United States Endangered Species Act 1973 ESA; 16 U.S.C. § 1531 et seq.) and narrowly distributed endemic perennial forb, found at the western edge of the Great Basin Desert (Figure 1; USFWS, 2014), in order to assess the effects of both natural history and landscape viscosity on dispersal and gene flow in this species. The geological history, basin and range topography and significant microclimatic gradients of the Great Basin Desert (Cassel et al., 2009; Kraft et al., 2010), together with the effects of historical climatic cycles, have shaped the distribution of native species in the Great Basin Desert resulting in many local distributed and endemic plant species. At the time of this study *I. webberi* was known from only 16 spatially discrete populations (additional populations were discovered after sampling occurred, see Figure 1) found at mid-elevation sites in Great Basin habitat in a narrow band along the eastern edge of the Sierra Nevada, together with other native Great Basin plant species including *Artemisia arbuscula* and *Balsamorhiza hookeri*. These sites have been impacted by severe historical and current anthropogenic disturbance including livestock grazing, wildfires, urbanization, off-highway vehicle (OHV) use and climate change, and are currently threatened by encroachment of invasive plants, such as *Bromus tectorum*, *Taeniatherum caput-medusae* and *Poa bulbosa* (USFWS, 2014).

We employ nuclear microsatellite genetic markers to: (a) measure levels of genetic diversity, effective population size (*N*e) and rates and direction of gene flow for *I. webberi* populations; (b) estimate the effect of Euclidean distance, landscape features, and ecological dissimilarity on the genetic structure in the sampled populations; and (c) investigate a relationship between *I. webberi* genetic diversity and floristic diversity in the vegetative communities. Due to the spatial configuration of these populations, we also (d) test the central-marginal hypothesis (CMH), which predicts decreased gene flow and increased pairwise genetic differentiation among populations towards the edge of the species range (Eckert et al., 2008; Pfenninger et al., 2011; Micheletti and Storfer, 2015). We predict a spatial genetic diversity pattern related to landscape features as well as effective resistance to gene flow by anthropogenic disturbance among the sampled *I. webberi* populations. Following the predictions of the central-marginal hypothesis, populations closer to the species' range center should have the highest genetic variation. We also predict genetic bottlenecks among *I. webberi* populations due to the past and current anthropogenic landscape perturbations

MATERIALS AND METHODS

Study species

I. webberi is a perennial forb that produces clusters of small greenish-gray leaves (i.e., 25 cm diameter) that grow at ground level and small bright yellow flowers. Flowering occurs between May and June. Occupied sites are sparsely vegetated flat, bench or terrace locations in shallow, rocky and with clay soils. All sampled *I. webberi* populations are located between 1364 and 1900 meters within the transition zone between the eastern edge of the northern Sierra Nevada and the northwestern edge of the Great Basin (United States Fish and Wildlife Service, https://www.fws.gov/nevada/nv_species/webber_ivesia.html).

I. webberi reproduces vegetatively from the dormant root caudex as well as sexually from seeds. The showy yellow flowers produced by *I. webberi* are visited by native Hymenopterans, Dipterans, and Lepidopterans and therefore, the species is thought to be entomophilous. However, little is known about the dispersal dynamics of this species (USFWS, 2014). The species produces dry indehiscent achene fruits, which are not adapted for long-range dispersal, but abscise into rock crevices that characterize the soil surface in all sites (Witham, 2000; USFWS, 2014). There are no known seed dispersers for this species, but there is localized seed dispersal to bare-soil microsites due to gravity-assisted surface runoff from summer precipitation that results in recruitment from seedlings and colonization of unoccupied bare-soil sites. Therefore, gene flow among *I. webberi* populations is thought to be more likely from pollen movement among populations than from seed dispersal (Ennos, 1994). However, it is not yet established if the *I. webberi* floral insect visitors are pollinators. Nevertheless, foraging distance and hence potential pollen dispersal in some Hymenopterans ranged from 200 m to 6 km (Pasquet et al., 2008; Albrecht et al., 2009).

Sample collection, DNA extraction, PCR amplification, and genotyping

Five leaves were collected per plant from 24 randomly selected plants in each of the 16 sampled *I. webberi* populations (Table 1). The leaves were stored in paper collection bags with silica gel to facilitate drying of samples at room temperature. GPS coordinates of each sample were also recorded using Garmin eTrex 20x.

Five mg of leaf tissue from each plant sample (n = 384) were processed using a TissueLyser II (QIAGEN Inc., Valencia, CA, USA). Genomic DNA was extracted using the protocol described in the DNeasy96 Plant Extraction Mini kit (QIAGEN). DNA per sample was quantified at the Nevada Genomics Center (https://www.unr.edu/genomics) using the PicoGreen dsDNA assay (ThermoFisher, Waltham, MA, USA). DNA concentration was determined using a standard curve equation following DNA detection under the Fluoroskan Microplate Fluorometer (ThermoFisher, Waltham, MA, USA).

No microsatellite loci have been developed for *I. webberi* nor for any species in this genus. We tested 20 microsatellite loci developed from *Potentilla pusilla* (Dobeš and Scheffknecht, 2012) for use with *I. webberi* (Table 2). *Potentilla* is phylogenetically related to *Ivesia* (Töpel et al., 2012) and the developed markers were reported to be polymorphic and cross-amplified with other species, at success rates ranging from 86 to 97% (Dobeš and Scheffknecht, 2012). Six microsatellite loci amplified consistently in *I. webberi* and were further optimized for this study. PCR amplification was carried out in a Labnet International Inc. MultiGene^{TM} OptiMax thermal cycler (115V model) in 10.0 µl reaction volumes in a 96-well format using the Qiagen Multiplex PCR kit, which contains HotStarTaq DNA polymerase, dNTPs, and PCR buffer at a 2× concentration. The six primer pairs were amplified in single or multiplexed PCR reactions with a final concentration of 0.05 µM of each tailed forward primer and 0.1 µM of each reverse primer. Each PCR reaction included 5 µl of Multiplex Mix, 20-50 ng of DNA, between 0.1-0.2 µl of primer and approximately 4.5 µl of ultra pure molecular grade water with some reactions being combined post-PCR. PCR parameters included a 15 minute hot start at 95 ºC, then 41 cycles of 95 ºC for 30 seconds, followed by a touchdown annealing temperature that ranged between 65 to 55 ºC for 90 seconds with a final elongation step of 72 ºC for 30 seconds. The touchdown annealing temperature begins with 7 cycles at 65 ºC, 7 cycles at 61 ºC, 7 cycles at 58 ºC and 20 cycles are 55 ºC.

PCR products were diluted to an appropriate concentration and 1 µl of diluted PCR product was added to 19 µl of Hi-Di Formamide/LIZ500 size standard (Applied Biosystems) by adding 5 µl of size standard for each 1 ml of Hi-Di Formamide. Fragment analysis was done on an Applied Biosystems (ABI) Prism 3730 DNA analyser at the Nevada Genomics Center (https://naes.unr.edu/genomics), and all alleles generated were scored, binned and genotyped using the ABI GeneMapper software (version 5; Applied Biosystems, ThermoFisher Scientific). We also re-amplified 30% of the sample size (~115 samples) to validate genotyping reliability. Individual leaf samples that failed to amplify were removed from the analysis, thus reducing the sample size from 384 to 314 (Table 1).

Genetic analyses

Population genetic diversity

We used FSTAT 2.9.4 (Goudet, 1995) to test for Hardy–Weinberg equilibrium (HWE) across all loci, (Wright, 1969), calculate the number of alleles (Na), allelic richness (R_S), the inbreeding coefficient (F_{IS}), and determine whether linkage disequilibrium among loci was present within populations. We estimated genetic diversity (He, Ho) using Microsatellite Toolkit in Excel. MICROCHECKER v.2.2.3 (van Oosterhout et al., 2004) was used to test for departures from the Hardy-Weinberg equilibrium for each locus in each population in order to estimate the frequency of allelic dropout and null alleles. MICROCHECKER uses multiple null allele estimators, including Chakraborty et al. (1992) estimator for null alleles, two Brookfield (1996) estimators, and the van Oosterhout (2004) estimator. Preferential amplification of shorter alleles (Wattier et al., 1998) can result in what appears as a deficit of heterozygotes, which is used to indicate large allelic dropout. Relatedness (*r*) among individuals within populations was calculated using the Ritland and Lynch equations (1999) in GENALEX v.6.5 (Peakall and Smouse, 2012). We tested for genetic bottlenecks per population using BOTTLENECK v.1.2.02 (Piry et al., 1999) under the single step (SSM) and the two-phase (TPM) mutation models.

Population genetic structure

We used GENAlEx to estimate pairwise genetic differentiation among populations (F_{ST}) and STRUCTURE (v.2.3.4; Pritchard et al., 2007) to estimate the number of Bayesian genotype clusters (*k*) across all *I. webberi* populations, using a 100,000 iteration burn-in followed by 10, 500,000 Markov Chain Monte Carlo (MCMC) replications per *k*. The optimal number of genotype clusters was determined using the Δ*k* method (Evanno et al., 2005). AMOVA and principal coordinate analysis (PCoA) in GENAlEx were used to characterize the partitioning of genetic variation on the landscape. Effective population size (*N*e) was calculated for each population and Bayesian genotype clusters were identified using the linkage disequilibrium (LD) method in NeEstimator v.2.0 (Do et al., 2014).

Geographical distance, landscape barriers and ecological dissimilarity

We assessed the influence of geographical distance (isolation by distance; IBD), land cover and inverse of habitat suitability (isolation by resistance; IBR), and ecological dissimilarity (isolation by environment; IBE) on pairwise genetic distance among the 16 *I. webberi* populations. Both IBD and IBR models were fitted using a linear mixed effects model framework in ResistanceGA R package v. 4.1-11 (Peterman, 2018). Slatkin's linearized pairwise F_{ST} values, which accounts for microsatellite mutation following the single step model (Di Rienzo et al., 1994; Slatkin, 1995), were used as genetic distance (response variable), while pairwise geographical distance was estimated from the GPS coordinates of the polygon centroid for each population. Land cover was derived from the Multi-Resolution Land Characteristics (MRLC) development of the U.S. National Land Cover Database (NLCD) 2016 (Xian et al., 2013), while the habitat suitability map was produced from ensemble projection of niche modeling replicates from six algorithms with $\mathrm{TSS} \geq 0.7$ (Appendix I; see Chapter 1). The choice of land cover layer was based on the hypothesis that gene flow in *I. webberi* is mainly pollen-based, since the achene seeds are not adapted for long range dispersal. Field observations show that *I. webberi* flowers are frequently visited by native Lepidopteran, Dipteran, and Hymenopteran insects. Studies show that the movement of these insects across the landscape is effectively impeded by roads and human settlements (Andersson et al., 2017; Corcos et al., 2019), thus a land cover layer is expected to illustrate landscape resistance.

ResistanceGA uses a genetic algorithm from the GA R package to optimize the transformation and impact of resistance surfaces (Scrucca 2013, 2017; Peterman, 2018). Optimization of resistance surfaces in ResistanceGA include a transformation of continuous surfaces (e.g., raster layers) or an assignment of resistance values to the categorical landscape features (e.g., land cover), calculation of pairwise effective distance (e.g., least cost path, random walk, etc.), fitting maximum likelihood population effects

(MLPE) on pairwise genetic distance using the pairwise effective distance as predictor, and performing model selection to determine the best-parametrized resistance to gene flow (Peterman et al., 2019). The habitat suitability map was resampled from 30 m to 250 m and converted to a resistance surface using inverse monomolecular method, which assumes a negative relationship between gene flow and landscape resistance (Peterman, 2018). The land cover layer was also resampled to 250 m and reduced to 15 feature classes each of which was automatically assigned a resistance value. We are aware of the potential effect of spatial resolutions on landscape connectivity modeling results, but this resampling is inevitable due to the computation limitations in running ResistanceGA (Cushman and Landguth, 2010; O'Connell et al., 2019). A composite resistance surface layer which combined both the optimized land cover layer and inverse habitat suitability map was also used.

Functional connectivity in the landscape was calculated using *commuteDistance* function which is similar to the resistance estimates calculated using CIRCUITSCAPE (McRae et al., 2008). For optimal computing efficiency with parallel processing, ResistanceGA was interfaced with CIRCUITSCAPE v.5.7.1 (Anantharaman et al., 2020). Random-walk commute-distance estimates are preferred over least cost path which assume that gene flow is maximized in the lowest cost path because individuals have knowledge of all possible paths (Adriaensen et al., 2013). We used default parameterizations and 10 iterations in ResistanceGA for the independent optimization of the two resistance surfaces (that is, habitat suitability map and land cover layer).

The maximum likelihood population effects (MLPE) model used the linearized pairwise F_{ST} as the response variable, the 16 population codes as the random effect term, while the fixed effect terms included pairwise geographical distance among the populations, landcover resistance and the transformed habitat suitability map. The MLPE model fitted a null model (*I. webberi* population ID), an IBD model (using pairwise geographical distance and population ID), and IBR model (comprising population ID, pairwise geographical distance and the resistance surfaces used individually and in combination). Following the 10 MLPE model replicates, we conducted bootstrapping to assess the sensitivity of the MLPE models to the spatial distribution of *I. webberi* populations. Here, we randomly subset 75% of the data without replacement, fitted the MLPE models again using 10,000 iterations, and selected the best models using the average AICc values. The percentage contribution of each surface within the multi-surface optimization was calculated by dividing each transformed resistance surface by the sum of the composite resistance surface (Peterman, 2018).

The effect of ecological dissimilarity (IBE) among the population sites on pairwise genetic distances was estimated by generating a distance matrix of the ecological conditions across the 16 sampled *I. webberi* sites from GIS raster layers of cumulative actual evapotranspiration, summer precipitation, native perennial herbaceous cover, mean minimum temperature, cosine aspect and Topographic Position Index (Appendix II). These environmental predictors (Appendix III) were the most important uncorrelated (r>0.6) variables from a suite of 72 assembled predictors, following three consecutive feature reduction analyses (Appendix IV). A Mantel test was used to assess the relationship between the pairwise F_{ST} genetic distance and the matrices of each of the six ecological variables. Accounting for the effect of geographical distance, multiple regression on distance matrices (MRM; Lichstein, 2007) was conducted between the pairwise F_{ST} values and the ecological variables. Mantel tests were conducted in ECODIST R package (Goslee and Urban, 2007), while MRM analysis was conducted in PHYTOOLS R package (Revell, 2012). Both the Mantel and MRM analyses were conducted with 10,000 permutations.

Central-marginal hypothesis

The range center of the *I. webberi* species distribution was estimated using the range center index (RCI; Enquist et al., 1995) method based on the latitudinal decimal degrees of the population sites. In the RCI, sites closer to the species' range center have values closer to zero, the northernmost site was assigned the value of 1, while the southernmost site was assigned a value of -1. Pearson correlation between *I. webberi* RCI and allelic richness or mean observed heterozygosity (H_O) was used to test the predictions of the central-marginal hypothesis. Additionally, the Mantel test was used to investigate the relationship between a matrix of the latitudinal degrees and the pairwise genetic distance (F_{ST}) among the sampled populations.

Relationship between plant community diversity and Ivesia webberi genetic diversity

We also tested the species-genetic diversity hypothesis that there is a relationship between *I. webberi* genetic diversity and the floristic dissimilarity across the 16 sites (Whitlock, 2014; Kahilainen et al., 2014). In a separate study (Borokini et al., 2021), species richness, abundance, and diversity of both the aboveground plant communities and the soil seed bank of 10 of the 16 sites were quantified (Appendix V), and from which the floristic dissimilarity matrix (β-diversity) across sites was generated using the Bray-Curtis method. Here, we assessed a relationship between linearized pairwise F_{ST}, our genetic distance metric, and the floristic dissimilarity matrix in both the aboveground vegetation and the soil seed bank for the 10 sampled sites, using a Mantel test with 10,000 permutations. In addition, we also conducted Pearson correlation between genetic diversity (e.g., allelic richness and mean observed heterozygosity) and species richness and diversity of both the aboveground vegetation and the soil seed bank in each of the 10 sites. Species diversity was the exponential conversion of the Shannon-Weiner H' index for each site (that is, effective number of species; Jost, 2006).

RESULTS

Population level genetic diversity metrics

We genotyped 314 *Ivesia webberi* individuals at six nuclear microsatellite loci (Table 1). Allelic diversity per locus (Na) ranged from 3–13 alleles, while allelic richness per locus (R_S) ranged from 2.002–4.073 (Appendix VI). No locus showed evidence of null alleles or allelic dropout. Average levels of heterozygosity ranged from Ho=0.192–0.605 and He=0.200–0.441 (Appendix VI). Two loci were out of HWE in single or multiple populations. Locus PMS1694 had a significant positive F_{IS} in CST the northernmost population sampled (F_{IS}=0.898, p=0.00052), indicating a heterozygote deficit, and locus PMS1438 had significant negative F_{IS} values in multiple populations indicating heterozygous excess, which is consistent with a signature of recent genetic bottlenecks (F_{IS} range=-0.8 to -1.0; p=0.00052) (Appendix VI). Many of the populations with significant negative F_{IS} values are also peripheral populations (SVE, EHJ, DVA, HGV, PPL and DMR; Figure 1). Genetic bottlenecks were observed for both the TPM and SMM mutation models in five of the populations, four of which had significant negative F_{IS} values (**bolded**) (**EHJ** – TPM *p*=0.017, SMM *p*=0.017; **MER** – TPM *p*=0.042, SMM *p*=0.047; **BSP** – TPM *p*=0.037, SMM *p*=0.039; **DMR** – TPM *p*=0.02, SMM *p*=0.02; STL – TPM, *p*=0.016, SMM, *p*=0.023).

The number of alleles per locus and allelic richness per population were the highest (*N*a=3.33, R_T=15.63) in the WLO population, which is centrally located in the cluster of populations at the center of *I. webberi* range (Figure 1), while the lowest values were found in the isolated southernmost population sampled (DMR, *N*a=1.83, R_T=9.34; Table 1). Similarly, the northernmost and isolated population sampled (CST) also had low allelic diversity (*N*a=2.17, R_T=10.31; Table 1). In addition, DMR and CST had the highest levels of within-population relatedness (*r*=0.38 and *r*=0.245 respectively; Figure 2), while most of the centrally located and spatially proximate populations had low levels of *r* (Figures 1&2). We could not calculate the 95% CIs for most of the population *N*e estimates (69%) and so do not report those values here. For the populations that we could calculate an *N*e and 95% CI the values ranged from 0.9–11.6 (Table 3). We also calculated *N*e for the genotype clusters identified below (Table 3).

Population genetic structure

Pairwise F_{ST} values among the sampled *I. webberi* populations (Table 4) tended to be high and statistically significant (corrected *p*=0.0004). The non-significant values were found primarily among the spatially proximate populations at the center of the range. The most isolated population sampled (DMR) was significantly differentiated from all remaining populations. Analysis of molecular variation (AMOVA) showed that

71% of the molecular variance was within individuals, while 11% and 18% of the molecular variance were among individuals and populations respectively.

Five genotype clusters (*k*) were identified as the best fit of the data [Average LnP(D)=-2801.42, SD±=2.936, Δ*k*=37.098] (Figure 3b&d). To visualize the spatial extent of the identified genotype clusters on the landscape, we culled the dataset to include only individuals with Q≥80% proportional membership per cluster (N = 207; Table 5). Populations were assigned to the cluster in which they had the highest number of individuals with Q≥80%. In several cases, however, individuals from a single population are assigned to multiple genotype clusters (see Table 5). We overlaid the spatial extent of the genotype clusters onto the study site map by grouping populations by cluster membership (Figure 3c).

The two genotype clusters with the greatest proportional membership included individuals from the spatially proximate populations at the center of the *I. webberi* range and these clusters showed little spatial overlap in this analysis (yellow, N = 46; green, N = 47; Figure 3c; Table 5). However, when considering the complete dataset, populations that assign primarily to the green cluster have admixed individuals from the yellow cluster suggesting directional gene flow from west to east (Figure 3a). All individuals in DMR, the southernmost population, assigned to a single genotype cluster (orange) that interestingly also included individuals from the northernmost population sampled CST (Figure 3a; Table 5). The populations with the highest assignment to the blue cluster formed a narrow northwest to southeast band, which included individuals from CST, the northernmost population and DLF, the southernmost of the central populations (Table 5). The gray genotype cluster membership was diffuse with individuals from 13 separate populations assigning to this cluster. However, the five populations with the highest number of individuals that are assigned to this cluster form a narrow band trending west to east (Figure 3c; Table 5). When examining the complete dataset, there are admixed individuals found throughout the range as well as individuals that assign to different genotype clusters with high proportional membership within single populations (Figure 3a; Table 5).

PCoA was conducted on the genotype clusters as described above (individuals with $Q \geq 80\%$). The first 3 axes explain 50% of the variance (Figure 4). There was little separation among clusters on axis 1 (21.86% variance), but genotype cluster 1 (orange) was clearly separated from cluster 3 (yellow) on axis 2 (14.91% variance) and cluster 4 (blue) was largely separated from all other clusters by axis 3 (13.28% variance; Figure 4). Effective population size was the largest in genotype cluster 3 (yellow; $N\mathrm{e}=40.5$) and lowest in genotype cluster 1 (orange; $N\mathrm{e}=2.6$; Table 3).

Spatial correlates of genetic diversity

Pairwise linearized F_{ST} shows a significant geographical pattern among the 16 sampled *I. webberi* populations indicating isolation by distance (Table 6). Latitudinal degrees and pairwise genetic distance among the 16 populations also showed a significant positive relationship (Table 6). However, we did not observe a significant relationship between range center index (RCI) and allelic richness ($r=0.393$, $p=0.132$) or observed heterozygosity ($r=0.257$, $p=0.337$) despite positive trends. These results indicate a significant spatial genetic structure in the sampled *I. webberi* populations and provide partial support for the predictions of the central marginal hypothesis.

Relationship between floristic diversity and genetic diversity in *Ivesia webberi*

Pearson correlation tests show a positive, but nonsignificant relationship between aboveground community species richness and *I. webberi* allelic richness (r=0.383, p=0.274) and observed heterozygosity (r=0.207, p=0.567). There was also no relationship between genetic diversity and species richness in the soil seed bank or aboveground species dissimilarity matrix, but there was a significant positive relationship between pairwise genetic distance (F_{ST}) and soil seed bank species dissimilarity (Table 6).

Models of isolation by resistance and environment

The result of the bootstrap analysis on the maximum likelihood population effects (MLPE) models show that isolation by distance explained most of the variance in the patterns of gene flow in *I. webberi*, followed by the inverse projected habitat suitability map (Table 7). Furthermore, pairwise genetic distance among the populations has a positive relationship with pairwise difference in cumulative actual evapotranspiration and mean annual precipitation, but not with cosine aspect, minimum annual temperature, perennial herbaceous cover, and Topographic Position Index (Table 6).

DISCUSSION

Results of this study reveal contrasting patterns of significant population genetic structure and isolation in addition to dispersal and gene flow among the sampled *I. webberi* populations. We found evidence of isolation by distance, by environment and by resistance as well as environmental correlates of standing genetic variation. These patterns appear to be largely driven by geographic distance, latitude and to a smaller degree inverse of habitat suitability, but also climatically influenced evapotranspiration and precipitation. Population levels of mean observed heterozygosity tended to be low $(\overline{X = 0.390})$ ranging from 0.192 to 0.605, with the exception of two neighboring populations at the center of the range (MER and IVF), which had higher observed heterozygosities (0.559 and 0.605 respectively). Not surprisingly, the highest levels of heterozygosity and allelic richness as well as non-significant pairwise F_{ST} estimates were found among spatially proximate populations at the center of the range.

However, the Bayesian genotype clustering analysis reveals a more complex movement pattern. We observed directional gene flow and admixture primarily from west to east between the two largest genotype clusters. Most admixed individuals (i.e. green and yellow genotype cluster ancestry; Figure 3) were found in populations that were assigned primarily to the easternmost genotype cluster (green). There was very little admixture observed among the other genotype clusters, but cluster dispersion spanned multiple populations creating distinct spatial patterns (see Figure 3c). The dispersion of the genotype clusters in PCoA space also suggests a landscape level influence on the source populations. Despite the fact that the blue and gray genotype clusters were found in multiple populations, the spatial distribution of these clusters was narrow. Although the populations with highest core membership in these genotyped clusters are the likely source of dispersers, we cannot definitively ascertain dispersal direction in these cases, but latitudinal degrees was one of the significant predictors of gene flow suggesting movement from higher to lower latitudes. These patterns also suggest that two dispersal modes – both pollen and seed dispersal may be in play here with landscape features influencing which dispersal mode is prevalent among populations.

Gene flow via pollen transfer may occur by native Dipterans, Lepidopterans and Hymenopterans, observed to be visiting the flowers frequently during field surveys (Dick et al., 2008; Auffret et al., 2017). The isolation by distance patterns may therefore be partially explained by the flight ranges and foraging behavior exhibited by potential pollen vectors (Matter et al., 2013; Mokany et al., 2014). However, it is unknown at this point, if the floral visitors on *I. webberi* are effective pollinators. Gamete dispersal (pollen) would result in pollination and hence admixture, whereas seed dispersal would not. Only through future sexual reproduction would dispersed seeds colonizing a new population lead to admixture. Once seeds are established and if the adult plant reproduces vegetatively no admixture would be observed and distinct genotype cluster assignments within populations would persist. *I. webberi* is known to reproduce vegetatively, which could explain the high proportional membership of individuals in the same population to distinct genotype clusters. In fact, negative F_{IS} values in some of the loci, indicating a heterozygous excess, together with high within individual genetic variance is consistent with vegetative regeneration and clonality in *I. webberi* (Balloux et al., 2005). The levels of genetic diversity observed in this study are also similar to those observed in mixed-mating plants and outcrossing species (e.g. Culley and Wolfe, 2001; Meeus et al., 2012), which suggests there is both successful sexual reproduction as well as vegetative reproduction in *I. webberi* (Genton et al., 2005; Dlugosch and Parker, 2008; Muller et al., 2011).

Localized seed movement due to gravity-assisted surface runoff is observed during field surveys, but vector(s) for potential long distance seed dispersal in this species remain unknown. Similar water-assisted seed dispersal patterns via spring snowmelt and summer precipitation is reported for other *Ivesia* species that do not reproduce vegetatively (e.g., *I. tweedyi*, Moseley, 1993; *I. lycopodioides*, Pollak, 1997), but neither vectors nor seed dispersal distances have been quantified for any *Ivesia* species. The genetic data here suggest that seed movement may occur over 10s of kilometers, but such dispersal distances may be the result of incremental movement of seeds across the landscape over multiple generations followed by vegetative reproduction. Patterns of heterozygote excess and negative F_{IS} values such as observed for the PMS1438 locus are also suggestive of genetic bottlenecks or small founder events. Three of five populations with signatures of significant genetic bottlenecks had very small populations, while four of the populations had individuals that assign to multiple genotype clusters with high proportional assignment ($Q \geq 80\%$) and little evidence of admixture.

A significant linear relationship between the pairwise geographical distance and genetic differentiation among the populations indicate a spatial genetic structure and support the predictions of the isolation by distance theory (Dias et al., 2016; Minasiewicz et al., 2018). However, nonsignificant positive correlation between genetic diversity estimates and the range center index and latitudinal position, but a strong linear relationship between the pairwise latitudinal degrees distance and genetic distance provide partial support for the predictions of the central-marginal hypothesis (e.g. Langin et al., 2017). The results indicate an increase in pairwise population genetic differentiation towards the edge of the species' range. This is not surprising given that many of the sampled populations are clustered near the species range center which may experience higher levels of gene flow. Indeed, the DMR population at the southern edge of the species range is geographically isolated from the rest of the populations and as a result has the greatest pairwise genetic differentiation, lowest allelic richness and expected heterozygosity and highest within population genetic relatedness.

Among the six environmental predictor variables used, genetic differentiation has a significant relationship with pairwise differences in actual evapotranspiration and precipitation. This highlights significant ecological dissimilarity among the sites which correlates with genetic distance, and may indicate isolation by environment. Both the actual evapotranspiration and precipitation represent water availability and climatic stress to which the native flora in the Great Basin Desert must adapt. The differences in water availability among these sites may be attributed to their varying elevation and topographic positions which determines the duration of their exposure to sunlight. Field observations suggest that *I. webberi* have adapted to these varying microclimatic conditions across the sites through varying phenology. For example, populations at lower elevations were observed to regenerate earlier, hence, flower earlier than those in the higher elevations and this could result in a temporal mismatch in flowering which can impede successful gene flow via pollen transfer among the populations. Previous studies also show a significant positive relationship between water availability and genetic distance (Allen et al., 2002; Oliveira et al., 2018; Tso and Allen, 2019). Moreover, climatic resistance to gene flow has been reported for plant species (Alvarado-Serrano et al., 2019), but may also indicate climatic impacts on the physiology of probable pollen vectors of *I. webberi*. Previous studies focusing on connectivity among the populations of animal species report on strong movement costs of climatic resistance surfaces which were attributed to the physiological tolerance limits (Sexton et al., 2014; Castillo et al., 2014; Hohnen et al., 2016; Flores-Manzanero et al., 2019).

Floristic richness and diversity in the aboveground vegetation and the soil seed bank in the sampled 10 population sites both have a significant linear relationship with effective population sizes in *I. webberi*. Though the species regenerates vegetatively, effective population sizes may be associated with seedling recruitment into the vegetative community. Most of the sites harboring *I. webberi* have been invaded by nonnative species. Field observations suggest that the abundance of the invasive plant species does not prevent the annual vegetative regeneration of established matured *I. webberi* individuals, however, invasive alien species can hinder new recruitment of native plants in the vegetative community by outcompeting the young and delicate native seedlings (Chambers et al., 2007; Borokini et al., 2021). Moreover, the dense tufts of invasive weeds may trigger Allee effects which prevent pollinators from detecting *I. webberi* and reduce chance pollen grain transfer to other individuals by wind. Therefore, a strong relationship between floristic richness and diversity in both the aboveground vegetation and the soil seed bank and the effective population size in *I. webberi* may be attributed to ecological interactions that facilitate or impede localized gene flow and *I. webberi* seedling recruitment. Furthermore, the significant relationship of the soil seed bank with effective population sizes in *I. webberi* underscores the role of the soil seed bank in maintaining the genetic diversity of native species (Mandák et al., 2012; Schulz et al., 2018). This finding is congruent with previous studies that show a significant and positive relationship between species-genetic diversity (Hughes et al., 2008; Kahilainen et al., 2014; Vellend et al., 2014). In a meta-analysis, Whitlock (2014) found a positive relationship between adaptive genetic diversity and species richness and with biomass productivity, representing community structure and function, respectively, but not with species diversity and evenness. Also, a significant positive relationship was observed between neutral genetic diversity and community structure (Vellend and Geber, 2005; Whitlock, 2014). Interspecific competition in niche space within an ecological community could impact both neutral and adaptive genetic diversity in populations over time and trigger varying selection across different populations within the species (Vellend, 2005; Bailey et al., 2009; Whitlock, 2014). Intraspecific genetic diversity can influence community responses to environmental changes and determine the velocity of shifts in community structure and functions (Broadhurst et al., 2008; Whitlock, 2014).

The results of this study reveal a complex interaction among Euclidean distance and environment parameters on the population genetic structure of *I. webberi*. A meta-analysis of 70 studies showed that gene flow among plants was more patterned along a combination of isolation by distance and by environment (Sexton et al., 2014). A narrowly distributed endemic found primarily in small spatially discrete populations, *I. webberi* is currently threatened by urbanization, changing fire regimes and invasive plant species. Any insect-assisted pollen transfer among *I. webberi* populations may be hindered as insects avoid human-altered landscapes such as the ones in which *I. webberi* occurs (Làzaro et al., 2020; Delnevo et al., 2020). Anthropogenic landscape features result in potential habitat loss and fragmentation, which could increase extirpation risks and resistance to gene flow among the populations. The extreme isolation of the DMR population, which also contains unique alleles, is of concern; given its small spatial extent, efforts to expand the population may be warranted. Although individuals which assign to same genotype cluster as the DMR population were found in other populations suggesting past dispersal and the possibility of unidentified populations in the habitat separating DMR from the core of the species range. Furthermore, the Great Basin Desert is projected to experience milder winters and hot summers as a result of climate change (Chambers, 2008). Therefore, conservation efforts on *I. webberi* should include genetic characterization of newly discovered sites, further characterization of dispersal dynamics, protection and monitoring of all population sites and potential connectivity corridors, and control of invasive alien species.

REFERENCES

Adriaensen, F., Chardon, J. P., De Blust, G., Swinnen, E., Villalba, S., Gulinck, H., & Matthysen, E. (2003). The application of 'least-cost' modelling as a functional landscape model. *Landscape and Urban Planning*, **64**(4), 233-247. https://doi.org/10.1016/S0169-2046(02)00242-6

Albrecht, M., Duelli, P., Obrist, M. K., Kleijn, D., & Schmid, B. (2009). Effective long-distance pollen dispersal in *Centaurea jacea*. *PLoS ONE*, **4**(8), e6751. https://doi.org/10.1371/journal.pone.0006751

Allen, A. P., Brown, J. H., & Gillooly, J. F. (2002) Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science*, **297**(5586), 1545-1548. doi: 10.1126/science.1072380

Alvarado-Serrano, D. F., Van Etten, M. L., Chang, S. M., & Baucom, R. S. (2019). The relative contribution of natural landscapes and human-mediated factors on the connectivity of a noxious invasive weed. *Heredity*, **122**, 29–40. https://doi.org/10.1038/s41437-018-0106-x

Anantharaman, R., Hall, K., Shah, V., & Edelman, A. (2020). Circuitscape in Julia: High performance connectivity modelling to support conservation decisions. *JuliaCon Proceedings*, **1**(1), 58. https://doi.org/10.21105/jcon.00058

Andersson, P., Koffman, A., Sjödin, N. E., & Johansson, V. (2017). Roads may act as barriers to flying insects: species composition of bees and wasps differs on two sides of a large highway. *Nature Conservation*, **18**, 47-59. https://doi.org/10.3897/natureconservation.18.12314

Auffret, A. G., Rico, Y., Bullock, J. M., Hooftman, D. A., Pakeman, R. J., Soons, M. B., Suárez-Esteban, A., Traveset, A., Wagner, H. H., & Cousins, S. A. (2017). Plant functional connectivity-integrating landscape structure and effective dispersal. *Journal of Ecology*, **105**(6), 1648-1656. http://dx.doi.org/10.1111/1365-2745.12742

Bailey, J. K., Schweitzer, J. A., Úbeda, F., Koricheva, J., LeRoy, C. J., Madritch, M. D., Rehill, B., Bangert, R. K., Fischer, D. G., Allan, G. J., & Whitham, T. G. (2009). From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, **364**(1523), 1607– 1616. https://doi.org/10.1098/rstb.2008.0336

Balloux, F., Lehmann, L., & de Meeûs, T. (2005). The Population genetics of clonal and partially clonal diploids. *Genetics*, **164**(4), 1635-1644.

Borokini, I. T., Weisberg, P. J., & Peacock, M. M. (2021). Quantifying the relationship between soil seed bank and plant community assemblage in sites harboring the threatened *Ivesia webberi* in the western Great Basin Desert. *Applied Vegetation Science* **24**(1): e12547. https://doi.org/10.1111/avsc.12547

Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., McDonald, M., Vesk, P. A., & Yates, C. (2008). Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications*, **1**(4), 587– 597. https://doi.org/10.1111/j.1752-4571.2008.00045.x

Brookfield, J. F. Y (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology*, **5**(3), 453-455. https://doi.org/10.1046/j.1365-294X.1996.00098.x

Brown, N. L., Peacock, M. M., & Ritchie, M. E. (2016). Genetic variation and population structure in a threatened species, the Utah prairie dog *Cynomys parvidens*: the use of genetic data to inform conservation actions. *Ecology and Evolution*, **6**(2), 426–446. https://doi.org/10.1002/ece3.1874

Canales-Delgadillo, J. C., Scott-Morales, L., & Korb, J. (2012). The influence of habitat fragmentation on genetic diversity of a rare bird species that commonly faces environmental fluctuations. *Journal of Avian Biology*, **43**(2), 168–176. https://doi.org/10.1111/j.1600-048X.2011.05372.x

Cassel, E. J., Calvert, A. T., & Graham, S. A. (2009). Age, geochemical composition, and distribution of Oligocene ignimbrites in the northern Sierra Nevada, California: implications for landscape morphology, elevation, and drainage divide geography of the Nevadaplano. *International Geology Review*, **51**(7-8), 723–742. https://doi.org/10.1080/00206810902880370

Castillo, J. A., Epps, C. W., Davis, A. R., & Cushman, S. A. (2014). Landscape effects on gene flow for climate-sensitive montane species, the American pika. *Molecular Ecology*, **23**(4), 843–856. https://doi.org/10.1111/mec.12650

Chakraborty, R., De Andrade, M., Daiger, S. P., & Budowle, B. (1992). Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Annals of Human Genetics*, **56**(1), 45-47. https://doi.org/10.1111/j.1469-1809.1992.tb01128.x

Chambers, J. C. (2008). Climate Change and the Great Basin. In: Chambers J.C., Devoe N., & Evenden A. (eds.). *Collaborative management and research in the Great Basin - examining the issues and developing a framework for action*. Gen. Tech. Rep. RMRS-GTR-204. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. p. 29-32

Chambers, J. C., Roundy, B. A., Blank, R. R., Meyer, S. E., & Whittaker, A. (2007). What makes Great Basin sagebrush ecosystems invasible by *Bromus tectorum*? *Ecological Monographs*, **77**(1), 117-145. https://doi.org/10.1890/05-1991

Consuegra, S., Verspoor, E., Knox, D., & de Leániz, C. G. (2005). Asymmetric gene flow and the evolutionary maintenance of genetic diversity in small, peripheral Atlantic salmon populations. *Conservation Genetics*, **6,** 823–842. https://doi.org/10.1007/s10592-005-9042-4

Corcos, D., Cerretti, P., Caruso, V., Mei, M., Falco, M., & Marini, L. (2019). Impact of urbanization on predator and parasitoid insects at multiple spatial scales. *PLoS ONE*, **14**(4), e0214068. https://doi.org/10.1371/journal.pone.0214068

Cruzan, M. B. (2001). Population size and fragmentation thresholds for the maintenance of genetic diversity in the herbaceous endemic *Scutellaria montana* (Lamiaceae). *Evolution*, **55**(8): 1569–1580. https://doi.org/10.1111/j.0014-3820.2001.tb00676.x

Culley, T. M., & Wolfe, A. D. (2001). Population genetic structure of the cleistogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity*, **86**, 545–556. https://doi.org/10.1046/j.1365-2540.2001.00875.x

Cushman, S. A., & Landguth, E. L. (2010). Scale dependent inference in landscape genetics. *Landscape Ecology*, **25**, 967– 979. https://doi.org/10.1007/s10980-010-9467-0

Delnevo, N., van Etten, E. J., Byrne, M., Petraglia, A., Carbognani, M., & Stock, W. D. (2020). Habitat fragmentation restricts insect pollinators and pollen quality in a threatened Proteaceae species. *Biological Conservation*, **252**, 108824. https://doi.org/10.1016/j.biocon.2020.108824.

Di Rienzo, A., Peterson, A. C., Garza, J. C., Valdes, A. M., Slatkin, M., & Freimer, N. B. (1994). Mutational processes of simple-sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences of the United States of America*, **91**(8), 3166-3170. https://doi.org/10.1073/pnas.91.8.3166

Dias, E. F., Moura, M., Schaefer, H., & Silva, L. (2016). Geographical distance and barriers explain population genetic patterns in an endangered island perennial. *AoB PLANTS* **8**, plw072. https://doi.org/10.1093/aobpla/plw072

Dick, C. W., Hardy, O. J., Jones, F. A., & Petit, R. J. (2008) Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Tropical Plant Biology*, **1**, 20– 33. https://doi.org/10.1007/s12042-007-9006-6

Dirnböck, T., Essl, F., & Rabitsch, W. (2011). Disproportional risk for habitat loss of high-altitude endemic species under climate change. *Global Change Biology*, **17**(2), 990-996. https://doi.org/10.1111/j.1365-2486.2010.02266.x

Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**(1), 431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NEESTIMATOR v2: re-implementation of software for the estimation of contemporary effective population size (*N*e) from genetic data. *Molecular Ecology Resources,* **14**(1), 209–214. https://doi.org/10.1111/1755-0998.12157

Dobeš, C., & Scheffknecht, S. (2012). Isolation and characterization of microsatellite loci for the *Potentilla* core group (Rosaceae) using 454 sequencing. *Molecular Ecology Resources,* **12**(4), 726–739. https://doi.org/10.1111/j.1755-0998.2012.03134.x

Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology,* **17**(5)**,** 1170-1188. https://doi.org/10.1111/j.1365-294X.2007.03659.x

Ellstrand, N. C. (2014). Is gene flow the most important evolutionary force in plants? *American Journal of Botany*, **101**(5), 737–753. https://doi.org/10.3732/ajb.1400024

Ennos, R. A. (1994). Estimating the relative rates of pollen and seed migration among plant-populations. *Heredity,* **72,** 250–259. https://doi.org/10.1038/hdy.1994.35

Enquist, B. J, Jordan, M. A., & Brown, J. H. (1995). Connections between ecology, biogeography. and paleobiology: relationship between local abundance and geographic distribution in fossil and recent molluscs. *Evolutionary Ecology,* **9**, 586-604. https://doi.org/10.1007/BF01237657

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* **14**(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Flores-Manzanero, A., Luna-Bárcenas, M. A., Dyer, R. J., & Vázquez-Domínguez, E. (2019). Functional connectivity and home range inferred at a microgeographic landscape genetics scale in a desert-dwelling rodent. *Ecology and Evolution*, **9**(1), 437-453. https://doi.org/10.1002/ece3.4762

Genton, B. J., Shykoff, J. A., & Giraud, T. (2005). High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. *Molecular Ecology*, **14**(14), 4275–4285. https://doi.org/10.1111/j.1365-294X.2005.02750.x

Goslee, S. C., & Urban, D. L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software,* **22**, 1-19. doi: 10.18637/jss.v022.i07

Goudet, J. (1995). FSTAT version (1.2): A Computer program to calculate F-Statistics. *Journal of Heredity*, **86**(6), 485-486. https://doi.org/10.1093/oxfordjournals.jhered.a111627

Hohnen, R., Tuft, K. D., Legge, S., Hillyer, M., Spencer, P. B., Radford, I. J., Johnson C.N., & Burridge, C. P. (2016). Rainfall and topography predict gene flow among populations of the declining northern quoll (*Dasyurus hallucatus*). *Conservation Genetics,* **17**, 1213–1228. https://doi.org/10.1007/s10592-016-0856-z

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008) Ecological consequences of genetic diversity. *Ecology Letters,* **11**(6), 609– 623. https://doi.org/10.1111/j.1461-0248.2008.01179.x

Jost, L. (2006). Entropy and diversity. *Oikos*, **113**(2), 363-375. https://doi.org/10.1111/j.2006.0030-1299.14714.x

Kahilainen, A., Puurtinen, M., & Kotiaho, J. S. (2014). Conservation implications of species–genetic diversity correlations. *Global Ecology and Conservation,* **2**, 315-323. https://doi.org/10.1016/j.gecco.2014.10.013

Kraft, N. J. B, Baldwin, B. G., & Ackerly, D. D. (2010). Range size, taxon age and hotspots of neoendemism in the California flora. *Diversity and Distributions*, **16**(3), 403–413. https://doi.org/10.1111/j.1472-4642.2010.00640.x

Langin, K., Sillett, T., Funk, W., Morrison S. A., & Ghalambor C. K. (2017). Partial support for the central–marginal hypothesis within a population: reduced genetic diversity but not increased differentiation at the range edge of an island endemic bird. *Heredity,* **119**, 8–15. https://doi.org/10.1038/hdy.2017.10

Làzaro, A., Fuster, F., Alomar, D., & Totland, Ø. (2020). Disentangling direct and indirect effects of habitat fragmentation on wild plants' pollinator visits and seed production. *Ecological Applications*, **30**(5), e02099. https://doi.org/10.1002/eap.2099

Lichstein, J. W. (2007). Multiple regression on distance matrices: a multivariate spatial analysis tool. *Plant Ecology,* **188**, 117–131. https://doi.org/10.1007/s11258-006-9126-3

Mandák, B., Zákravský, P., Mahelka, V., & Plačková, I. (2012). Can soil seed banks serve as genetic memory? A study of three species with contrasting life history strategies. *PLoS ONE*, **7**(11), e49471. https://doi.org/10.1371/journal.pone.0049471

Matter, P., Kettle, C. J., Ghazoul, J., Hahn, T., & Pluess, A. R. (2013). Evaluating contemporary pollen dispersal in two common grassland species *Ranunculus bulbosus* L. (Ranunculaceae) and *Trifolium montanum* L. (Fabaceae) using an experimental approach. *Plant Biology*, **15**(3), 583-592. https://doi.org/10.1111/j.1438-8677.2012.00667.x

McRae, B. H. (2006). Isolation by resistance. *Evolution*, **60**(8): 1551-1561. https://doi.org/10.1111/j.0014-3820.2006.tb00500.x

McRae, B. H., & Beier, P. (2007). Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences,* **104**(5), 19885-19890. https://doi.org/10.1073/pnas.0706568104

McRae, B. H., Dickson, B. G., Keitt, T. H., & Shah, V. B. (2008). Using circuit theory to model connectivity in ecology, evolution, and conservation. *Ecology*, **89**(10), 2712–2724. https://doi.org/10.1890/07-1861.1

Meeus, S., Honnay, O., Brys, R., & Jacquemyn, H. (2012). Biased morph ratios and skewed mating success contribute to loss of genetic diversity in the distylous *Pulmonaria officinalis*. *Annals of Botany*, **109**(1), 227–235. https://doi.org/10.1093/aob/mcr272

Micheletti, S. J., & Storfer, A. (2015). A test of the central-marginal hypothesis using population genetics and ecological niche modeling in an endemic salamander (*Ambystoma barbouri*). *Molecular Ecology,* **24**(5), 967-979. https://doi.org/10.1111/mec.13083

Minasiewicz, J., Znaniecka, J. M., Górniak, M., & Kawiński, A. (2018). Spatial genetic structure of an endangered orchid *Cypripedium calceolus* (Orchidaceae) at a regional scale: limited gene flow in a fragmented landscape. *Conservation Genetics,* **19**, 1449–1460. https://doi.org/10.1007/s10592-018-1113-4

Mokany, K., Prasad, S., & Westcott, D. A. (2014). Loss of frugivore seed dispersal services under climate change. *Nature Communications,* **5**, 3971. https://doi.org/10.1038/ncomms4971

Moseley, R. K. (1993). *Floristic inventory of subalpine parks in the Coeur d'Alene river drainage, northern Idaho*. Report to Cooperative Challenge Cost-share Project Idaho Panhandle National Forests and Idaho Department of Fish and Game; 62 pp.

Muller, M. H., Latreille, M., & Tollon, C. (2011). The origin and evolution of a recent agricultural weed: population genetic diversity of weedy populations of sunflower (*Helianthus annuus* L.) in Spain and France. *Evolutionary Applications*, **4**(3), 499–514. https://doi.org/10.1111/j.1752-4571.2010.00163.x

Murphy, M. A., Dezzani, R., Pilliod, D. S., & Storfer, A. (2010). Landscape genetics of high mountain frog metapopulations. *Molecular Ecology,* **19**(17), 3634–3649. https://doi.org/10.1111/j.1365-294X.2010.04723.x

Nevill, P. G., Robinson, T. P., Di Virgilio, G., & Wardell-Johnson, G. (2017). Beyond isolation by distance: what best explains functional connectivity among populations of three sympatric plant species in an ancient terrestrial island system? *Diversity and Distributions,* **25**(10), 1551-1563. https://doi.org/10.1111/ddi.12959

Neville, H., Dauwalter, D., & Peacock, M. (2016). Monitoring demographic and genetic responses of a threatened inland trout to habitat reconnection. *Transactions of the American Fisheries Society,* **145**(3), 610–626. https://doi.org/10.1080/00028487.2015.1131747

O'Connell, K. A., Mulder, K. P., Maldonado, J., Currie, K. L., & Ferraro, D. M. (2019). Sampling related individuals within ponds biases estimates of population structure in a pond-breeding amphibian. *Ecology and Evolution,* **9**(6), 3620-3636. https://doi.org/10.1002/ece3.4994

Oliveira, E. F., Martinez, P. A., São-Pedro, V. A., Gehara, M., Burbrink, F. T., Mesquita, D. O., Garda, A. A., Colli, G. R., & Costa, G. C. (2018). Climatic suitability, isolation by distance and river resistance explain genetic variation in a Brazilian whiptail lizard. *Heredity,* **120**(3), 251-265. https://doi.org/10.1038/s41437-017-0017-2

Pasquet, R. S., Peltier, A., Hufford, M. B., Oudin, E., Saulnier, J., Paul, L., Knudsen, J. T., Herren, H. R., & Gepts, P. (2008). Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proceedings of the National Academy of Sciences of the United States of America*, **105**(36), 13456–13461. https://doi.org/10.1073/pnas.0806040105

Peacock, M. M., & Dochtermann, N. A. (2012). Evolutionary potential but not extinction risk of Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) is associated with stream characteristics. *Canadian Journal of Fisheries and Aquatic Sciences,* **69**(4), 615–626. https://doi.org/10.1139/f2012-006

Peacock, M. M., & Smith, A. T. (1997). The effect of habitat fragmentation on dispersal patterns, mating behavior, and genetic variation in a pika (*Ochotona princeps*) metapopulation. *Oecologia,* **112**, 524–533. https://doi.org/10.1007/s004420050341

Peakall, R. & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* **28**(19), 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Peterman, W. E. (2018). ResistanceGA: An R package for the optimization of resistance surfaces using genetic algorithms. *Methods in Ecology and Evolution*, **9**(6), 1638-1647. https://doi.org/10.1111/2041-210X.12984

Peterman, W. E., Winiarski, K. J., Moore, C. E., da Silva Carvalho, C., Gilbert, A. L., & Spear, S. F. (2019). A comparison of popular approaches to optimize landscape resistance surfaces. *Landscape Ecology*, **34**, 2197–2208. https://doi.org/10.1007/s10980-019-00870-3

Pfenninger, M., Salinger, M., Haun, T., & Feldmeyer, B. (2011). Factors and processes shaping the population structure and distribution of genetic variation across the species range of the freshwater snail *Radix balthica* (Pulmonata, Basommatophora). *BMC Evolutionary Biology*, **11**, 135. https://doi.org/10.1186/1471-2148-11-135

Piry, S., Luikart, G., & Cornuet, J. M. (1999). BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**(4), 502–503. https://doi.org/10.1093/jhered/90.4.502

Pollak, O. (1997). Morphology and dynamics of alpine populations of *Ivesia lycopodioides* ssp. *scandularis* from the White Mountains of California. In: University of California White Mountain Research Station Symposium 1:97–116.

Pritchard, J. K., Wen, X. Q., & Falush, D. (2007). STRUCTURE (version 2.2). Chicago, IL: University of Chicago. http://pritch.bsd.uchicago.edu/software

R Development Core Team (2020). *R*: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.Rproject.org/.

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**(2), 217-223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Schulz, B., Durka, W., Danihelka, J., & Eckstein, R. L. (2018). Differential role of a persistent seed bank for genetic variation in early vs. late successional stages. *PLoS ONE*, **13**(12): e0209840. https://doi.org/10.1371/journal.pone.0209840

Scrucca, L. (2013). GA: A package for genetic algorithms in R. *Journal of Statistical Software*, **53**, 1– 37. doi: 10.18637/jss.v053.i04

Scrucca, L. (2017). On some extensions to GA package: hybrid optimisation, parallelisation and islands evolution. *The R Journal*, **9**(1), 187–206. doi:10.32614/RJ-2017-008.

Sexton, J. P., Hangartner, S. B., & Hoffmann, A. A. (2014). Genetic isolation by environment or distance: Which pattern of gene flow is most common? *Evolution*, **68**, 1–15. https://doi.org/10.1111/evo.12258

Shirk, A. J., Wallin D. O., Cushman, S. A., Rice, C. G., & Warheit, K. I. (2010). Inferring landscape effects on gene flow: a new model selection framework. *Molecular Ecology*, **19**(17), 3603–3619. https://doi.org/10.1111/j.1365-294X.2010.04745.x

Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**(1), 457–462.

Spear, S. F., Balkenhol, N., Fortin, M.-J., McRae, B. H., & Scribner, K. (2010). Use of resistance surfaces for landscape genetics studies: Considerations for parameterization and analysis. *Molecular Ecology*, **19**(17), 3576–3591. https://doi.org/10.1111/j.1365-294X.2010.04657.x

Templeton, A. R., Shaw, K., Routman E., & Davis, S. K. (1990). The Genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden*, **77**(1), 13-27. https://doi.org/10.2307/2399621

Töpel, M., Antonelli, A., Yesson, C., & Eriksen, B. (2012). Past climate change and plant evolution in Western North America: A case study in Rosaceae. *PLoS ONE*, **7**(12), e50358. https://doi.org/10.1371/journal.pone.0050358

Tso, K. L., & Allan, G. J. (2019). Environmental variation shapes genetic variation in *Bouteloua gracilis*: Implications for restoration management of natural populations and cultivated varieties in the southwestern United States. *Ecology and Evolution*, **9**(1), 482-499. https://doi.org/10.1002/ece3.4767

USFWS (2014). *Species Report for Ivesia webberi (Webber's ivesia)*. Nevada Fish and Wildlife Office, Reno. United States Fish and Wildlife Services, 49 pp.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Vellend, M. (2005). Species diversity and genetic diversity: parallel processes and correlated patterns. *American Naturalist*, **166**(2), 199– 215. https://doi.org/10.1086/431318

Vellend, M., & Geber, M.A. (2005). Connections between species diversity and genetic diversity. *Ecology Letters*, **8**(7), 767– 781. https://doi.org/10.1111/j.1461-0248.2005.00775.x

Vellend, M., Lajoie, G., Bourret, A., Múrria, C., Kembel, S. W., & Garant, D. (2014). Drawing ecological inferences from coincident patterns of population- and community-level biodiversity. *Molecular Ecology*, **23**(12), 2890-2901. https://doi.org/10.1111/mec.12756

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology*, **23**(23), 5649–5662. https://doi.org/10.1111/mec.12938

Wattier, R., Engel, C. R., Saumitou-Laprade, P., & Valero, M. (1998). Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). *Molecular Ecology*, **7**(11), 1569-1573. https://doi.org/10.1046/j.1365-294x.1998.00477.x

Whitlock, R. (2014). Relationships between adaptive and neutral genetic diversity and ecological structure and functioning: a meta-analysis. *Journal of Ecology*, **102**(4): 857-872. https://doi.org/10.1111/1365-2745.12240

Witham, C. W. (2000). *Current Knowledge and Conservation Status of Ivesia webberi A. Gray (Rosaceae), the Webber ivesia, in Nevada*. Status Report prepared for the Nevada Natural Heritage Program, Dept. of Conservation and Natural Resources, Carson City, NV. 52pp.

Wright, S. (1965). The interpretation of population structure by F-Statistics with special regard to systems of mating. *Evolution*, **19**(3), 395–420. https://doi.org/10.1111/j.1558-5646.1965.tb01731.x

Zeller, K. A., McGarigal, K., & Whiteley, A. R. (2012). Estimating landscape resistance to movement: a review. *Landscape Ecology* **27,** 777–797. https://doi.org/10.1007/s10980-012-9737-0

Zwick, P. (1992). Stream habitat fragmentation — a threat to biodiversity. *Biodiversity Conservation,* **1,** 80–97. https://doi.org/10.1007/BF00731036

LIST OF APPENDICES

TABLES

Table 1. *Ivesia webberi* populations sampled for this study, abbreviated (abr) site names, patch size (acres), sample size (N), mean number of alleles per locus (*N*a), allelic richness over all loci per population (R_T) and mean observed (*H*o) and expected (*H*e) heterozygosity per population.

		Patch		Averages			
Population	Abr	size	N	*N*a	R_T	*H*o	*H*e
Sierra Valley	SVE	44.8	21	2.67	13.45	0.400	0.344
Constantia	CST	1.91	20	2.17	10.31	0.192	0.204
Evans Canyon, East of Hallelujah junction wildlife area (HJWA)	EHJ	0.14	24	2.17	11.60	0.419	0.305
HJWA	HJA	0.05	18	2.67	14.56	0.410	0.363
Dog Valley meadow	DVA	71.58	22	2.67	12.83	0.359	0.317
White Lake overlook	WLO	13.56	22	3.33	15.63	0.487	0.405
Mules Ear Flat	MER	0.14	20	3.00	15.28	0.559	0.441
Ivesia flat	IVF	0.73	20	2.83	14.29	0.605	0.435
Stateline road 1	STL	7.03	9	2.50	14.29	0.495	0.379
Stateline road 2	STN	4.03	13	2.33	12.85	0.316	0.346
Hungry valley	HGV	0.16	24	2.50	12.28	0.492	0.369
Black springs	BSP	6.31	18	2.33	11.85	0.315	0.271
Raleigh heights	RAH	9.55	23	3.17	13.88	0.423	0.355
Dutch Louie flat	DLF	1.35	19	2.83	12.51	0.237	0.242
The Pines power line	PPL	0.14	18	2.17	10.79	0.265	0.216
Dante Mine Road	DMR	0.56	23	1.83	9.34	0.274	0.200

Table 2. Locus, primer sequences, repeat motif and PCR melting temperatures for the six microsatellite loci used in this study

Locus	Primer 5'-3'	Repeat Motif	Melting temperature (°C)
PMS 1080	F: AAATAGGCCATCCCAATTCC R: TGCCCATCTTTCTTCTGGTT	$(TAG)_{14}$	66.7 57.9
PMS 1180	F: CGATCGTAACCGTTCTCCAT R: ACCGCTCTTCTTCTCCGATT	$(TC)_4$, $(GGC)_7$	65.3 58.5
PMS 1438	F: GGACTTGGGACTTTGTTGGA R: TCCCAAATGCAATCGTGTAA	$(AC)_{10}$	68.5 56.6
PMS 1665	F: CCAAGTGAAGGAAGCCAAAC R: GCCGACGAAGAAGGAAGAC	$(AG)_6$	68.2 59.7
PMS 1694	F: CCTCGAGGAACAACCTGTTT R: CATGGACTGAGGAAGAACACAA	$(AT)_{13}$	67.3 58.8
PMS 2190	F: ATAAAGGCAACGCAAGATCA R: CGTATAATCTTACCAATCAATTAAACA	$(CA)_5/(TA)_{16}$	65.5 54.8

*The loci were developed from *Potentilla pusilla* and cross amplified with *I. webberi* (Dobeš and Scheffknecht 2012). See Table S1 for the number of genotyped individuals, alleles observed, allelic richness, expected and observed heterozygosity, and inbreeding coefficient per locus across the 16 *I. webberi* populations

Table 3. Effective population size for populations where we could calculate a 95% CI and genotype clusters (including only individuals with Q≥0.8 per cluster)

Populations	*N*e	95% CI	
HJA	0.9	0.6	1.3
DVA	5.2	3.3	8.5
STN	3.3	1.6	7.7
DLF	8.3	4.4	20
RAH	11.7	6.8	24.1
Clusters			
1 (orange)	2.6	1.7	4
2 (gray)	27.9	12.6	148.1
3 (yellow)	40.5	19.5	162.1
4 (blue)	18.3	10.5	35.9
5 (green)	20.4	12.3	37.6

Table 4. Pairwise genetic differentiation (F_{ST}) values among the 16 *Ivesia webberi* populations. Bold Pairwise F_{ST} values indicate statistical different (corrected *p*=0.0004)

Population	SVE	CST	EHJ	HJA	DVA	WLO	MER	IVF	STL	STN	HGV	BSP	RAH	DLF	PPL
SVE															
CST	**0.657**														
EHJ	**0.135**	**0.770**													
HJA	**0.114**	**0.612**	0.045												
DVA	**0.156**	**0.354**	**0.121**	**0.104**											
WLO	0.041	**0.541**	**0.109**	0.084	**0.106**										
MER	**0.227**	**0.337**	**0.285**	**0.248**	**0.090**	**0.156**									
IVF	**0.130**	**0.317**	**0.220**	**0.160**	**0.102**	0.090	0.048								
STL	**0.195**	0.440	**0.109**	**0.105**	0.020	0.087	0.107	0.137							
STN	**0.143**	**0.287**	**0.159**	0.126	0.049	0.108	0.120	0.092	0.021						
HGV	**0.252**	**0.335**	**0.257**	**0.188**	**0.146**	**0.188**	**0.131**	**0.028**	**0.186**	**0.124**					
BSP	**0.167**	0.352	**0.164**	**0.121**	0.008	**0.110**	0.099	**0.070**	**0.072**	0.040	**0.102**				
RAH	**0.184**	**0.224**	**0.228**	**0.170**	0.059	**0.141**	0.073	0.015	0.116	0.053	0.025	0.027			
DLF	**0.352**	0.201	**0.407**	**0.282**	0.068	**0.262**	0.107	**0.132**	**0.154**	0.102	**0.167**	**0.053**	0.066		
PPL	**0.421**	0.216	**0.465**	**0.352**	0.101	**0.316**	0.104	**0.148**	0.220	0.153	**0.185**	0.080	0.077	-0.002	
DMR	**0.770**	**0.667**	**0.992**	**0.861**	**0.631**	**0.709**	**0.606**	**0.531**	**0.609**	**0.319**	**0.561**	**0.687**	**0.477**	**0.612**	**0.738**

Table 5. Genotype cluster assignment for individuals with Q≥80%. Highlighted numbers represent the populations per cluster with the highest number of individuals assigned (see text) and are included in Figure 6c

Population	**cluster1**	**cluster2**	**cluster3**	**cluster4**	**cluster5**	**Total**
CST	4	1		7	3	15
MER		2		8	4	14
STN	2	2	1	4	1	10
DLF	2	1		4	3	10
WLO		2	4	8	1	15
HJA			11	4		15
DVA		1	4	2	3	10
EHJ		6	16			22
SVE		5	5		1	11
STL	1	2		1		4
IVF		4		3	6	13
RAH		3			10	13
HGV		1	1	1	9	12
BSP			3		5	8
PPL	1	1	1	5	7	15
DMR	20					20
	30	31	46	47	53	207

Table 6. Results of the Mantel tests and multiple regression on distance matrices (MRM) analysis between pairwise genetic distance (F_{ST}) and predictors of gene flow among the 16 sampled *Ivesia webberi* populations. MRM analysis accounts for the effect of geographical distance between pairwise genetic distance (F_{ST}) and predictors of gene flow. All Mantel test were run in ECODIST R package, while MRM analysis was conducted in PHYTOOLS R package, both implemented with 10,000 permutations

Predictors	**Mantel's *r***	***p***	**MRM F**	***P***
Geographical distance	0.8535	0.0001	n/a	n/a
Latitudinal degrees	0.8608	0.0001	n/a	n/a
Aboveground species dissimilarity*	-0.0173	0.5001	1663.260	0.0001
Soil seed bank species dissimilarity*	0.9862	0.0001	6135.768	0.0001
Actual evapotranspiration	0.6188	0.0061	11119.242	0.0001
Cosine aspect	0.1955	0.1211	15294.608	0.0001
Summer precipitation	0.7066	0.0030	15708.377	0.0001
Minimum annual temperature	-0.0448	0.5717	15561.648	0.0001
Native herb cover	-0.0077	0.4154	12012.579	0.0001
Topographic position index	-0.2259	0.9516	214.772	0.0001

*Species dissimilarity in both the aboveground vegetation and the soil seed bank was computed from 10 of the 16 *I. webberi* populations (Borokini et al. 2021). Therefore, pairwise genetic distance (F_{ST}) corresponding to the sampled 10 populations was used.

Table 7. Summary table from the bootstrap analysis on the MLPE models with 10,000 iterations in RESISTANCE GA R package. *k* is the number of parameters fitted in the bootstrap analysis, AIC and AICc represent average values of the two parameters in the bootstrap analysis, LL is the average log likelihood of the bootstrap analysis. Weight represents the average contribution of each predictor to the model relative to all predictors included. R^2m is the average marginal R^2 value of the bootstrap analysis on the MLPE model

Parameters	Land cover:niche	Land cover	Niche	Distance	Null
k	19	16	4	2	1
AIC	-64.1803	-70.3673	-91.0559	-96.2079	n/a
AICc	695.8197	473.6327	-85.3417	-94.8746	n/a
LL	51.0902	51.1836	49.5279	50.1039	n/a
R^2m	0.55184	0.5616	0.4934	0.4855	n/a
Weight	0.0000	0.0000	0.0488	0.9512	n/a

FIGURE LEGENDS

Figure 1. Map of the global distribution of *Ivesia webberi*. Symbols represent the geographic center of extant, mapped occurrences. Locations represented by circles show the sampled populations used for this study; circles depicted in the same color represent occurrences that were grouped together by the USFWS as populations. New locations discovered after sample collection and thus not included in this study are represented by diamonds.

Figure 2. Ritland and Lynch (1999) mean relatedness (*r*) ± SD for each the 16 sampled *Ivesia webberi* populations.

Figure 3. (A) STRUCTURE output showing proportional membership per genotype cluster (*k*=5) per individual**.** (B) The natural log of the probability of the data [LnP(D)] values per *k* for *k*=1–10. (C) Spatial extent of genotype clusters for individuals with Q≥80%. (D) Mean LnP(K)±SD, Ln’(*k*), [Ln”(*k*)] and Δ*k* for *k*=1–10.

Figure 4. PCoA plot genotype clusters (*k*=5) for axes (A)1vs2, (B) 2vs3, and (C) 1vs3.

FIGURES

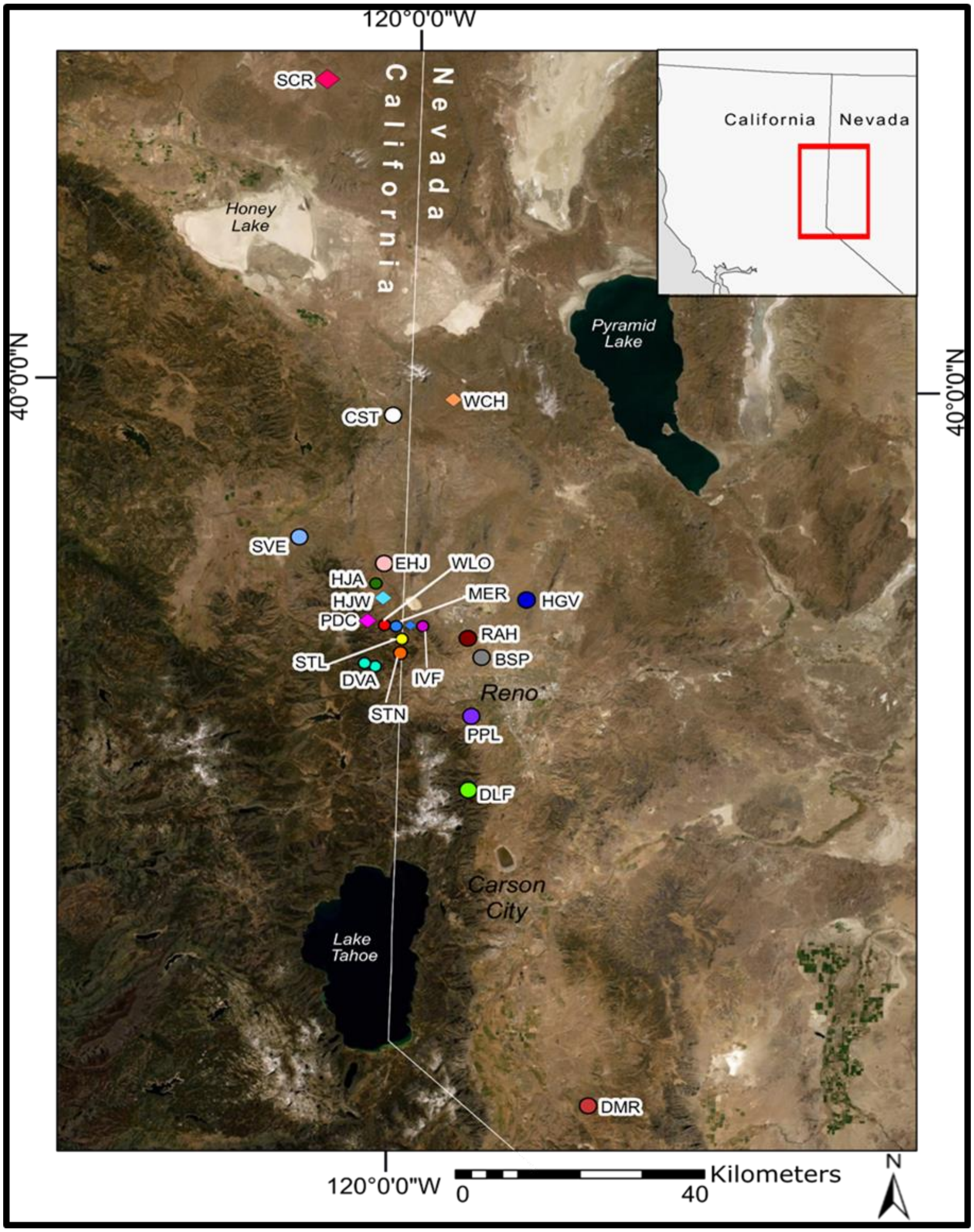


Figure 1.

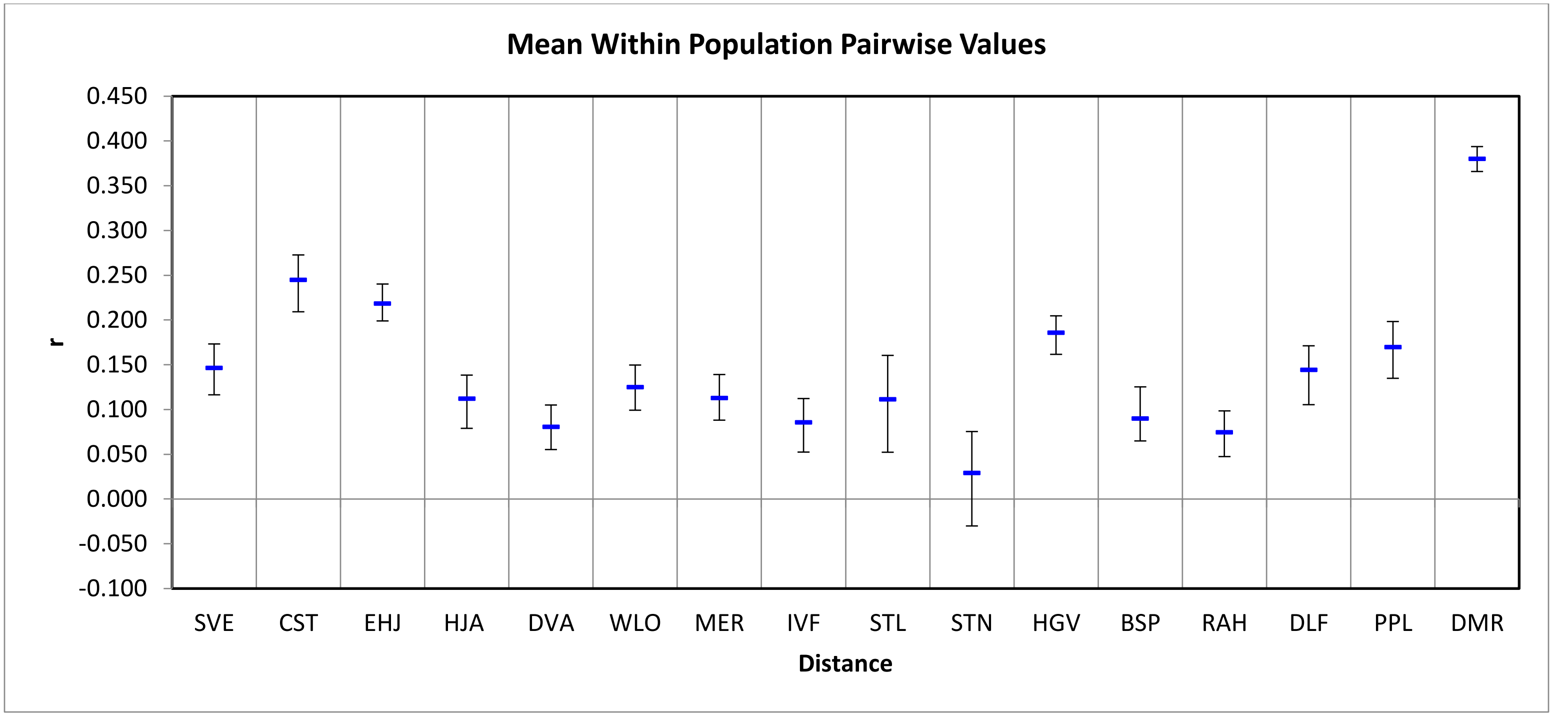
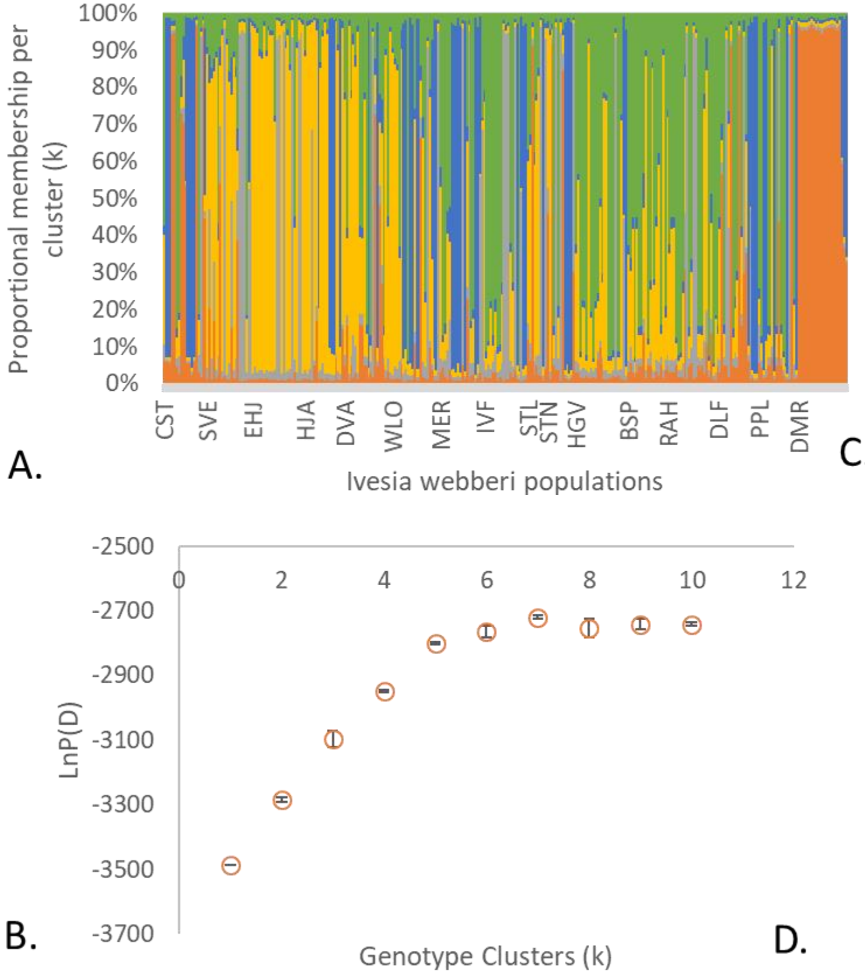
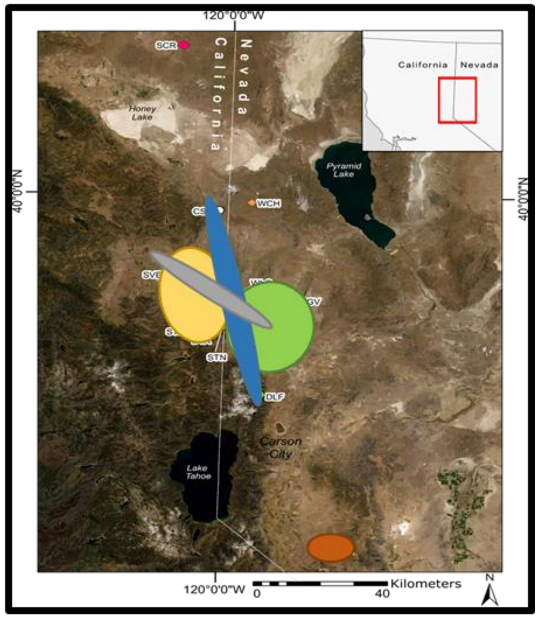

Mean Within Population Pairwise Values
r
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
-0.050
-0.100
SVE
CST
EHJ
HJA
DVA
WLO
MER
IVF
STL
STN
HGV
BSP
RAH
DLF
PPL
DMR
Distance

Figure 2.

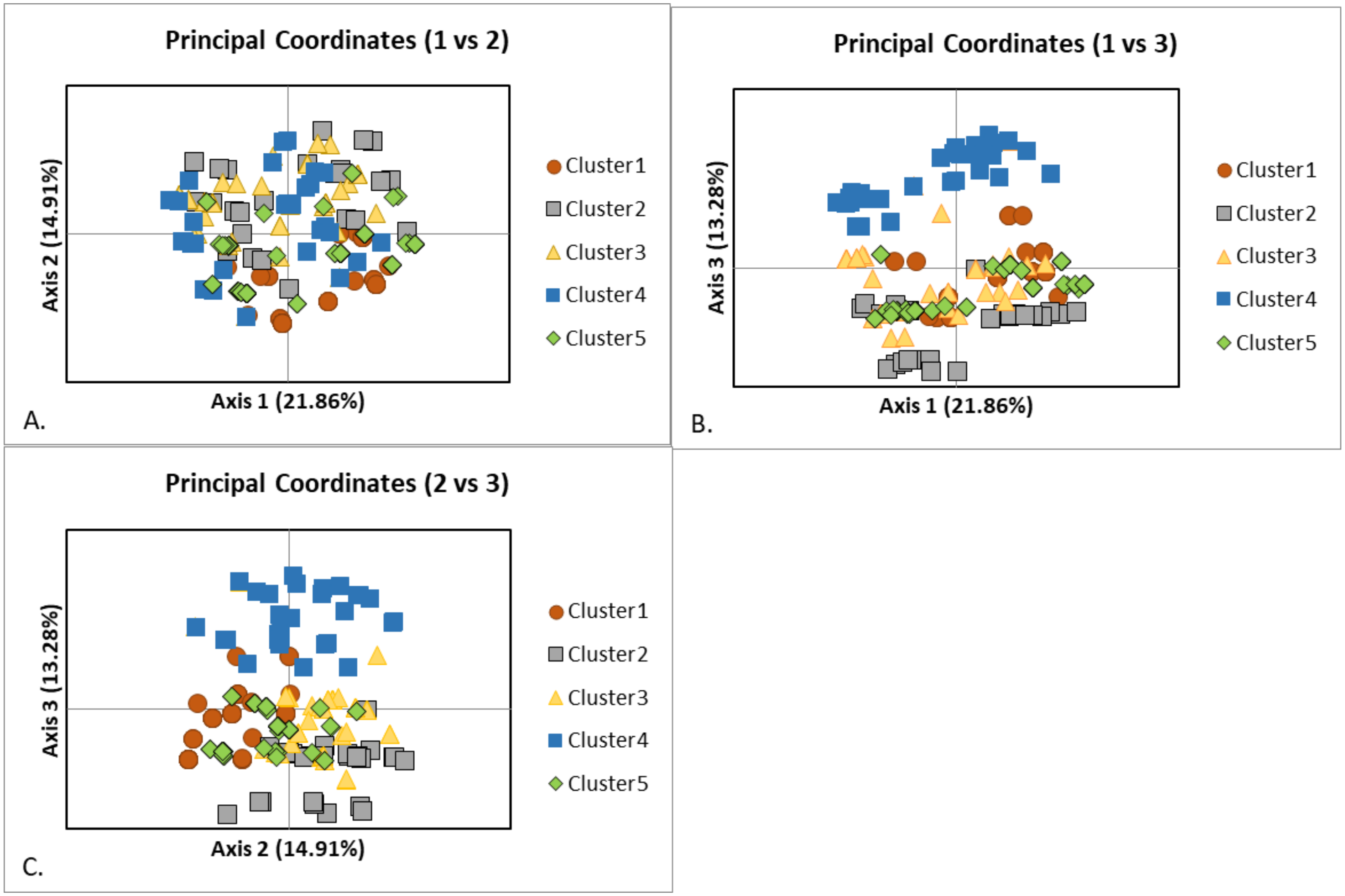




D.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-3484.64	0.052	–	–	–
2	10	-3284.27	6.917	200.37	12.27	1.774
3	10	-3096.17	26.728	188.1	40.78	1.526
4	10	-2948.85	4.226	147.32	0.11	0.026
5	10	-2801.42	2.936	147.43	108.93	37.098
6	10	-2762.92	18.382	38.5	4.94	0.269
7	10	-2719.48	4.321	43.44	76.51	17.707
8	10	-2752.55	28.688	-33.07	44.68	1.557
9	10	-2740.94	14.627	11.61	11.88	0.812
10	10	-2741.21	6.459	-0.27	–	–

Figure 3.


Principal Coordinates (1 vs 2)

Principal Coordinates (1 vs 3)

Principal Coordinates (2 vs 3)

Figure 4.

ACKNOWLEDGEMENTS: This research was funded through a grant from the U.S. Fish and Wildlife Service CFDA program (award number F15AC00784), with additional funding support from the Northern California Botanists (NCB) Botany research scholarship program and the Nevada Native Plant Society Margaret Williams research grant, and the Society for Conservation Biology (SCB) graduate student research fellowship. The authors are grateful to Dr. William Peterman, Dr. Javan Bauder, Dr. Scott Buchanan, Dr. Andrew Shirk, and Dr. Samuel Cushman for their expert advice on the isolation by resistance modeling. The kind support of Ms. Sarah Kulpa of the U.S. Fish and Wildlife Service, Reno Field Office, Ms. Janel Johnson of the Nevada Natural Heritage Program, and Ms. Charlene Duncan during the field collection is appreciated.

APPENDICES

Appendix I. Fitting of the ecological niche modeling for *Ivesia webberi*

Niche model was fitted for *I. webberi* using 31 occurrence points and 102 spatially thinned true absence points, using six algorithms, including Generalized linear models (GLM), Generalized additive models (GAM), Boosted Regression Trees (BRT), Random Forests (RF), Maximum Entropy (MAXENT), and Artificial Neural Networks (ANN). The spatial dataset was a product of iterative niche modeling and successive field validation to the projected suitable sites from 2015 to 2020. The models were fitted in BIOMOD2 R package (Thuiller et al., 2009), using the default settings. GLM were generated using quadratic terms in stepwise approach, and using the Akaike information Criterion (AIC) for model selection. BRT models were generated by fitting 2500 trees and three cross-validations, while GAM was analyzed with a spline smoothing function. We used five cross-validations to select the optimal hidden layers in ANN models, we fitted 500 random forest models, and ran MAXENT models with 10,000 background points, using linear, quadratic and product features but with logistic model output. Due to the small size of occurrence points, spatial data was not partitioned to independent training and test data, rather all niche models were fitted with 10 replicates each for the six algorithms using 80% of the data for cross-validation (Araújo et al., 2005; Thuiller et al., 2009).

Model performance was measured using True statistic skill (TSS; Allouche et al. 2006), area under the curve (AUC) of the receiver operated characteristics (ROC) plot (Hanley & McNeil, 1982), specificity, and Continuous Boyce index (CBI; Boyce, Vernier, Nielsen, & Schmiegelow, 2002) to produce model predictions with maximal accuracy and minimal omission errors. Model replicates with (TSS≥0.7) were used for ensemble modeling from which projection of habitat suitability maps were produced.

References

Allouche, O., Tsoar, A., & Kadmon, R. (2006). Assessing the accuracy of species distribution models: Prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology*, **43**(6), 1223–1232. https://doi.org/10.1111/j.1365-2664.2006.01214.x

Araújo, M. B., Whittaker, R. J., Ladle, R. J., & Erhard, M. (2005). Reducing uncertainty in projections of extinction risk from climate change. *Global Ecology and Biogeography*, **14**(6), 529–538. https://doi.org/10.1111/j.1466-822X.2005.00182.x

Boyce, M. S., Vernier, P. R., Nielsen, S. E., & Schmiegelow, F. K. A. (2002). Evaluating resource selection functions. *Ecological Modelling*, **157**(2-3), 281-300. https://doi.org/10.1016/S0304-3800(02)00200-4

Hanley, J. A., & McNeil, B. J. (1982). The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology*, **143**(1), 29–36. https://doi.org/10.1148/radiology.143.1.7063747

Thuiller, W., Lafourcade, B., Engler, R., & Araújo, M. B. (2009). BIOMOD – a platform for ensemble forecasting of species distributions. *Ecography*, **32**(3), 1-5. https://doi.org/10.1111/j.1600-0587.2008.05742.x

Appendix II. Ecological conditions in the 16 *Ivesia webberi* populations based on the six ecological variables

Unit*	Population	AET (mm)	Cosine aspect	Summer precipitation (mm)	Temperature (°C)	Herbaceous cover	Topographic position index
1	SVE	243.57	-0.52	25.15	-7.40	32.16	-45.26
2	CST	248.11	0.64	22.42	-6.13	24.71	-111.29
3	EHJ	184.19	0.95	27.27	-7.16	27.74	-131.05
4	HJA	189.89	0.99	29.32	-7.42	35.11	-102.13
5	DVA	274.64	0.12	28.03	-7.36	17.13	-179.72
6	WLO	218.31	0.35	28.29	-6.76	15.70	-65.21
7	MER	240.00	0.89	25.99	-6.69	18.06	-114.72
8	IVF	244.10	0.51	26.05	-6.78	30.40	0.08
9	STL	235.90	0.74	26.61	-6.63	24.01	-99.67
10	STN	248.80	-0.52	27.35	-7.11	22.77	-80.85
11	HGV	215.68	0.86	22.65	-5.04	23.71	44.45
12	BSP	229.91	0.72	25.65	-6.31	32.09	25.93
13	RAH	223.13	0.96	24.39	-5.90	23.46	25.12
14	DLF	284.36	-0.81	27.51	-6.34	41.44	-184.36
15	PPL	241.22	0.93	26.01	-5.88	50.19	-42.74
16	DMR	321.21	-0.64	34.72	-6.75	16.96	-103.10

AET stands for cumulative actual evapotranspiration, summer precipitation represents mean summer precipitation, temperature stands for mean minimum temperature, while herbaceous cover describes perennial herbaceous cover layer.

Appendix III. A description of the six selected predictor variables used to test the isolation of environment pattern in the gene flow among the 16 sampled *Ivesia webberi* populations

Predictor name	**Description and source**
Cumulative actual evapotranspiration	Water balance variable calculated from 800 m 1971-2000 PRISM climate normals (Daly et al., 2008), available water capacity (Chaney et al., 2016), and the 1-arcsecond digital elevation model (DEM; USGS, 2017) combined in the Climatic Water Deficit Toolbox for ArcGIS (Dilts 2014, Dilts et al. 2015). AET represents the simultaneous availability of water and energy to support plant productivity.
Minimum monthly temperature	The bioclimatic variables were downscaled from the PRISM climatic data (1970-2001) normals (Daly, Halbleib, Smith, Gibson, Doggett, Taylor, … Pasteris, 2008) from 4 km to 30 m spatial resolution, using BIOCLIM methods (Booth, Nix, Busby, & Hutchinson, 2014) and the climatic water deficit toolbox (Dilts, 2014; Dilts, Weisberg, Dencker, & Chambers, 2015)
Summer precipitation	
Cosine aspect	Cosine aspect was calculated in ArcMap version 10.6.1 using USGS (2017) DEM and the slope layers, the formula: $= \theta \times \cos(\alpha)$, where $= \theta$ is slope (in percentage), and α is aspect (in radians)

Topographic position index	Calculated from USGS (2017) DEM in ArcMap version 10.6.1, using formula introduced by Weiss (2001), and a 333 m neighborhood. At 333 m scale, the landscape is classified into either a valley or a mountain range
Perennial herbaceous cover	It is a vegetation type raster layer sourced from the Multi-Resolution Land Characteristics (MRLC) development of the U.S. National Land Cover Database (NLCD) 2016 Shrub component products (Xian, Homer, Meyer, & Granneman, 2013)

References

Booth, T. H., Nix, H. A., Busby, J. R., & Hutchinson, M. F. (2014). BIOCLIM: the first species distribution modelling package, its early applications and relevance to most current MaxEnt studies. *Diversity and Distributions*, **20**(1), 1-9. https://doi.org/10.1111/ddi.12144

Chaney, N. W., Wood, E. F., McBratney, A. B., Hempel, J. W., Nauman, T. W., Brungard, C. W., & Odgers, N. P. (2016). POLARIS: A 30-meter probabilistic soil series map of the contiguous United States. *Geoderma*, **274**, 54–67. https://doi.org/10.1029/2018WR022797

Daly, C., Halbleib, M., Smith, J. I., Gibson, W. P., Doggett, M. K., Taylor, G. H., Curtis, J., & Pasteris, P. A. (2008). Physiographically-sensitive mapping of temperature and precipitation across the conterminous United States. *International Journal of Climatology,* **28**(15), 2031-2064. https://doi.org/10.1002/joc.1688

Dilts, T. E., Weisberg, P. J., Dencker, C. M., & Chambers, J. C. (2015). Functionally relevant climate variables for arid lands: a climatic water deficit approach for modelling desert shrub distributions. *Journal of Biogeography*, **42**(10), 1986-1997. https://doi.org/10.1111/jbi.12561

Dilts, T.E. (2014). Climatic Water Deficit Toolbox for ArcGIS 10.1. University of Nevada Reno. Available at: http://www.arcgis.com/home/item.html?id=de9ca57d43c041148b815da7ce4aa3a0

U.S. Geological Survey [USGS] (2017). 1 Arc-second Digital Elevation Models (DEMs) - USGS National Map 3DEP Downloadable Data Collection. U.S. Geological

Survey. Accessed at https://www.usgs.gov/core-science-systems/ngp/tnm-delivery/

Weiss, A. (2001). Topographic position and landform analysis. Poster presentation, ESRI User Conference, San Diego, California, USA.

Xian, G., Homer, C., Meyer, D., & Granneman, B. (2013). An approach for characterizing the distribution of shrubland ecosystem components as continuous fields as part of NLCD. *ISPRS Journal of Photogrammetry and Remote Sensing*, **86**, 136-149. https://doi.org/10.1016/j.isprsjprs.2013.09.009

Appendix IV. Feature reduction process for the ecological predictor variables

A total of 72 predictor variables describing edaphic, topographic, land cover, vegetative cover, climatic and biotic factors were assembled for fitting niche models for *Ivesia webberi*. However, in order to avoid multicollinearity, the predictor variables were reduced to six uncorrelated predictors using a combination of Kendall r correlation coefficient, and feature selection runs in *Boruta* R package (Kursa & Rudnicki, 2010) and recursive feature elimination algorithm in *caret* R package (Kuhn, 2008). The six selected predictor variables are described in the Tables S1 and S2 below.

References

Kursa, M. B., & Rudnicki, W. R. (2010). Feature selection with the *Boruta* package. *Journal of Statistical Software,* **36**(11), 1-13. 10.18637/jss.v036.i11

Kuhn, M. (2008). Building Predictive models in R using the *caret* package. *Journal of Statistical Software*, **28**(5), 1-26. 10.18637/jss.v028.i05

Appendix V. Floristic richness and diversity in the aboveground and the soil seed bank of the 10 *Ivesia webberi* sampled populations (adapted from Borokini et al., 2021)

Unit*	Site name	Code	Aboveground community		Soil seed bank	
			Species richness	Species diversity	Species richness	Species diversity
2	Constantia	CST	18	3.4109	18	1.3749
3	Evans Canyon, East of Hallelujah junction wildlife area (HJWA)	EHJ	20	3.5312	14	1.7979
5	Dog Valley meadow	DVA	34	12.3489	14	8.7159
6	White Lake overlook	WLO	26	10.3199	16	1.7157
7	Mules Ear Flat	MER	26	4.0713	16	2.3309
8	Ivesia flat	IVF	22	6.2757	16	3.0484
11	Hungry valley	HGV	24	1.8893	21	3.0377
12	Black springs	BSP	25	3.6016	20	1.3293
13	Raleigh heights	RAH	20	6.2627	18	1.7913
16	Dante Mine Road	DMR	21	8.8097	16	2.6453

*Unit follows the unit number assigned by US Fish and Wildlife Service (USFWS); Species diversity of the exponent conversion of Shannon-Weiner H’ index into natural numbers (effective number of species; Jost 2006)

References

Borokini I.T., Weisberg P.J., and Peacock M.M. (2021). Quantifying the relationship between soil seed bank and plant community assemblage in sites harboring the threatened *Ivesia webberi* in the western Great Basin Desert. *Applied Vegetation Science* 24(1): e12547 https://doi.org/10.1111/avsc.12547

Jost L. (2006). Entropy and diversity. *Oikos* 113(2): 363–375. https://doi.org/10.1111/j.2006.0030-1299.14714.x

Appendix VI. Number of individuals genotyped (N), number of alleles observed (A), allelic richness (R_S), expected (He) and observed (Ho) heterozygosities, and the inbreeding coefficient (F_{IS}) for each locus across the 16 *Ivesia webberi* populations. F_{IS} values with asterisk are statistically significant (adjusted $P = 0.00052$, based on 1920 randomizations). NA means no analysis.

Locus		SVE	CST	EHJ	HJA	DVA	WLO	MER	IVF	STL	STN	HGV	BSP	RAH	DLF	PPL	DMR
PMS 1080	N	20	12	24	13	20	13	11	16	8	9	22	18	23	14	13	20
	A	2	1	2	2	2	3	3	2	2	2	2	2	2	1	1	2
	R_S	1.35	1.00	2.00	1.92	1.97	2.34	2.64	1.44	2.00	2.00	1.54	1.63	1.52	1.00	1.00	1.74
	H_E	0.05	0	0.454	0.212	0.296	0.218	0.481	0.063	0.5	0.294	0.089	0.108	0.085	0	0	0.142
	H_O	0.05	0	0.667	0.231	0.15	0.231	0.636	0.063	0.75	0.333	0.091	0.111	0.087	0	0	0.15
	F_{IS}	0	NA	-0.48	-0.09	0.5	-0.06	-0.35	0	-0.56	-0.14	-0.02	-0.03	-0.02	NA	NA	-0.06
PMS 1180	N	21	19	24	18	22	22	19	20	9	12	24	18	23	17	17	22
	A	4	3	3	4	4	4	4	5	4	4	5	4	4	6	3	2
	R_S	2.60	1.98	2.47	3.56	3.28	3.23	3.14	3.90	4.00	3.52	3.45	3.27	2.82	3.71	2.31	2.00
	H_E	0.331	0.2	0.377	0.6	0.659	0.593	0.44	0.64	0.791	0.659	0.592	0.602	0.503	0.414	0.266	0.46
	H_O	0.286	0.2	0.375	0.5	0.591	0.545	0.211	0.8	0.889	0.333	0.542	0.5	0.522	0.471	0.235	0.409
	F_{IS}	0.14	-0	0.005	0.171	0.105	0.082	0.528	-0.26	-0.13	0.506	0.087	0.173	-0.04	-0.14	0.117	0.113
PMS 1438	N	21	18	24	15	22	22	20	20	9	13	24	18	23	19	17	23

	A	2	3	2	3	2	2	2	2	3	2	2	2	2	2	2	2
	R_S	2	2.4	2	2.724	2	2	2	2	2.778	2	2	2	2	2	2	2
	H_E	0.512	0.5	0.507	0.559	0.511	0.512	0.513	0.513	0.582	0.517	0.511	0.513	0.507	0.491	0.515	0.511
	H_O	1	0.9	0.917	0.867	0.955	1	1	1	1	0.923	1	0.944	0.913	0.789	1	1
	F_{IS}	***-1***	-0.7	***-0.84***	-0.58	***-0.91***	***-1.0***	***-1.0***	***-1.0***	-0.8	-0.85	***-1***	***-0.89***	***-0.83***	-0.64	***-1.0***	***-1.0***
PMS 1665	*N*	20	20	24	18	22	21	19	19	9	13	24	18	21	19	16	23
	A	3	1	1	1	3	4	3	3	1	1	1	1	4	2	2	1
	R_S	2.94	1.00	1.00	1.00	1.64	3.19	2.61	2.60	1.00	1.00	1.00	1.00	2.23	1.37	1.44	1.00
	H_E	0.586	0	0	0	0.09	0.517	0.494	0.448	0	0	0	0	0.182	0.053	0.063	0
	H_O	0.5	0	0	0	0.091	0.476	0.632	0.368	0	0	0	0	0.143	0.053	0.063	0
	F_{IS}	0.15	NA	NA	NA	-0.01	0.08	-0.29	0.182	NA	NA	NA	NA	0.221	0	0	NA
PMS 1694	*N*	21	20	24	18	22	19	19	20	9	13	24	18	23	18	18	23
	A	3	3	2	3	3	4	3	3	4	4	3	3	4	4	3	2
	R_S	2.84	2.58	1.95	2.71	2.23	2.69	2.46	2.35	3.52	3.34	2.29	2.32	2.83	2.74	2.35	1.30
	H_E	0.459	0.5	0.284	0.379	0.212	0.36	0.284	0.512	0.399	0.606	0.529	0.294	0.544	0.376	0.332	0.043
	H_O	0.429	0.1	0.333	0.333	0.227	0.421	0.316	0.8	0.333	0.308	0.5	0.222	0.522	0.111	0.167	0.043
	F_{IS}	0.067	***0.89***	-0.18	0.124	-0.07	-0.18	-0.11	-0.59	0.172	0.503	0.056	0.249	0.042	0.711	0.505	0

PMS 2190	*N*	15	19	18	17	21	20	16	15	7	10	22	18	20	16	16	23
	A	2	2	3	3	2	3	3	2	1	1	2	2	3	2	2	2
	R_S	1.72	1.37	2.17	2.65	1.72	2.19	2.43	2.00	1.00	1.00	2.00	1.63	2.48	1.69	1.69	1.30
	H_E	0.129	0.1	0.208	0.426	0.136	0.229	0.433	0.434	0	0	0.495	0.108	0.309	0.121	0.121	0.043
	H_O	0.133	0.1	0.222	0.529	0.143	0.25	0.563	0.6	0	0	0.818	0.111	0.35	0	0.125	0.043
	F_{IS}	-0.04	0	-0.07	-0.25	-0.05	-0.09	-0.31	-0.4	NA	NA	-0.68	-0.03	-0.14	1	-0.03	0

CHAPTER 4 Potentially adaptive significance of genome size diversity across ecological gradients and seed size in *Ivesia* (Rosaceae)*

Israel T. Borokini[1*], Shaun R. Broderick[2], Zhi Gao[3], James A. Birchler[3], Joshua M. Hallas[1], Mary M. Peacock[1,4]

[1]Program in Ecology, Evolution and Conservation Biology, Department of Biology, University of Nevada, Reno, Reno NV 89557, United States of America

[2]Mississippi Agricultural and Forestry Experiment Station (MAFES) Truck Crops Branch, Mississippi State University, Crystal Springs MS 39059, United States of America

[3]Division of Biological Sciences, University of Missouri, Columbia MO 65211, United States of America

[4]Department of Biology, University of Nevada, Reno, Reno NV 89557, United States of America

*Submitted to *Annals of Botany*

ABSTRACT

Background and Aims: Variation in genome-size across taxa have been explained using neutral and non-adaptive theories, however genome size variation among taxonomic groups can also be shaped by natural selection and have adaptive value if it correlates with other plant functional traits. Currently, little work has been published on the genome size of *Ivesia* and its evolutionary history in the desert ecosystems in western North America. Here, we estimated the genome sizes of 33 taxa, including 31 *Ivesia* taxa and additional two taxa from *Potentilla* and one from *Horkelia*, and determined if the genome sizes have an adaptive value.

Methods: We collected leaf samples from 31 of *Ivesia*'s 38 taxa, in addition to two taxa from the closely related *Potentilla* and one from *Horkelia* genera. Using flow cytometry, we obtained genome size estimates from every accession. We investigated intraspecific variation of genome size within 11 *I. webberi* populations. We also tested the predictions of the nucleotype theory, which states that genome size has adaptive significance.

Key Results: All *Ivesia* taxa were diploid ($2n$=2x=28 chromosomes), despite an 8.1-fold variance in genome size, ranging from 0.73 pg/2C in *I. baileyi* var. *beneolens* to 5.91 pg/2C in *I. lycopodioides* ssp. *megalopetala*. Only six taxa, with a genome size >1.5 pg/2C, significantly differed from the remaining taxa. We also observed a longitudinal gradient in genome size in *Ivesia*; genome size was significantly correlated with seed size and actual evapotranspiration both within *I. webberi* and among *Ivesia* taxa. *I. webberi* populations near the species range centre have the largest genomes, which reduces towards the marginal populations.

Conclusions: Seed size and actual evapotranspiration correlate with smaller genomes found among desert-dwelling *Ivesia*, which supports the predictions of the nucleotype theory and suggests an adaptive significance of genome size in the genus. Furthermore, intraspecific genome size variation in *I. webberi* could be attributed to gene flow and other evolutionary processes. However, low phylogenetic resolution limits inferences on evolutionary events in the genus.

Keywords: nucleotype theory, genome size, flow cytometry, adaptive radiation, ploidy, genome size constancy hypothesis, genome size evolution, central-marginal hypothesis, functional traits, actual evapotranspiration, *Ivesia*, Rosaceae, North America.

INTRODUCTION

Genome size (GS) is the total amount of DNA in an organism's unreplicated haploid nucleus (Greilhuber *et al.*, 2005). Up to 2,400-fold variation in genome size has been reported among angiosperms (Pellicer *et al*., 2018), while variation among intraspecific accessions is generally much lower (Huang *et al.*, 2013; Pascual-Díaz *et al.*, 2020). GS data have been used in genotyping studies (Garner, 2002), and can also be integrated into a phylogenetic tree to investigate the relationship between GS and speciation (e.g., Leitch *et al.*, 2010; Burleigh *et al.*, 2012). Previous studies have demonstrated a significant and positive relationship between the rates of genome size evolution and species diversification at the family and subfamily levels in angiosperms (e.g., Jiao *et al.*, 2011; Puttick *et al.*, 2015; Soltis and Soltis, 2021; but see Bromham *et al.*, 2015). Furthermore, closely related taxa have been shown to have relatively small differences in genome sizes (Loureiro *et al.*, 2010; Lanfear *et al.*, 2014), while ancestral taxa were reported to have smaller genomes in some plant families (Leitch *et al.*, 2005; Du *et al.*, 2017). Conversely, genome size reduction following speciation has been observed in other plant groups indicating that both ancestral and young taxa can have smaller genomes (Baniaga *et al.*, 2016). Overall, studies suggest a multi-directional role of GS on species diversification among plant clades, which is driven by the rates and mechanisms of GS evolution, not changes in the absolute GS (Kraaijveld, 2010; Puttick *et al.*, 2015; Pellicer *et al.* 2018).

The mechanisms of GS evolution have been explained using different hypotheses that describe GS evolution as either neutral, maladaptive, or adaptive (Whitney *et al.*, 2010). The mutational equilibrium hypothesis (MEH), a neutral model of GS evolution, posits that GS variations are a result of the balance between insertions and deletions into the genome (Petrov, 2002), while the mutational hazard hypothesis (MHH), a maladaptive and nearly neutral model, states that excess DNA is slightly deleterious and will be removed by selection in populations and species with a larger effective population size (*N*e) but are fixed by genetic drift in taxonomic units with smaller *N*e, thus resulting in a larger GS (Lynch and Conery, 2003). Adaptive models such as the nucleotype theory (Bennett, 1976) and nucleoskeletal hypothesis (Cavalier-Smith, 2005) posits that GS evolution can influence functional phenotypic traits that support species life history strategy, physiology, and geographical distribution. The nucleoskeletal hypothesis suggests that plants with larger GS are selected against in environments with low nitrogen and phosphorus (Bales and Hersch-Green, 2019) because these elements are important building blocks of nucleotides. Empirical evidence of a correlation between GS and plant functional traits such as nucleus weight, RNA and protein content, cell size, flower and fruit size, and minimum generation time (Bennett, 1987), seed size (Beaulieu *et al.,* 2007), guard cell and epidermal cell sizes (Beaulieu *et al.,* 2008; Hoang *et al.,* 2019), stomata size (Jordan *et al.,* 2015), photosynthetic rates (Roddy *et al.,* 2020), and mitotic cell cycle duration (Hodgson *et al.,* 2010) support the predictions of the nucleotype theory. Conversely, selection against larger genomes has been reported in plants in harsh environments, higher altitudes, and latitudes (Knight *et al.*, 2005; Guignard *et al.*, 2016). Empirical studies testing the predictions of these hypotheses are equivocal (Oliver *et al.*, 2007; Whitney *et al.*, 2010; Schrider *et al.*, 2013; Leushkin *et al.*, 2013; Bilinski *et al.*, 2018). These GS evolution models are best tested in species with intraspecific GS variation (Stelzer *et al.*, 2019; Blommaert, 2020), and congenerics with shared evolutionary history such as those that originated from adaptive radiations.

Ivesia Torrey and A. Gray (Rosoideae, Rosaceae) is a genus distributed in the western United States and northern Mexico and contains 30 described perennial forb species, which are further divided into eight subspecific taxonomic groupings (Ertter, 1989). The ancestral species of this genus is thought to have originated from the eastern Great Basin and the western slopes of the Rocky Mountains. From there, it spread westward across the prehistorically wet Basin and Range region of western United States. The collapse of the Nevadaplano caused valleys to drop in the Great Basin Desert. The reduced elevation of the valleys relative to the Sierra Nevada Mountains created a rain shadow effect, leading to arid conditions across the Great Basin Desert (Cassel *et al.,* 2009). Climatic changes and topographic, geologic and edaphic heterogeneity (resulting from these geological transformations) may have led to the extinction of some ancestral *Ivesia* taxa. Many of the remaining taxa are now restricted to mountain, hydrologic, and edaphic habitat islands (Ertter, 1989), which may have facilitated *in situ* speciation and adaptive radiations of new *Ivesia* taxa (Töpel *et al.*, 2012). Therefore, this genus is composed of both surviving ancient taxa and relatively young radiations, many of which exhibit substrate specialization (Kraft *et al.,* 2010; Ertter and Reveal, 2014).

The evolutionary history of the taxa in *Ivesia* and their geographical distribution in arid environments make them an excellent choice for testing the adaptive or maladaptive effects of GS evolution. This study investigates variation in genome size within and among *Ivesia* taxa. Here, we considered minimum-rank taxa (MRT), which are the smallest taxonomic units (including subspecies and varieties), as individual taxa. Using the genome sizes obtained from all sampled *Ivesia* taxa (in addition to two *Potentilla* spp., and one *Horkelia* sp., which were included to serve as reference taxa from closely related genera [Table 1]), we asked the following questions: (1) is there a relationship between genome size and environmental variables and seed size according to the predictions of the nucleotype theory? (2) Did genome size increase in *Ivesia* as species radiated out in a westward direction from the hypothesized origin of the ancestral *Ivesia* taxa? (3) Does intra-specific genome size variation follow the genome size constancy hypothesis (Greilhuber *et al.*, 2005)? We also built a phylogenetic tree with chloroplast and nuclear DNA sequences available from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) to assess our ability to detect evolutionary signals in the GS variations among *Ivesia*. Furthermore, we made an inference on the ploidy of the sampled *Ivesia* taxa using flow cytometry and karyotypic data. Based on previous studies that showed that ancestral taxa have relatively small genome sizes (e.g., Du *et al.*, 2017), we predict that ancestral *Ivesia* taxa would have smaller genomes and thus expect a westward increase in genome sizes of the sampled *Ivesia* taxa.

MATERIALS AND METHODS

Study species and field sampling

Thirty-one out of 38 taxa (Table 1), representing 82% of all *Ivesia* taxa across the western United States (Table 1, Figure 1, see also Supplemental Table S1), were sampled for flow cytometric analysis. These taxa grow primarily on the soil, but 12 of them are chasmophytes, growing on rocky outcrops (Table 1). The substrates on which these taxa grow have either volcanic or limestone origin, while two grow on alkali flats (Table 1). Based on previous studies documented in the Flora of North America, 15 *Ivesia* taxa were reported to have 2*n*=28 chromosomes (Ertter and Reveal, 2014). Many of the species are found in disjunct populations growing on specific substrates, restricted to the mountainous sky islands, and are suspected to be relics of the ancestral *Ivesia*.

Fresh leaf samples were collected from six individuals from one population for each of the sampled *Ivesia* taxa. In a few taxa such as *I. webberi* (Figure 2), *I. lycopodioides* var. *megalopetala* and *Horkelia fusca* var. *parviflora*, samples were collected from multiple populations. Leaves from each plant were stored between moist tissue paper in a labelled plastic bag and shipped to the Benaroya Research Institute (Seattle, Washington State, United States) for analysis. In all cases, the samples arrived at the laboratory within 48 hours and were prepared for flow cytometry. Flow cytometric analysis was conducted for only fresh, intact samples; degraded samples were excluded (See Table 1). Field collections were made between June 2018 and November 2019.

Flow cytometry

Flow cytometry is a high throughput and non-destructive method for determining the nuclear genome size with applications in systematics, ploidy level estimation, and the base composition (proportion of AT-GC base pairs) of the genome (Kron *et al.*, 2007). Flow cytometric analysis was conducted using the following protocol (Arumuganathan and Earle, 1991). Each 50 mg of leaf tissue samples were separately ground in $MgSO_4$ buffer, mixed with DNA of an internal reference standard, and stained with propidium iodide (PI) in a solution containing DNase-free-RNase to remove any RNA from the sample. Nuclei from chicken red blood cells (2.5 pg./2C; Biosure Inc., Grass Valley CA; CEN singlets catalogue #1013) were used as internal reference standards. The amount of nuclear DNA was estimated by comparing fluorescence intensities of the stained nuclei of *Ivesia* leaf samples with those of the reference standard using a FACScalibur flow cytometer (Becton-Dickinson, San Jose, CA). For each taxon, 10,000 counts of PI-stained nuclei from three samples were recorded and analysed using the CellQuest software (Becton-Dickinson, San Jose, CA). The mean position of the G0/G1 nuclei peak of the sample and the internal standard was also determined using CellQuest software. Only histograms with coefficients of variation (CVs) of the G0/G1 peak of the analysed sample below 5% were considered (Dušková *et al.*, 2010). Samples that did not produce histograms with double peaks for both internal standard and the *Ivesia* taxa were excluded (Greilhuber *et al.*, 2005).

Karyotyping

Chromosome analysis was conducted using a 4’,6-diamidino-2-phenylindole (DAPI) stain, as described in Findley *et al.* (2010). Root tips were obtained from freshly germinated *I. webberi* seeds and digested in nitrous oxide to stop cell division at metaphase. Meristematic tissues from seven root tips were fixed in 100% acetic acid, mounted on a slide, and viewed under the microscope. Images were taken at 100× with an Olympus BX61 using FISHView EXPO 4.5 software (Applied Spectral Imaging) and a Cool-1300QS CCD camera.

Phylogenetic analysis

We acquired all available DNA sequences from chloroplast, mitochondria and nuclear loci of *Ivesia* and *Horkelia fusca* ssp. *parviflora* from GenBank. We used both nuclear (ETS and ITS) and chloroplast (matK and trnL) sequences for the phylogenetic analysis (Supplemental Table S2). To limit noise within our phylogenetic estimate, only one individual per taxon that had at least two sequences from the same individual were analysed. *Potentilla chinensis* was chosen as an outgroup because it has been shown to have a close phylogenetic relationship with the ivesoid clade (Eriksson *et al.*, 2003; Potter *et al.*, 2007; Sun *et al.*, 2016). We aligned and edited fragments using E-INS-i algorithm in MAFFT (Katoh *et al.*, 2009) in Geneious Pro v. 9.1.8 (https://www.geneious.com). We concatenated and partitioned our alignment by the genetic sequence for phylogenetic analysis. We took a maximum likelihood approach to generate our phylogenetic estimate using RAxML v 8.2.12 (Stamatakis, 2006). We set our evolutionary model to GTR-I^{-} and executed 2×10^{4} fast-bootstrapping runs. A bootstrap threshold of ≥ 50 was interpreted as relationships having significant support (Hillis and Bull, 1993).

Data analysis

We fitted a fixed-effects model to determine statistical differences in the nuclear genome size of all sampled *Ivesia* taxa and the representative samples from *Potentilla* and *Horkelia* genera. Geographical patterns in genome size variation in *Ivesia* were investigated using the Kendall r correlation of the longitudinal degrees and the genome size, and with spatial autocorrelation in the genome size of the 31 sampled *Ivesia* taxa using Mantel *r* Kendall correlation. The range of each taxon was estimated in ArcGIS version 10.7.1 using the convex hull function. Furthermore, to assess a relationship between genome size in *Ivesia* and the selected environmental factors, which included elevation, actual and potential evapotranspiration (AET and PET respectively), climatic water deficit (CWD), substrate type (soil or rock), and substrate geology, we fitted a generalized linear mixed model, accounting for species as a fixed effect. We also investigated the relationship between genome and mean seed sizes of taxa for which seeds were available, using linear regression.

Similar analyses were conducted on samples collected from 11 *Ivesia webberi* populations. A fixed-effects model was used to investigate the predictions of genome size constancy within a species. Genome sizes of sampled *I. webberi* individuals were modelled as a function of the representative populations. We also checked for spatial autocorrelation (Mantel's *r*) and fitted generalized linear mixed-effects models to test for the effect of elevation, AET, PET, CWD, and seed size on nuclear DNA content of *I. webberi* samples, using populations as a fixed effect. To test if genome size in *I. webberi* populations is structured along the central-marginal hypothesis (CMH), we tested for correlation between population genome size and distance from the range centre of *I. webberi*. Distance from the species' range centre was computed as the latitudinal range central index (Enquist *et al.*, 1995; Fenberg and Rivadeneira, 2011).

RESULTS

Genome size variation among *Ivesia* taxa

Genome size varied widely among the sampled *Ivesia* taxa, ranging between 0.73 pg/2C in *I. baileyi* var. *beneolens* to 5.91 pg/2C in *I. lycopodioides* var. *megalopetala* (Table 1). Genome size varied across the sampled taxa (F=510.2, *P*<0.01), but Tukey post-hoc pairwise comparisons showed that genome sizes of six *Ivesia* taxa including *I. lycopodioides* ssp. *megalopetala, I. lycopodioides* var. *scandularis, I. shockleyi* var. *shockleyi, I. aperta* var. *aperta*, *I. aperta* var. *canina*, and *I. sericoleuca,* with ≥1.8 pg/2C were significantly different (adjusted *P*<0.01) from the remaining taxa. Additionally, we observed overlap in genome size (0.8 pg/2C-1.3 pg/2C) for multiple *Ivesia* taxa (Table 1).

When compared to the reference samples from *Potentilla* and *Horkelia* genera, the second logistic regression model (F=506.6, *P*<0.01) and post-hoc test showed that *P. gracilis* var. *fastigiata* was different from all sampled *Ivesia* taxa (adjusted *P*<0.01), but *Potentilla* sp. and *H. fusca* ssp. *parviflora* were different from only 13 and 12 *Ivesia* taxa (adjusted *P*<0.01), respectively.

Geographical gradients in *Ivesia* genome size variations

We observed an inverse but nonsignificant relationship between genome size in *Ivesia* and longitude (Kendall *r*=-0.08, *P*=0.53), which indicates a westward increase in genome size. The six taxa with the largest genome sizes were restricted to the western edge of the range of the genera in or near the Sierra Nevada Range but a few taxa, such as *I. callida* endemic to the Sierra Nevada also had smaller genomes (Table 1). There was no spatial autocorrelation (Mantel's *r*=-0.15, *P*>0.99) in genome size variation among *Ivesia* taxa.

Genome size and the environment

Ivesia taxa growing on the soil have larger genome sizes in general than chasmophytic *Ivesia* (Fig. 3a) however, this difference was not significant (*P*>0.05). Similarly, the geology of the substrates on which the sampled *Ivesia* taxa grow has no significant effect on their genome size (Fig. 3b). *Ivesia* with larger genomes produced relatively large seeds (Fig. 3c) and are found in higher elevation habitats (Fig. 3d). Furthermore, *Ivesia* taxa with larger nuclear genomes were located in areas with lower AET (Fig. 3e). Also, potential evapotranspiration (Fig. 3f) and climatic water deficit (Fig. 3g) have no significant relationship with genome size in *Ivesia*. However, regression models show a significant relationship between genome size in *Ivesia* taxa and seed size (GLM; t=2.66, *P*<0.05), elevation (GLMM; z=4.74, *P*<0.001) and actual evapotranspiration (GLMM; z=-2.06, *P*<0.05).

Intraspecific genome size variation and chromosome count of *Ivesia webberi*

To measure intraspecific genome size variation, we analysed 11 *I. webberi* populations (See Figure 2). Genome size variation was significantly and positively correlated with the distance from the species' range centre (Pearson's *r*=0.65, *P*=0.03). Thus, genome size was the lowest in population unit 16, an isolated population, and highest in unit 8, a centrally located population (Figure 4). However, there was positive but weak and nonsignificant spatial autocorrelation (Mantel's *r*=0.2, *P*=0.07) in genome size variations across sampled *I. webberi* populations. A post-hoc pairwise test on the fixed-effect model showed that the genome size of samples from *I. webberi* populations in unit 16 was different from six other populations (units 2, 3, 5, 6, 7 and 8; adjusted

P<0.01). Unit 8 was also different from three populations (units 11, 12 and 15; adjusted *P*<0.01), while unit 15 was different from units 6 and 7 (adjusted *P*<0.01).

Sampled *I. webberi* populations with larger genome sizes were located in higher elevation sites (Fig. 5a), lower AET (Fig. 5b), lower PET (Fig. 5c), but slightly higher climatic water deficit (Fig. 5d), and they produced larger seeds (Fig. 5e). However, genome size variation across these 11 *I. webberi* populations had a significant negative relationship with AET (GLMM; z=-2.181, *P*<0.05), and a significant linear relationship with seed size (GLM; t=2.43, *P*<0.05).

Karyotyping showed that *I. webberi* had 28 chromosomes, which is similar to karyotyping results of other *Ivesia* taxa as reported in the Flora of North America. Furthermore, since the ploidy level of some of the taxa is already known (Table 1), it could be inferred from the genome size that all of the 31 sampled taxa are diploid with *2n*=2x=28 chromosomes.

Phylogenetic analysis

Nuclear and chloroplast sequences were used to estimate a phylogeny for comparison with the genome size variation. The edited and concatenated alignment length was 4,670 bp, of which only 55 were parsimony-informative. Phylogenetic relationships among *Ivesia* were not well supported (bootstrap values ≤50), and the topology of the phylogenetic estimate recovered many polytomies (Supplemental Figure S1). Due to this lack of resolution and confidence in our estimate, we could not use the tree for further analysis with the genome size results.

DISCUSSION

This study is the first to report on the genome size variation in *Ivesia*. An 8.1-fold variation in genome size was observed among the sampled *Ivesia* taxa, ranging from 0.73 pg/2C to 5.91 pg/2C; however, based on Leitch *et al.* (1998) GS classifications (small: 1C < 1.40 pg, large: 1C > 14 pg, and very large: 1C > 35 pg genomes), all of the sampled *Ivesia* taxa have relatively small genomes. Despite the GS variation, all sampled *Ivesia* taxa are believed to be diploid with 28 chromosomes. Relatively small genome sizes ranging from 0.2 pg/2C to 3.09 pg/2C have been reported for other taxa in Rosaceae, but a genome size of 5.91 pg/2C for *I. lycopodioides* var. *megalopetala* is the largest for any taxa in the *Potentilleae* Tribe, and among the largest reported genome size for diploid taxa in Rosaceae (Zonneveld *et al.*, 2005). Smaller genomes prevalent among the desert-dwelling *Ivesia* taxa support adaptive models of GS evolution that predicts selection against larger genomes in marginal ecosystems. Previous but few studies also demonstrated smaller genomes among C_3 xerophytes and associated this with faster metabolic rates (Sliwinska *et al.*, 2009; Roddy *et al.*, 2020). Further support for this was found in the significant relationship of GS with actual evapotranspiration (AET) for both the sampled *I. webberi* populations and the 31 *Ivesia* taxa. *Ivesia lycopodioides* varieties, which have the largest genomes, were found in areas with the lowest AET. *I. lycopodioides ssp. megalopetala* is particularly found in wet alpine meadows, which have higher water availability and lower temperatures, and consequently, lower AET. In comparison, *I. pickeringii* and *I. longibracteata* for example, were sampled in locations with the highest AET, both of which also have relatively small genome sizes. The significant association of larger genome sizes in sampled *Ivesia* taxa located in habitats with lower AET suggests that genome size variation in *Ivesia* may have adaptive significance, thus supporting the predictions of the nucleotype theory (Bennett, 1987). Moreover, previous studies have reported a positive relationship between GS and traits associated with transpiration (Jordan *et al.*, 2015) and other ecological gradients (Kang *et al.* 2014; Du *et al.*, 2017).

Generally, speciation events are associated with genome size evolution resulting from whole genome duplications, changes in non-coding repetitive DNA, transposable elements, introns, and gene duplications (Du *et al.*, 2017; Vitales *et al.*, 2019). Even though we observed a westward but nonsignificant increase in genome size in *Ivesia*, phylogenetic analysis would be needed to identify geographic gradients in speciation events and to test the hypothesis that ancestral *Ivesia* taxa originated from the eastern edge of the range of the genera in the western slopes of the Rocky Mountains and eastern Great Basin Desert (Ertter and Reveal, 2014). All of the sampled *Ivesia* with significantly larger GS were distributed near or in the Sierra Nevada Range, which is the western edge of the genus geographic range. In plant lineages where speciation events correlate with genome size increases, ancestral taxa were observed to have smaller genomes (Soltis *et al.,* 2003; Garnatje *et al.,* 2007; Šmarda *et al.*, 2008; Du *et al.*, 2017). Our attempt to further corroborate this hypothesis with phylogenetic analysis was not successful. The molecular markers used in this study lack the phylogenetic signal to resolve relationships among *Ivesia* (Eriksson *et al.,* 1998, 2003; Töpel *et al.,* 2011, 2012). Consequently, our estimates and previous phylogenetic estimates reported for *Ivesia*, *Potentilleae* Tribe and

Rosaceae (Eriksson *et al.,* 2003; Potter *et al.,* 2002, 2007; Dobeš and Paule, 2010; Töpel *et al.,* 2011, 2012; Feng *et al.,* 2017) resulted in uninformative polytomies and low bootstrap node support. However, GS variations have been successfully used with a phylogenetic analysis of several taxonomic groups, including monocotyledonous plants (Leitch *et al.,* 2010), *Lilium* (Du *et al.*, 2017), Asteraceae (Vitales *et al.,* 2019), and bryophytes (Bainard *et al*., 2020). The limitations on genus-level phylogeny for *Ivesia* may be due to added noise in taxon sampling and fewer variable regions in the currently available sequences (Sanmartín and Meseguer, 2016; Hallas *et al.,* 2017). Therefore, next-generation sequencing methodologies that access more of the genome are needed to resolve phylogenetic relationships within this genus.

Nine of the 11 *I. webberi* populations showed nonsignificant genome size variations, which may support the genome size constancy hypothesis (Greilhuber *et al.*, 2005). The genome size constancy hypothesis predicts that GS is conserved within a species but varies significantly among congenerics (Greilhuber *et al.*, 2005). Populations close to the latitudinal range centre of the species (units 6, 7, and 8) have relatively large GS, while the most geographically isolated population (unit 16) has the smallest genome. Intraspecific *I. webberi* GS variation is congruent with previous studies, which show that small and isolated populations should have smaller GS because they have no way of receiving novel transposable elements proliferating within the genome (Wright and Schoen, 1999; Morgan, 2001). Additionally, geographical patterns of intraspecific GS variations in *I. webberi* support the central marginal hypothesis, which predicts that genetic diversity may be the highest in the populations of a species near the geographical centre of its global distribution and decreases towards the marginal population (Eckert *et* *al.,* 2008). Geographical patterns in intraspecific genome size variation have been reported earlier in other plant species (Bottini *et al.,* 2000; Mráz *et al.,* 2009). Regardless, the relationship between marginality and intraspecific variation of genome size in plants has not been well studied. A reduction in transposable elements in isolated populations of *Aegilops speltoides* (Hosid *et al.,* 2012) and *Veronica* (Albach and Greilhuber, 2004) have been reported, while populations with high gene flow levels were reported to show small genome size variations (Díez *et al.*, 2013). This suggests that gene flow may play an important role in intraspecific GS variations. However, studies also show that GS variations within a species could be attributed to the nucleotype effect due to variations in prevailing environmental conditions across the populations of a species (Šmarda and Bureš, 2010) and varying selective pressures (Bilinski *et al*., 2018).

This study showed that seed size increases with increasing genome size, supporting the predictions of the nucleotype theory. This is congruent with previous studies that show a positive correlation between GS and seed size (Krahulcová *et al.,* 2017). The molecular divergence of seed size was found to be positively correlated with genome size diversification (Beaulieu *et al.,* 2007), both of which play a positive and significant role in speciation (Puttick *et al.,* 2015; Igea *et al.,* 2017). Moreover, GS influences physiological developments that determine seed size in many plant species (Beaulieu *et al.*, 2007). Seed size is correlated with the mode of dispersal, seed persistence in soil bank, seedling survival, plant growth form and specific leaf area, among others (Westoby *et al.,* 1996), and could be used as an indicator of habitat quality and environmental stress (Loureiro *et al.,* 2010). Seed size was also shown to affect plant fitness, population dynamics, and interactions with seed predators (Gómez, 2004; DeSoto *et al.*, 2016). The possible adaptive values of GS in *Ivesia* and its association with functional traits such as the seed size can elucidate eco-evolutionary dynamics in the genus (Vitales *et al*., 2019).

We demonstrated in this study that genome size variation in *Ivesia* and within *I. webberi* is potentially adaptive, given their relatively small GS, and correlation with energy availability and seed size. Furthermore, GS variation in *Ivesia* taxa is structured along an elevational gradient which may also be correlated with bioclimatic variables. Similar GS trends within *Ivesia webberi* further strengthens a potential adaptive significance of genome size evolution. Moreover, larger GS of the sampled *I. webberi* populations close to the species known range suggest that evolutionary processes such as gene flow or natural selection may be influential on genome size variation within the species. It is believed that the ancestral *Ivesia* taxa originated in the Rocky Mountains and eastern Great Basin Desert; therefore, smaller GS for the sampled *Ivesia* taxa near these regions may suggest that GS evolution may be potentially associated with speciation events in the genus. However, a phylogenetic analysis may help identify ancestral taxa and verify a potential relationship between speciation events and GS evolution in *Ivesia*.

LITERATURE CITED

Albach DC, Greilhuber J. 2004. Genome size variation and evolution in *Veronica*. *Annals of Botany* **94***:* 897–911.

Arumuganathan K, Earle ED. 1991. Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter* **9*:*** 208-218.

Bainard JD, Newmaster SG, Budke JM. 2020. Genome size and endopolyploidy evolution across the moss phylogeny. *Annals of Botany* **125**: 543–555.

Bales AL, Hersch-Green EI. 2019. Effects of soil nitrogen on diploid advantage in fireweed, *Chamerion angustifolium* (Onagraceae). *Ecology and Evolution* **9**: 1095–1109.

Baniaga AE, Arrigo N, Barker MS 2016. The small nuclear genomes of Selaginella are associated with a low rate of genome size evolution. *Genome Biology and Evolution* **8**: 1516–1525.

Beaulieu JM, Leitch IJ, Patel S, Pendharkar A, Knight CA. 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist* **179**: 975–986.

Beaulieu JM, Moles AT, Leitch IJ, Bennett MD, Dickie JB, Knight CA. 2007. Correlated evolution of genome size and seed mass. *New Phytologist* **173**: 422–437.

Beaulieu JM, Smith SA, Leitch IJ. 2010. On the tempo of Genome Size evolution in Angiosperms. *Journal of Botany* **2010:** 989152.

Bennett MD. 1976. DNA amount, latitude and crop plant distribution. *Environmental and Experimental Botany* **16**: 93-98.

Bennett MD. 1987. Variation in genomic form in plants and its ecological implications. *New Phytologist* **106**: 177-200.

Bilinski P, Albert PS, Berg JJ, *et al.* 2018. Parallel altitudinal clines reveal trends in adaptive evolution of genome size in *Zea mays*. *PLoS Genetics* **14**: e1007162.

Blommaert J. 2020. Genome size evolution: towards new model systems for old questions. *Proceedings of the Royal Society Series B* **287**: 20201441.

Bottini MCJ, Greizerstein EJ, Aulicino MB, Poggio L. 2000. Relationships among genome size, environmental conditions and geographical distribution in natural populations of NW Patagonian species of *Berberis* L. (Berberidaceae). *Annals of Botany* **86**: 565-573.

Broderick SR, Stevens MR, Geary B, *et al.* 2011. A survey of Penstemon’s genome size. *Genome* **54**: 160-173

Bromham L, Hua X, Lanfear R, Cowman PF. 2015. Exploring the relationships between mutation rates, life history, genome size, environment, and species richness in flowering plants. *The American Naturalist* **185**: 507–524.

Burleigh JG, Barbazuk WB, Davis JM, Morse AM, Soltis PS. 2012. Exploring diversification and genome size evolution in extant gymnosperms through phylogenetic synthesis. *Journal of Botany* **2012**: Article ID 292857.

Cassel EJ, Calvert AT, Graham SA. 2009. Age, geochemical composition, and distribution of Oligocene ignimbrites in the northern Sierra Nevada, California: implications for landscape morphology, elevation, and drainage divide geography of the Nevadaplano. *International Geology Review* **51**: 723–742.

Cavalier-Smith T. 2005. Economy, speed and size matter: evolutionary forces driving nuclear genome miniaturization and expansion. *Annals of Botany* **95**: 147–175

DeSoto L, Tutor D, Torices R, Rodríguez-Echeverría S, Nabais C. 2016. Pre-dispersal predation effect on seed packaging strategies and seed viability. *Oecologia* **180**: 91–102.

Díez CM, Gaut BS, Meca E, *et al.* 2013. Genome size variation in wild and cultivated maize along altitudinal gradients. *New Phytologist* **199**: 264–276.

Dobeš C, Paule J. 2010. A comprehensive chloroplast DNA-based phylogeny of the genus *Potentilla* (Rosaceae): Implications for its geographic origin, phylogeography and generic circumscription. *Molecular Phylogenetics and Evolution* **56**: 156–175.

Du Y-P, Bi Y, Zhang M-F, Yang F-P, Jia G-X, Zhang X-H. 2017. Genome size diversity in *Lilium* (Liliaceae) is correlated with karyotype and environmental traits. *Frontiers in Plant Science* **8**: 1303.

Dušková E, Kolář F, Sklenář P, *et al.* 2010. Genome size correlates with growth form, habitat and phylogeny in the Andean genus *Lasiocephalus* (*Asteraceae*). *Preslia* **82**: 127-148.

Eckert CG, Samis KE, Lougheed SC. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology* **17**: 1170–1188.

Enquist BJ, Jordan MA, Brown JH. 1995. Connections between ecology, biogeography, and paleobiology: relationship between local abundance and geographic distribution in fossil and recent molluscs. *Evolutionary Ecology* **9**: 586-604.

Eriksson T, Donoghue MJ, Hibbs MS. 1998. Phylogenetic analysis of *Potentilla* using DNA sequences of nuclear ribosomal internal transcribed spacers (ITS), and implications for the classification of Rosoideae (Rosaceae). *Plant Systematics and Evolution* **211**: 155–179.

Eriksson T, Hibbs MS, Yoder AD, Delwiche CF, Donoghue MJ. 2003. The phylogeny of Rosoideae (Rosaceae) based on sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and the trnL/F region of chloroplast DNA. *International Journal of Plant Science* **164**: 197–211.

Ertter B, Reveal JL. 2014. *Ivesia*. In: Flora of North America Editorial Committee, eds. *Flora of North America North of Mexico*, 19+ vols, New York and Oxford, Vol. 9, 219-246.

Ertter B. 1989. Revisionary studies in *Ivesia* (Rosaceae). *Systematic Botany* **14**: 231–244.

Fenberg PB, Rivadeneira MM. 2011. Range limits and geographic patterns of abundance of the rocky intertidal owl limpet, *Lottia gigantea*. *Journal of Biogeography* **38**: 2286–2298.

Feng T, Moore MJ, Yan M-H, *et al.* 2017. Phylogenetic study of the tribe Potentilleae (Rosaceae), with further insight into the disintegration of *Sibbaldia*. *Journal of Systematics and Evolution* **55**: 177-191.

Findley SD, Cannon S, Varala K, *et al.* 2010. A Fluorescence in Situ Hybridization system for karyotyping soybean. *Genetics* **185**: 727–744.

Garnatje T, Garcia S, Canela MÁ. 2007. Genome size variation from a phylogenetic perspective in the genus *Cheirolophus* Cass. (Asteraceae): biogeographic implications. *Plant Systematics and Evolution* **264**: 117-134.

Garner TW. 2002. Genome size and microsatellites: the effect of nuclear size on amplification potential. *Genome* **45:** 212–215.

Gómez JM. 2004. Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution* **58**: 71–80.

Greilhuber J, Doležel J, Lysák MA, Bennett MD. 2005. The origin, evolution and proposed stabilization of the terms “Genome size” and “C-value” to describe nuclear DNA contents. *Annals of Botany* **95**: 255–260.

Guignard MS, Nichols RA, Knell RJ, *et al.* 2016. Genome size and ploidy influence angiosperm species’ biomass under nitrogen and phosphorus limitation. *New Phytologist* **210:** 1195–1206

Hallas JM, Chichvarkhin A, Gosliner TM. 2017. Aligning evidence: concerns regarding multiple sequence alignments in estimating the phylogeny of the *Nudibranchia* suborder Doridina. *Royal Society Open Science* **4**: 171095.

Hillis D, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**: 182-192.

Hoang PTN, Schubert V, Meister A, Fuchs J, Schubert I. 2019. Variation in genome size, cell and nucleus volume, chromosome number and rDNA loci among duckweeds. *Scientific Reports* **9**: 3234.

Hodgson JG, Sharafi M, Jalili A, *et al.* 2010. Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? *Annals of Botany* **105**: 573–584.

Hosid E, Brodsky L, Kalendar R, Raskina O, Belyayev A. 2012. Diversity of long terminal repeat retrotransposon genome distribution in natural populations of the wild diploid wheat *Aegilops speltoides*. *Genetics* **190**: 263-274.

Huang H, Tong Y, Zhang Q-J, Gao L-Z. 2013. Genome size variation among and within *Camellia* species by using flow cytometric analysis. *PLoS One* **8**: e64981.

Igea J, Miller EF, Papadopulos AST, Tanentzap AJ. 2017. Seed size and its rate of evolution correlate with species diversification across angiosperms. *PLoS Biology* **15**: e2002792.

Jiao Y, Wickett N, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS, Soltis DE, Clifton SW, Schlarbaum SE, Schuster SC, Ma H, Leebens-Mack J, dePamphilis CW. 2011. Ancestral polyploidy in seed plants and angiosperms. *Nature* **473**: 97–100.

Jordan GJ, Carpenter RJ, Koutoulis A, Price A, Brodribb TJ. 2015. Environmental adaptation in stomatal size independent of the effects of genome size. *New Phytologist* **205**: 608–617.

Kang M, Tao J, Wang J, *et al.* 2014. Adaptive and nonadaptive genome size evolution in Karst endemic flora of China. *New Phytologist* **202**: 1371–1381.

Katoh K, Asimenos G, Toh H. 2009. Multiple alignment of DNA sequences with MAFFT. Multiple Alignment of DNA Sequences with MAFFT. In: Posada D. *eds. Bioinformatics for DNA Sequence Analysis. Methods in Molecular Biology (Methods and Protocols).* New Jersey: Humana Press, Vol 537, pp 39-64.

Knight CA, Molinari N, Petrov D. 2005. The large genome constraint hypothesis: evolution, ecology, and phenotype. *Annals of Botany* **5**: 177–190.

Kraaijeveld, K. (2010). Genome Size and Species Diversification. Evolutionary Biology 37: 227–233. https://doi.org/10.1007/s11692-010-9093-4

Kraft NJB, Baldwin BG, Ackerly DD. 2010. Range size, taxon age and hotspots of neoendemism in the California flora. *Diversity and Distributions* **16**: 403–413.

Krahulcová A, Trávnícek P, Krahulec F, Rejmánek M. 2017. Small genomes and large seeds: chromosome numbers, genome size and seed mass in diploid *Aesculus* species (Sapindaceae). *Annals of Botany* **119**: 957-964.

Kron P, Suda J, Husband BC. 2007. Applications of flow cytometry to evolutionary and population biology. *Annual Review of Ecology, Evolution, and Systematics* **38**: 847–876.

Lanfear R, Kokko H, Eyre-Walker A. 2014. Population size and the rate of evolution. *Trends in Ecology and Evolution* **29**: 33–41.

Leitch IJ, Beaulieu JM, Chase MW, Leitch AR, Fay MF. 2010. Genome size dynamics and evolution in monocots. *Journal of Botany* 2010: **862516**.

Leitch IJ, Chase MW, Bennett MD. 1998. Phylogenetic Analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. *Annals of Botany* **82**: 85-94.

Leitch IJ, Soltis DE, Soltis PS, Bennett MD. 2005. Evolution of DNA amounts across land plants (Embryophyta). *Annals of Botany* **95**: 207–217.

Leushkin EV, Bazykin GA, Kondrashov AS. 2013. Strong mutational bias toward deletions in the Drosophila melanogaster genome is compensated by selection. *Genome Biology and Evolution* **5**: 514–524.

Loureiro J, Trávníček P, Rauchová J, *et al.* 2010. The use of flow cytometry in the biosystematics, ecology and population biology of homoploid plants. *Preslia* **82**: 3-21.

Lynch M, Conery JS. 2003. The origins of genome complexity. *Science* **302**: 1401–1404.

Morgan MT. 2001. Transposable element number in mixed mating populations. *Genetics Research* **77**:261–275.

Mráz P, Chrtek J, Šingliarová B. 2009. Geographical parthenogenesis, genome size variation and pollen production in the arctic-alpine species *Hieracium alpinum*. *Botanica Helvetica* **119**: 41–51.

Oliver MJ, Petrov D, Ackerly D, *et al.* 2007. The mode and tempo of genome size evolution in eukaryotes. *Genome Research* **17**:594–601.

Pascual-Díaz JP, Serçe S, Hradecká I, *et al.* (2020). Genome size constancy in Antarctic populations of *Colobanthus quitensis* and *Deschampsia antarctica*. *Polar Biology* **43**: 1407–1413

Pellicer J, Hidalgo O, Dodsworth S, Leitch IJ. 2018. Genome size diversity and its impact on the evolution of land plants. *Genes* **9**: 88.

Petrov DA. 2002. Mutational equilibrium model of genome size evolution. *Theoretical Population Biology* **61**: 531–544.

Potter D, Eriksson T, Evans RC, *et al.* 2007. Phylogeny and classification of Rosaceae. *Plant Systematics and Evolution* **266**: 5–43.

Potter D, Gao F, Bortiri PE, Oh S, Baggett S. 2002. Phylogenetic relationships in Rosaceae inferred from chloroplast matK and trnL–trnF nucleotide sequence data. *Plant Systematics and Evolution* **231**: 77–89.

Puttick MN, Clark J, Donoghue PCJ. 2015. Size is not everything: rates of genome size evolution, not C-value, correlate with speciation in angiosperms. *Proceedings of the Royal Society B* **282**: 20152289.

Roddy AB, Théroux-Rancourt G, Abbo T, *et al.* 2020. The scaling of genome size and cell size limits maximum rates of photosynthesis with implications for ecological strategies. *International Journal of Plant Sciences* **181**: 75–87.

Sanmartín I, Meseguer AS. 2016. Extinction in phylogenetics and biogeography: from timetrees to patterns of biotic assemblage. *Frontiers in Genetics* **7**: 35.

Schrider DR, Houle D, Lynch M, *et al.* 2013. Rates and genomic consequences of spontaneous mutational events in Drosophila melanogaster. *Genetics* **194**: 937–954.

Sliwinska E, Pisarczyk I, Pawlik A, *et al.* 2009. Measuring genome size of desert plants using dry seeds. *Botany* **87**: 127-135.

Šmarda P, Bureš P, Horová L, Foggi B, Rossi G. 2008. Genome size and GC content evolution of *Festuca*: ancestral expansion and subsequent reduction. *Annals of Botany* **101**: 421–433.

Šmarda P, Bureš P. 2010. Understanding intraspecific variation in genome size in plants. *Preslia* **82**: 41-61

Soltis DE, Soltis PS, Bennett MD, Leitch IJ. 2003. Evolution of genome size in the angiosperms. *American Journal of Botany* **90**: 1596–1603.

Soltis PS, Soltis DE 2021. Plant genomes: Markers of evolutionary history and drivers of evolutionary change. *New Phytologist* **3**: 74-82.

Stamatakis A. 2006. Raxml-vi-hpc: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Stelzer C-P, Pichler M, Stadler P, *et al.* 2019. Within-population genome size variation is mediated by multiple genomic elements that segregate independently during meiosis. *Genome Biology and Evolution* **11**: 3424–3435

Sun M, Naeem R, Su J-X, *et al.* 2016. Phylogeny of the Rosidae: A dense taxon sampling analysis. *Journal of Systematics and Evolution* **54**: 363-391.

Töpel M, Lundberg M, Eriksson T, Eriksen B. 2011. Molecular data and ploidal levels indicate several putative allopolyploidization events in the genus *Potentilla* (Rosaceae). *PLoS Currents* **16**: RRN1237.

Töpel MH, Antonelli A, Yesson C, Eriksen B. 2012. Past climate change and plant evolution in western North America: A case study in Rosaceae. *PLoS One* **7**: e50358.

Vitales D, Fernández P, Garnatje T, Garcia S. 2019. Progress in the study of genome size evolution in Asteraceae: analysis of the last update. *Database* **2019**: baz098.

Westoby M, Leishman M, Lord J. 1996. Comparative ecology of seed size and dispersal. *Philosophical Transactions of the Royal Society B* **351**: 1309–1317.

Whitney KD, Baack EJ, Hamrick JL, *et al.* 2010. A role for nonadaptive processes in plant genome size evolution? *Evolution* **64**: 2097–2109

Wright SI, Schoen DJ. 1999. Transposon dynamics and the breeding system. *Genetica* **107**:139–148

Zonneveld BJM, Leitch IJ, Bennett MD. 2005. First Nuclear DNA Amounts in more than 300 Angiosperms. *Annals of Botany* **96**: 229–244.

Table 1. Nuclear genome size, reference ploidy, environmental attributes and the estimated geographical ranges of the 31 sampled *Ivesia* taxa across western United States

Taxa	**Section**[a]	**Substrate type**[a]	**Substrate geology**[a]	**Ploidy**[a]	**Species range (km^2)**[b]	**Sample size**	**2C Genome size (mean±SD)**	**2C Genome size range**	**Mean seed weight (g)**[c]
Ivesia aperta var. *aperta*	Unguiculatae	Soil	Volcanic	*2n* = 28	2189.508	2	2.47±0.02	2.42-2.51	0.0008/50
Ivesia aperta var. *canina*	Unguiculatae	Soil	Volcanic	*2n* = 28	62.281	2	2.11±0.03	2.09-2.13	0.0024/50
Ivesia arizonica var. *arizonica*	Setosae	Outcrop	Limestone	unknown	92270.580	3	0.97±0.02	0.95-0.99	n/a
Ivesia arizonica var. *saxosa*	Setosae	Outcrop	Volcanic	unknown	1812.572	2	1.33±0.02	1.29-1.36	n/a
Ivesia baileyi var. *baileyi*	Setosae	Outcrop	Volcanic	*2n* = 28	183703.313	2	1.2±0.03	1.17-1.22	0.00038/12
Ivesia baileyi var. *beneolens*	Setosae	Outcrop	Volcanic	*2n* = 28	150232.450	3	0.78±0.07	0.73-0.86	n/a
Ivesia callida	Setosae	Outcrop	Volcanic	unknown	0.114	3	0.94±0.01	0.93-0.95	n/a
Ivesia gordonii var. *alpicola*	Ivesia	Outcrop	Volcanic	*2n* = 28	209315.498	2	1.1±0.02	1.09-1.1	0.00071/10
Ivesia gordonii var. *ursinorum*	Ivesia	Soil	Volcanic	*2n* = 28	236297.467	3	0.94±0.00	0.94	0.00107/30
Ivesia gordonii var. *wasatchensis*	Ivesia	Soil	Limestone	*2n* = 28	15078.437	3	1.0±0.00	1.0	n/a
Ivesia jaegeri	Setosae	Outcrop	Limestone	unknown	2050.163	3	1.04±0.01	1.04-1.05	n/a
Ivesia kingii var. *eremica*	Unguiculatae	Soil	Alkali	unknown	0.001	2	1.33±0.03	1.32-1.34	n/a
Ivesia kingii var. *kingii*	Unguiculatae	Soil	Alkali	unknown	236134.174	2	1.4±0.03	1.35-1.45	0.00095/50
Ivesia longibracteata	Setosae	Outcrop	Volcanic	unknown	0.417	3	0.86±0.03	0.83-0.88	n/a
Ivesia lycopodioides var. *megalopetala unit 1*	Ivesia	Soil	Limestone	*2n* = 28	3419.988	3	5.31±0.2	5.02-5.73	0.0003/90
Ivesia lycopodioides var.	Ivesia	Soil	Limestone	*2n* = 28		3	5.82±0.3	5.70-5.91	0.0005/50

megalopetala unit 2									
Ivesia lycopodioides var. *scandularis*	Ivesia	Soil	Volcanic	*2n* = 28	9312.761	3	3.3±0.05	3.25-3.35	n/a
Ivesia multifoliolata	Comarella	Soil	Volcanic	*2n* = 28	5249.121	2	1.15±0.01	1.15	n/a
Ivesia paniculata	Setosae	Soil	Volcanic	unknown	67.169	2	1.04±0.02	1.00-1.08	0.00039/50
Ivesia pickeringii	Unguiculatae	Soil	Volcanic	unknown	553.319	3	1.23±0.04	1.09-1.37	n/a
Ivesia pityocharis	Unguiculatae	Soil	Volcanic	unknown	3.768	3	1.02±0.03	0.99-1.04	0.00046/50
Ivesia rhypara var. *rhypara*	Setosae	Soil	Volcanic	*2n* = 28	32194.802	3	0.85±0.02	0.86-0.91	0.00078/40
Ivesia rhypara var. *shellyi*	Setosae	Outcrop	Volcanic	*2n* = 28	38.510	3	0.9±0.01	0.89-0.9	0.00032/20
Ivesia sabulosa	Comarella	Soil	Limestone	unknown	115784.095	4	0.84±0.04	0.83-0.85	n/a
Ivesia santolinoides	Stellariopsis	Soil	Volcanic	*2n* = 28	68465.661	3	0.98±0.01	0.98-0.99	n/a
Ivesia saxosa	Setosae	Outcrop	Volcanic	unknown	154796.221	6	0.9±0.02	0.80-0.92	0.00034/50
Ivesia sericoleuca	Unguiculatae	Soil	Volcanic	*2n* = 28	1298.464	2	2.32±0.02	2.31-2.32	0.00123/7
Ivesia setosa	Setosae	Outcrop	Limestone	unknown	106880.547	6	0.99±0.02	0.96-1.01	n/a
Ivesia shockleyi var. *shockleyi*	Setosae	Outcrop	Volcanic	unknown	8161139.392	3	1.88±0.07	1.8-1.93	n/a
Ivesia tweedyi	Ivesia	Soil	Volcanic	unknown	25250.732	3	1.0±0.05	0.96-1.05	0.00102/50
Ivesia utahensis	Ivesia	Soil	Limestone	unknown	1022.194	3	1.03±0.05	0.97-1.07	n/a
Ivesia webberi unit 2	Ivesia	Soil	Volcanic	unknown	3214.223	3	0.85	0.85-0.86	0.00151/50
Ivesia webberi unit 3	Ivesia	Soil	Volcanic	unknown		3	0.84	0.83-0.85	0.00127/50
Ivesia webberi unit 5	Ivesia	Soil	Volcanic	unknown		3	0.85	0.85	0.00328/50
Ivesia webberi unit 6	Ivesia	Soil	Volcanic	unknown		3	0.87	0.85-0.89	0.0023/50
Ivesia webberi unit 7	Ivesia	Soil	Volcanic	unknown		3	0.88	0.87-0.9	n/a
Ivesia webberi unit 8	Ivesia	Soil	Volcanic	unknown		3	0.9	0.89-0.9	0.00222/50
Ivesia webberi unit 11	Ivesia	Soil	Volcanic	unknown		3	0.82	0.78-0.85	0.00145/10

Ivesia webberi unit 12	Ivesia	Soil	Volcanic	unknown	3	0.83	0.81-0.84	0.00218/50
Ivesia webberi unit 13	Ivesia	Soil	Volcanic	unknown	3	0.84	0.82-0.85	0.00177/50
Ivesia webberi unit 15	Ivesia	Soil	Volcanic	unknown	3	0.79	0.77-0.81	n/a
Ivesia webberi unit 16	Ivesia	Soil	Volcanic	unknown	3	0.77	0.76-0.78	0.00133/30
Potentilla gracilis var. *fastigiata*	-	Soil	Volcanic	*2n* = 52-109	3	2.71	2.69-2.74	n/a
Potentilla sp.[d]	-	Soil	Volcanic	unknown	2	1.25±0.01	1.24-1.26	n/a
Horkelia fusca ssp. *parviflora* unit 1		Soil	Volcanic	*2n* = 28	2	1.26±0.02	1.26-1.26	
Horkelia fusca ssp. *parviflora* unit 2	-	Soil	Volcanic	*2n* = 28	3	1.12±0.06	1.06-1.16	n/a

[a] *Information is based on the description of Ivesia in the Flora of North America (Ertter and Reveal 2014).*

[b] *Ranges of the sampled taxa was estimated from the convex hull boundary of their known occurrences*

[c] *The first value represents the mean seed weight, and the second value represents the total number of seeds weighed. n/a means seeds were not collected*

[d] *This taxon is suspected to be either P. morefieldii* or *P. pseudosericea*

FIGURE LEGENDS

Figure 1. Locations of representative samples of *Ivesia, Potentilla,* and *Horkelia* taxa collected for this study.

Figure 2. Locations of representative samples of 11 *Ivesia webberi* populations collected for this study.

Figure 3. Plots of the relationship between genome size of *Ivesia* taxa and the selected environmental predictors, including (A) substrate type, (B) substrate geology, (C) average seed size for the sampled *Ivesia* taxa, (D) the elevation of each collection, (E) mean actual evapotranspiration between 1981 and 2015, (F) mean potential evapotranspiration between 1981 and 2015, and (G) mean climatic water deficit between 1981 and 2015. In Figures 3c-g, the blue regions represent confidence intervals around the line of best fit

Figure 4. Genome size of plant samples collected from 11 *Ivesia webberi* populations.

Figure 5. Relationship between genome size of 11 *Ivesia webberi* populations and the selected environmental predictors, including (A) elevation of each collection, (B) mean actual evapotranspiration between 1981 and 2015, (C) mean potential evapotranspiration between 1981 and 2015, (D) mean climatic water deficit between 1981 and 2015, and (E) mean seed size from each population.

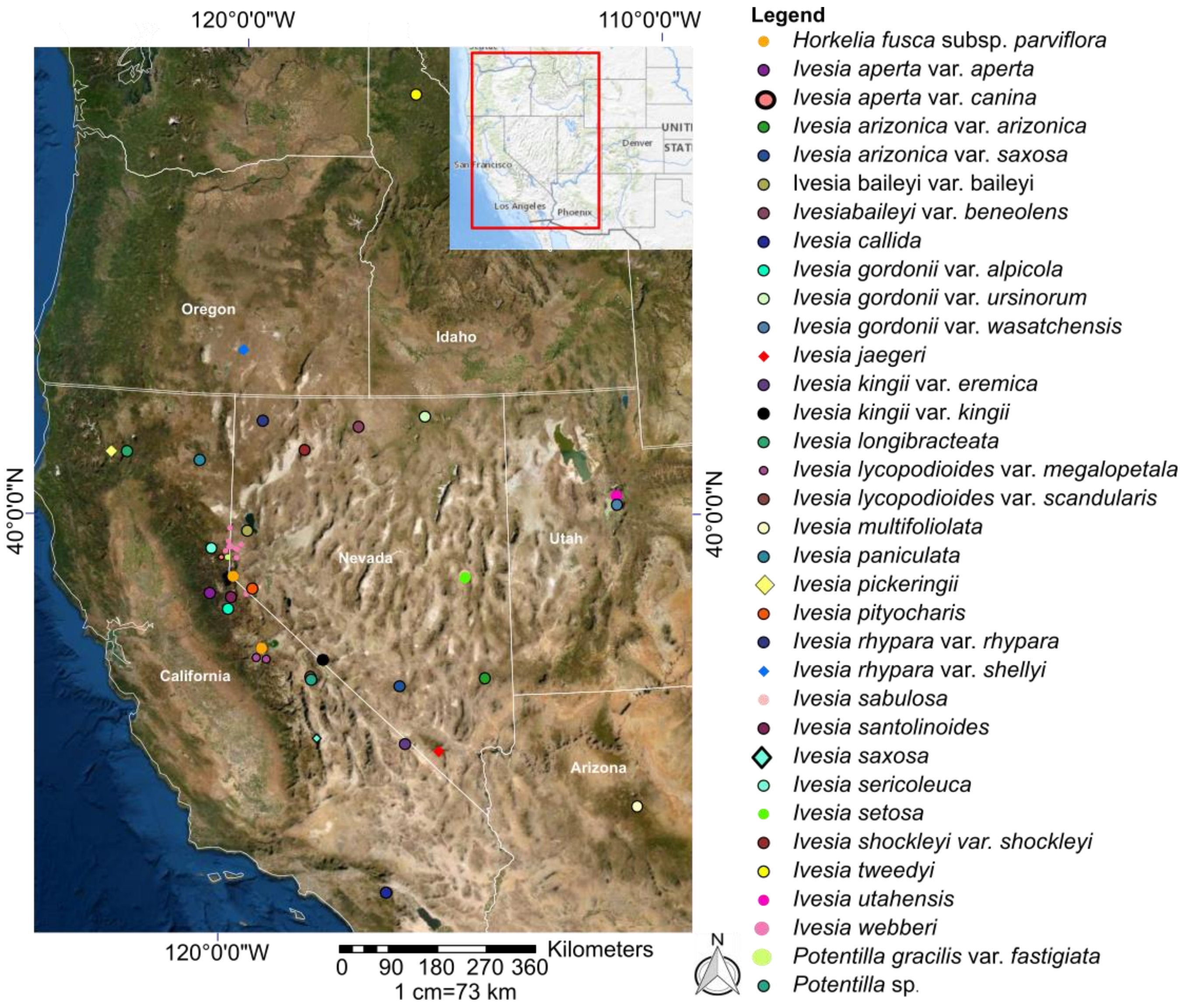

120°0'0"W
110°0'0"W
40°0'0"N
40°0'0"N
Oregon
Idaho
Nevada
Utah
California
Arizona
San Francisco
Los Angeles
Denver
Phoenix
UNITI
STATI
120°0'0"W
0 90 180 270 360
Kilometers
1 cm=73 km
N
Legend
Horkelia fusca subsp. parviflora
Ivesia aperta var. aperta
Ivesia aperta var. canina
Ivesia arizonica var. arizonica
Ivesia arizonica var. saxosa
Ivesia baileyi var. baileyi
Ivesiabaileyi var. beneolens
Ivesia callida
Ivesia gordonii var. alpicola
Ivesia gordonii var. ursinorum
Ivesia gordonii var. wasatchensis
Ivesia jaegeri
Ivesia kingii var. eremica
Ivesia kingii var. kingii
Ivesia longibracteata
Ivesia lycopodioides var. megalopetala
Ivesia lycopodioides var. scandularis
Ivesia multifoliolata
Ivesia paniculata
Ivesia pickeringii
Ivesia pityocharis
Ivesia rhypara var. rhypara
Ivesia rhypara var. shellyi
Ivesia sabulosa
Ivesia santolinoides
Ivesia saxosa
Ivesia sericoleuca
Ivesia setosa
Ivesia shockleyi var. shockleyi
Ivesia tweedyi
Ivesia utahensis
Ivesia webberi
Potentilla gracilis var. fastigiata
Potentilla sp.

Figure 1.


120°0'0"W
40°0'0"N
40°0'0"N
California
Nevada
all other units
Unit16
Unit 2
Nevada
California
Unit 3
Unit 11
Unit 6
Unit 7
Unit 8
Unit 13
Unit 12
Unit 5
Reno
Unit 15
120°0'0"W
0
24
Kilometers
N

Figure 2.

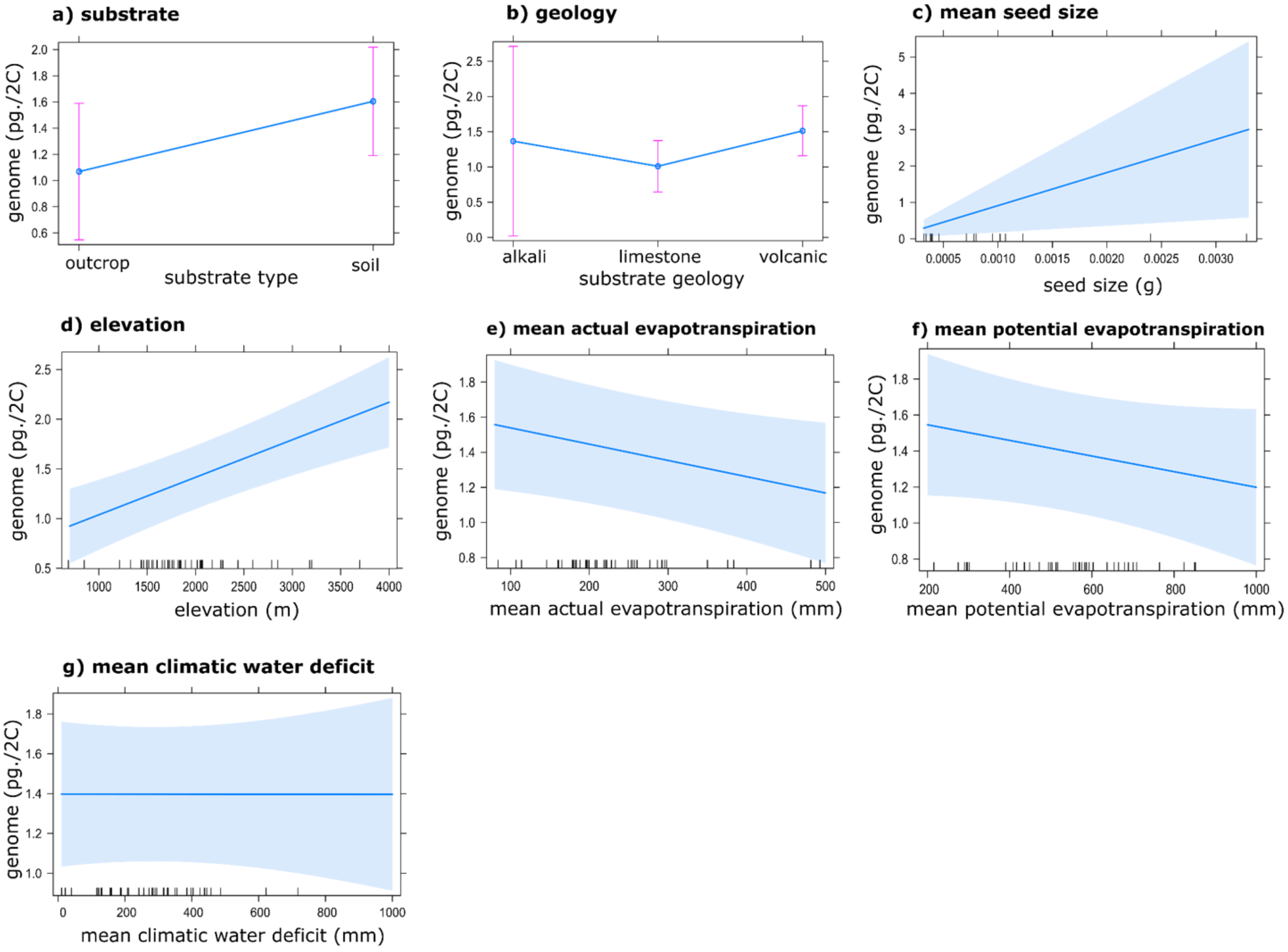

a) substrate
genome (pg./2C)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
outcrop
soil
substrate type
b) geology
genome (pg./2C)
2.5
2.0
1.5
1.0
0.5
0.0
alkali
limestone
volcanic
substrate geology
c) mean seed size
genome (pg./2C)
5
4
3
2
1
0
0.0005
0.0010
0.0015
0.0020
0.0025
0.0030
seed size (g)
d) elevation
genome (pg./2C)
2.5
2.0
1.5
1.0
0.5
1000
1500
2000
2500
3000
3500
4000
elevation (m)
e) mean actual evapotranspiration
genome (pg./2C)
1.8
1.6
1.4
1.2
1.0
0.8
100
200
300
400
500
mean actual evapotranspiration (mm)
f) mean potential evapotranspiration
genome (pg./2C)
1.8
1.6
1.4
1.2
1.0
0.8
200
400
600
800
1000
mean potential evapotranspiration (mm)
g) mean climatic water deficit
genome (pg./2C)
1.8
1.6
1.4
1.2
1.0
0
200
400
600
800
1000
mean climatic water deficit (mm)

Figure 3.

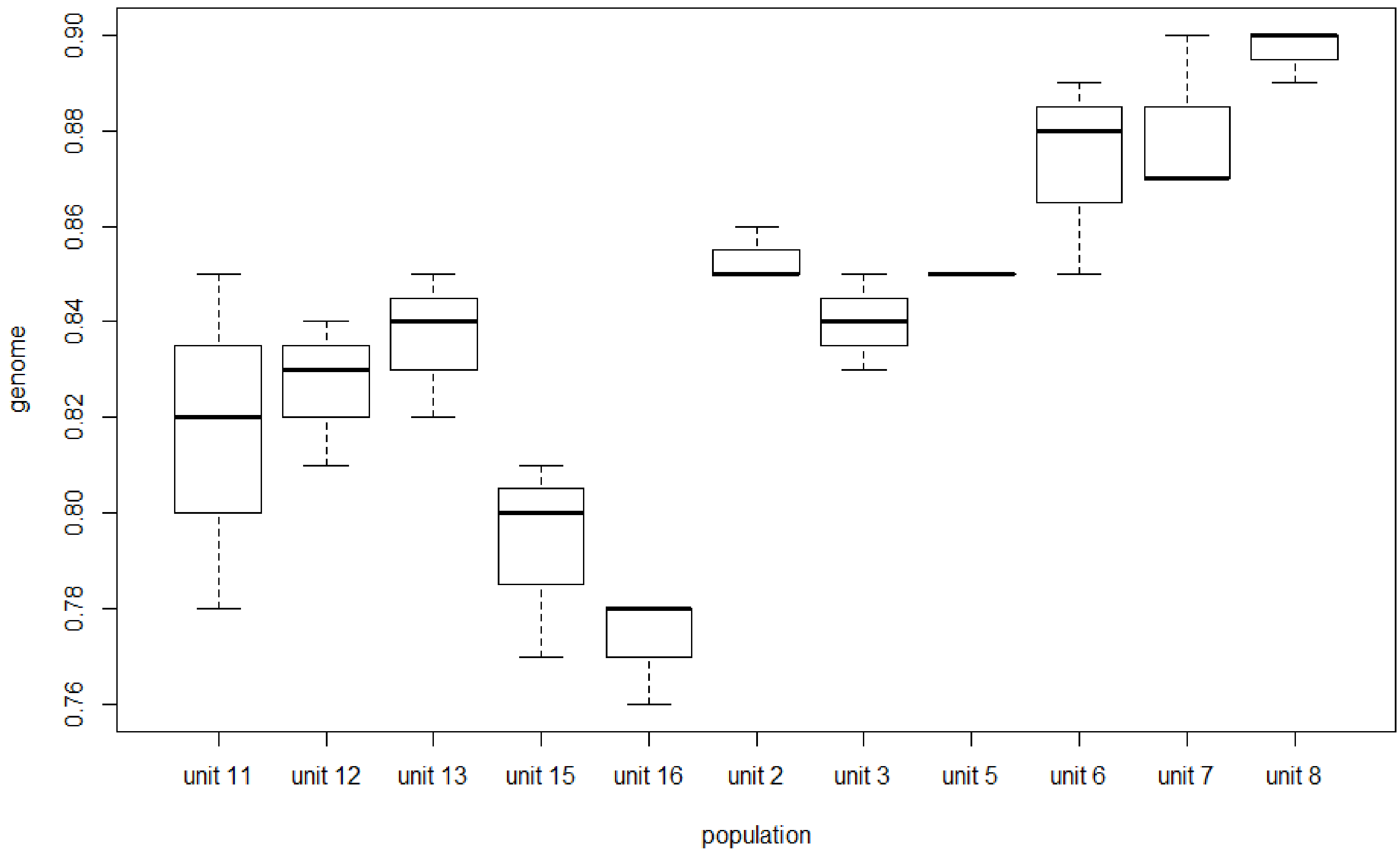
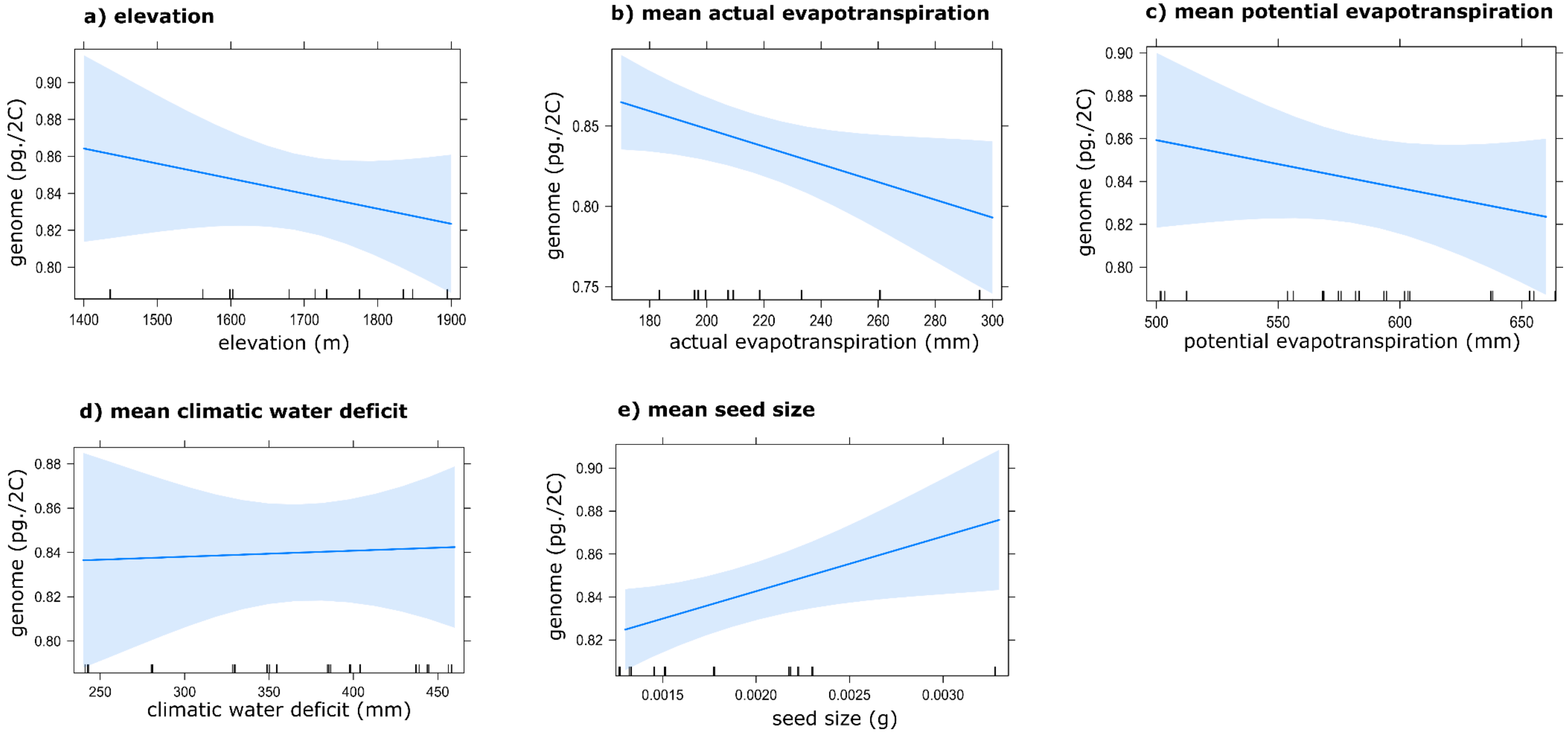

genome
0.76
0.78
0.80
0.82
0.84
0.86
0.88
0.90
unit 11
unit 12
unit 13
unit 15
unit 16
unit 2
unit 3
unit 5
unit 6
unit 7
unit 8
population

Figure 4.


a) elevation

b) mean actual evapotranspiration

c) mean potential evapotranspiration

d) mean climatic water deficit

e) mean seed size

Figure 5.

ACKNOWLEDGEMENTS

We thank the following people for their support during the field collections: Arnold Tiehm (Museum of Natural History, University of Nevada, Reno), Michael Hagebusch, Mr. John Dittes, Dr Margaret Widdowson, Dr Derek Antonelli, Prof Tina Ayers (Northern Arizona University), Greg Gust, Maura Olivos, Dr Duncan Bell and Dr Naomi Fraga (Rancho Santa Ana Botanical Garden), Dr Jennifer Wilkening (USFWS Southern Nevada Office), Dr Jeanette Perry and Dr Patricia Hardesty (Nevada National Security Site), Kathryn Birgy (University of Nevada Las Vegas Herbarium), Gary Reese, and Cayenne Engel (Nevada Department of Forestry), Jordan Brown (Oregon Native Plant Conservation Program, and Department of Botany, Oregon State University), Lindsey Wise (Oregon Biodiversity Information Centre).

DATA ACCESSIBILITY

All data used for this study (except the geographical coordinates) are publicly accessible at the Knowledge Network for Biocomplexity doi:10.5063/TH8K3G. The raw genome size will be submitted to the Plant DNA C-values database managed by the Royal Botanic Garden, Kew, U.K., following publication.

SUPPLEMENTARY INFORMATION

Supplemental Table 1. Description of collection sites for the 31 *Ivesia* taxa across western United States

Taxa	Code	Location	Elevation (m)	County	State	Herbarium repository
Ivesia aperta var. *aperta*	IVAPA	Near Feather River, Sierra valley	1491.9	Sierra	California	University of Nevada Herbarium (RENO:V): 103929
Ivesia aperta var. *canina*	IVAPC	Dog valley meadows	1758.1	Sierra	California	University of Nevada Herbarium (RENO:V): 103994
Ivesia arizonica var. *arizonica*	IVARA	Rainbow canyon, tertiary canyon, just north of Chokecherry canyon, growing on protected west facing rock wall	1329	Lincoln	Nevada	University of Nevada Herbarium (RENO:V): 100365
Ivesia arizonica var. *saxosa*	IVARS	Pahute Mesa, South Silent Canyon, near Pahute Mesa Road	1958	Nye	Nevada	University of Nevada Herbarium (RENO:V): 59321
Ivesia baileyi var. *baileyi*	IVBABA	Needle Rock, Virginia Range, along Pyramid Way, near Pauite tribe reservation	1463	Washoe	Nevada	University of Nevada Herbarium (RENO:V): 35739
Ivesia baileyi var. *beneolens*	IVBABE	Santa Rosa Range, base of vertical cliffs just east of Hinkey Summit, 20 km (12 mi) air distance north (358°) of Paradise Valley (town)	1719.3	Humboldt	Nevada	University of Nevada Herbarium (RENO:V): 37473
Ivesia callida	IVCA	Taquitz Peak, San Jacinto Mountains	2439	Riverside	California	Rancho Santa Ana Botanic Garden Herbarium (RSABG || POM): 005612
Ivesia gordonii var. *alpicola*	IVGOA	Rock clefts, near Winnemucca Lake and Round Top Mountain	2787.8	Alpine	California	University of Nevada Herbarium (RENO:V): 103931
Ivesia gordonii var. *ursinorum*	IVGOU	The Mahoganies, hills east-southeast of Entight Hills	2019.9	Elko	Nevada	University of Nevada Herbarium (RENO:V): 38394
Ivesia gordonii var. *wasatchensis*	IVGOW	Big Cottonwood Canyon, Mt. Baldy, near Lupine Trail	2848.5	Salt Lake	Utah	University of Nevada Herbarium (RENO:V): 103900

Ivesia jaegeri	IVJA	Limestone cliffs behind Spring Mountain visitor gateway, Mount Charleston	2045	Clark	Nevada	University of Nevada Herbarium (RENO:V): 15908
Ivesia kingii var. *eremica*	IVKIE	Ash Meadows National wildlife refuge	685	Nye	Nevada	University of Nevada Herbarium (RENO:V): 43885
Ivesia kingii var. *kingii*	IVKIK	Alkaline salt flat near Fish Lake Valley hot well, Silver Peak range, near Dyer NV	1442	Esmeralda	Nevada	University of Nevada, Las Vegas; Wesley E. Niles Herbarium (UNLV): 33393
Ivesia longibracteata	IVLO	On the rock clefts of Castle crags, Shasta-Trinity National Forest	1214.6	Shasta	California	University of Nevada Herbarium (RENO:V): 41907
Ivesia lycopodioides var. *megalopetala*	IVLYM	Tuolumne Meadows, Yosemite National Park	2592.1	Tuolumne	California	University of Nevada Herbarium (RENO:V): 103946
Ivesia lycopodioides var. *megalopetala*	IVLYM	In the meadows along trail to Gardisky Lake, Inyo National Forest, off Saddlebag Lake Road, off CA 120 highway towards Yosemite National Park	3179.6	Mono	California	University of California, Riverside Plant Herbarium(UCR): UCR-255565 (registered as var. *lycopodioides*)
Ivesia lycopodioides var. *scandularis*	IVLYS	Along the road to University of California Barcroft field station, White Mountains, Inyo National Forest	3696.8	Mono	California	University of Nevada Herbarium (RENO:V): 59318
Ivesia multifoliolata	IVMU	Walnut Canyon, Sandy's canyon, Coconino	2018	Coconino	Arizona	Deaver Herbarium (Northern Arizona University) (ASC): ASC00118859
Ivesia paniculata	IVPA	Ash creek valley, Modoc Plateau	1547.2	Modoc	California	University of Nevada Herbarium (RENO:V): 103933
Ivesia pickeringii	IVPI	Meadow 0.3 miles after Eagle creek site, near Trinity river, Shasta Mountain area	849.2	Trinity	California	University of Nevada Herbarium (RENO:V): 40953
Ivesia pityocharis	IVPIT	Pine Nut Mts., 1.2 air miles SE of Slaters Mine on the NE side of Mt. Siegel	2282	Douglas	Nevada	University of Nevada Herbarium (RENO:V): 9588

Ivesia rhypara var. *rhypara*	IVRHR	Sheldon National Wildlife Refuge: East side of Fish creek table, 0.3 road miles NW of Fish Creek campground	1820.6	Washoe	Nevada	University of Nevada Herbarium (RENO:V): 11230
Ivesia rhypara var. *shellyi*	IVRHS	First canyon south of Venator Canyon, ca. 7.3 air miles NNE of the town of Alkali Lake	1516.9	Lake	Oregon	University of Nevada Herbarium (RENO:V): 103901
Ivesia sabulosa	IVSAB	Egan range, ~0.6 miles by air south of Highway 50 intersection, just east of Ruth road	2073	White Pine	Nevada	New York Botanical Garden Steere Herbarium (NY): 834037
Ivesia santolinoides	IVSAN	Near Hope Valley, CA 88 roadside	2171.2	Alpine	California	University of Nevada Herbarium (RENO:V): 103997
Ivesia saxosa	IVSAX	Along switchbacks of Horseshoe Meadow road, 11.2 miles south-southwest of Jct. with Whitney Portal road, Inyo National Forest, off Lone Pine	2261.4	Inyo	California	University of Nevada Herbarium (RENO:V): 57877
Ivesia sericoleuca	IVSER	California road A23, opposite Calpine junction; California A49 roadside between Sierraville and Sattley	1502.9	Sierra	California	University of Nevada Herbarium (RENO:V): 103930
Ivesia setosa	IVSET	Egan range, ~0.5 miles by air south of Highway 50 intersection, just east of Ruth road	2067	White Pine	Nevada	New York Botanical Garden Steere Herbarium (NY): 834072
Ivesia shockleyi var. *shockleyi*	IVSHS	Jackson Mountains, 2.1 road miles northeast of the Jackson and Trout Creek road junction on the road to Iron King Mine	2055	Humboldt	Nevada	University of Nevada Herbarium (RENO:V): 44622
Ivesia tweedyi	IVTW	About 0.5 air miles south of Moon Peak, along the divide between Horseshoe creek and Moon creek, side of the divide	1653.9	Shoshone	Idaho	University of Nevada Herbarium (RENO:V): 103903
Ivesia utahensis	IVUT	Saddle below and west of Sugarloaf Mountain, Alta ski resort	3201.2	Salt Lake	Utah	Brigham Young University, S. L. Welsh Herbarium (BRY:V): BRYV0114721

Ivesia webberi	IVWE	population 2: near Doyle	1436	Lassen	California	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 3: East of Hallelujah Junction	1561.7	Lassen	California	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 5: Dog Valley meadows	1834.7	Sierra	California	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 6: White Lake Overlook	1715.2	Sierra	California	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 7: Mules Ear flat	1680	Sierra	California	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 8: Ivesia flat	1775.6	Washoe	Nevada	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 11: Hungry valley	1599	Washoe	Nevada	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 12: Black Springs	1730.2	Washoe	Nevada	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 13: Raleigh Heights	1602.6	Washoe	Nevada	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 15: Pines Powerlines	1847.6	Washoe	Nevada	Collection of voucher specimen not permitted
Ivesia webberi	IVWE	population 16: Dante Mine Road	1894.8	Douglas	Nevada	Collection of voucher specimen not permitted
Potentilla gracilis var. *fastigiata*	POGRF	Dog valley meadows	1758.1	Sierra	California	University of Nevada Herbarium (RENO:V): 103995
Potentilla sp. [either *P. morefieldii* or *P. pseudosericea*]	POSP	White Mountains, White Mountain road, 6.8 km (4.2 mi) north of Patriarch Grove turnoff, east side of road, 26.5 km (16.5 mi) air distance northeast (35°) of downtown Bishop.	3587	Mono	California	University of Nevada Herbarium (RENO:V): 61149
Horkelia fusca ssp. *parviflora*	HOFUP	Sawmill walk-in campground, off Tioga pass road to Yosemite National Park, near Lee Vining CA	2930.5	Mono	California	University of Nevada Herbarium (RENO:V): 103902
Horkelia fusca ssp. *parviflora*	HOFUP	Spooner Lake: SW corner, dry meadow edges	2127.7	Douglas	Nevada	University of Nevada Herbarium (RENO:V): 103904

Supplemental Table 2. List of sequences for *Ivesia, Horkelia* and *Potentilla* taxa used for the phylogenetic tree reconstruction

Taxa	**Taxa unique ID**	**ETS sequence NCBI accession**	**ITS sequence NCBI accession**	**trnL sequence NCBI accession**	**matK sequence NCBI accession**	**Reference**
Horkelia fusca ssp. *parviflora*	CM322771	KT985777.1	KT985648.1	KT991758.1		Koski & Ashman 2016
Ivesia aperta var. *aperta*	UC1559690	KT985781.1	KT985652.1			Koski & Ashman 2016
Ivesia argyrocoma	GB260			FR873003.1		Töpel et al. 2012
Ivesia arizonica var. *arizonica*	RSA508466	KT985782.1	KT985653.1	KT991759.1		Koski & Ashman 2016
Ivesia arizonica var. *saxosa*	UC1559755	KT985783.1	KT985654.1	KT991760.1		Koski & Ashman 2016
Ivesia baileyi var. *baileyi*	GB293			FR872947.1		Töpel et al. 2012
Ivesia baileyi var. *beneolens*	RSA508468	KT985784.1	KT985655.1	KT991761.1		Koski & Ashman 2016
Ivesia cryptocaulis	JEPS104			FR872968.1		Töpel et al. 2012
Ivesia gordonii	CM473284	KT985785.1	KT985656.1			Koski & Ashman 2016
Ivesia jaegeri	GB269			FR872983.1		Töpel et al. 2012
Ivesia kingii var. *kingii*	GB4782	FN421377.1	FN430787.1	FN561735.1		Töpel et al. 2011
Ivesia longibracteata	JEPS101			FR872937.1		Töpel et al. 2012
Ivesia lycopodioides var. *megalopetala*	RSA131949	KT985786.1	KT985657.1			Koski & Ashman 2016
Ivesia lycopodioides var. *scandularis*	RSA663921	KT985787.1	KT985658.1	KT991762.1		Koski & Ashman 2016
Ivesia paniculata	UC-Ptl6031			GQ384741.1		Dobes & Paule 2010
Ivesia pygmaea	GB341			FR872963.1	FR851335.1	Töpel et al. 2012
Ivesia pityocharis	UC1728514	KT985788.1	KT985659.1	KT991763.1		Koski & Ashman 2016
Ivesia rhypara var. *rhypara*	GB372			FR872953.1	FR851332.1	Töpel et al. 2012
Ivesia sabulosa	GB366			FR872956.1	FR851334.1	Töpel et al. 2012
Ivesia santolinoides	CM265071	KT985789.1	KT985660.1			Koski & Ashman 2016
Ivesia saxosa	UC1559752	KT985790.1	KT985661.1		FR851336.1	Koski & Ashman 2016

Ivesia sericoleuca	GB291			FR872989.1		Töpel et al. 2012
Ivesia setosa	CM480028	KT985791.1	KT985662.1	KT991764.1		Koski & Ashman 2016
Ivesia shockleyi var. *shockleyi*	GB228			FR872998.1		Töpel et al. 2012
Ivesia tweedyi	JEPS19303			FR872944.1		Töpel et al. 2012
Ivesia utahensis	CM361607	KT985792.1	KT985663.1			Koski & Ashman 2016
Ivesia unguiculata	JEPS19215			FR872952.1	FR851331.1	Töpel et al. 2012
Ivesia webberi	GB290			FR872988.1		Töpel et al. 2012
Potentilla chinensis	Feng110	KP875266.1	KP875298.1	KP875319.1		Feng et al. 2017

References

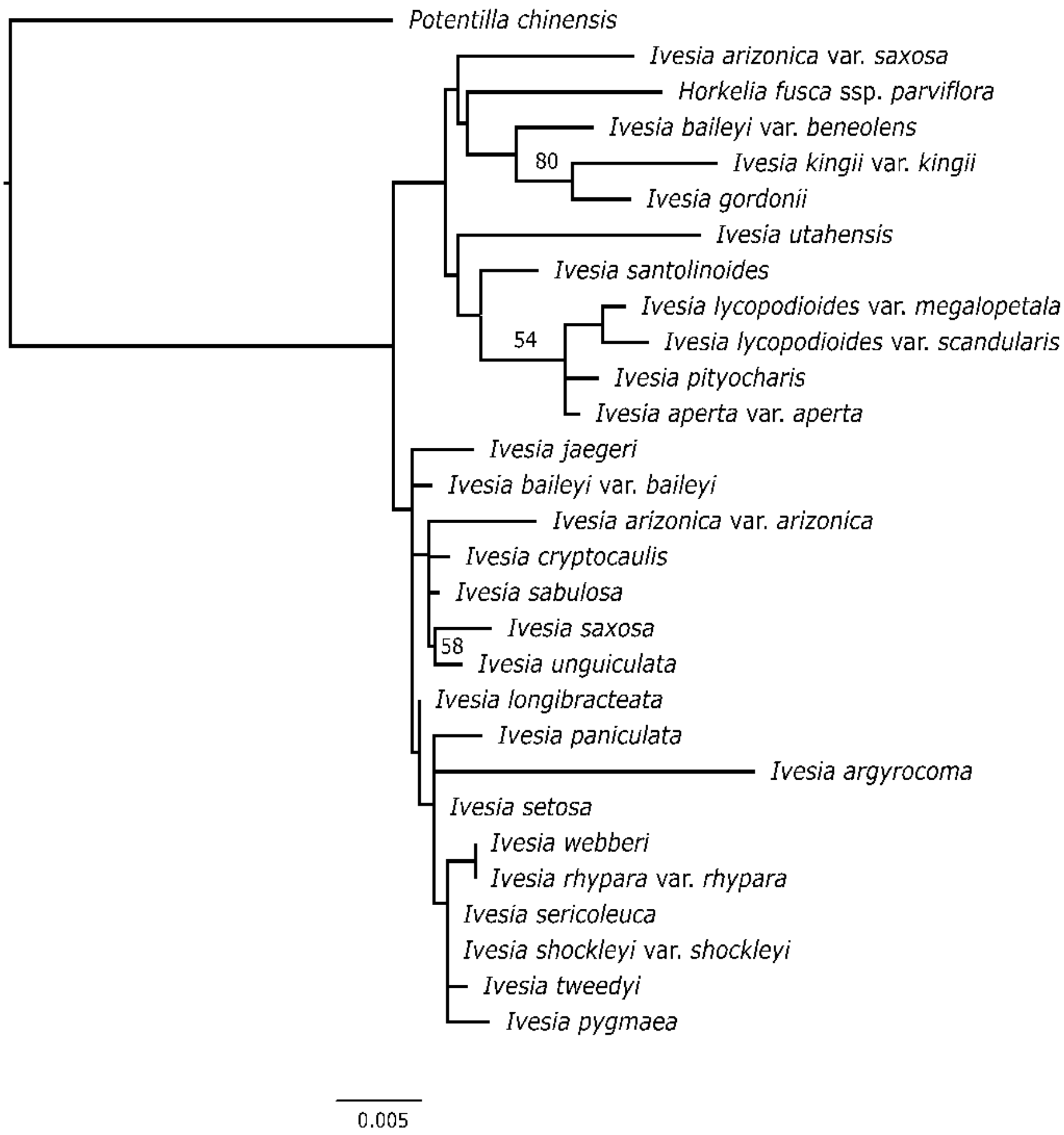
Dobeš, C., & Paule, J. (2010). A comprehensive chloroplast DNA-based phylogeny of the genus *Potentilla* (Rosaceae): Implications for its geographic origin, phylogeography and generic circumscription. *Molecular Phylogenetics and Evolution, 56*, 156–175. https://doi.org/10.1016/j.ympev.2010.03.005

Feng, T., Moore, M. J., Yan, M.-H., Sun, Y.-X., Zhang, H.-J., Meng, A.-P., Li, X.-D., Jian, S.-G., Li, J.-Q., & Wang, H.-C. (2017). Phylogenetic study of the tribe Potentilleae (Rosaceae), with further insight into the disintegration of *Sibbaldia*. *Journal of Systematics and Evolution*, *55*, 177-191. https://doi.org/10.1111/jse.12243

Koski, M. H., & Ashman, T.-L. (2016). Macroevolutionary patterns of ultraviolet floral pigmentation explained by geography and associated bioclimatic factors. *New Phytologist*, *211*, 708–718. https://doi.org/10.1111/nph.13921

Töpel, M. H., Antonelli, A., Yesson, C., & Eriksen, B. (2012). Past climate change and plant evolution in western North America: A case study in Rosaceae. *PLoS ONE*, *7*, e50358. https://doi.org/10.1371/journal.pone.0050358

Töpel, M., Lundberg, M., Eriksson, T., & Eriksen, B. (2011). Molecular data and ploidal levels indicate several putative allopolyploidization events in the genus *Potentilla* (Rosaceae). *PLoS Currents*, *16*, RRN1237. doi: 10.1371/currents.RRN1237.



Supplemental Figure 1. Supplemental Figure 1. *Ivesia* and *Horkelia fusca* ssp. *parviflora* maximum likelihood phylogenetic estimate from available nuclear (ETS and ITS) and chloroplast (matK and trnL) molecular markers. Non-parametric bootstraps greater than 50 were represented on the corresponding branches

CHAPTER 5 Geographical and inter-annual patterns of seed viability in the threatened cold desert perennial *Ivesia webberi*, and the prospect of non-destructive seed testing methods*

Israel T. Borokini[a], Kevin T. Shoemaker[b], Peter J. Weisberg[c], Mary M. Peacock[d]

[a]Program in Ecology, Evolution and Conservation Biology, Department of Biology, University of Nevada, Reno, Reno NV 89557

[b]Department of Natural Resources and Environmental Science, University of Nevada, Reno, Reno NV 89557

[c]Department of Natural Resources and Environmental Science, University of Nevada, Reno, Reno NV 89557

[d]Department of Biology, University of Nevada, Reno, Reno NV 89557

*Prepared for *Plant Ecology*

ABSTRACT

An estimated one-third of the globally threatened plant species have recalcitrant storage behavior, for which seed banking is relatively ineffective. Therefore, to understand seed storage behavior in *Ivesia webberi*, a threatened Great Basin Desert forb in the United States, we examined the effect of seed size and storage time on seed viability, evaluated inter-annual and inter-population variation in seed viability, and investigated the predictive accuracy of non-destructive seed viability testing methods (seed x-ray and multispectral imaging). The results showed a significant reduction in seed viability from three months to two years, suggesting that *I. webberi* seeds have recalcitrant storage behavior. Seed viability exhibited significant inter-annual, but not inter-population, variation across 11 *I. webberi* populations; seed size has no significant effect on seed viability. The x-ray and multispectral imaging methods had high classification accuracy (>80%) and could replace the widely used tetrazolium test, which destroys the seed germplasm, resulting in the cumulative reduction of stored seeds from periodic monitoring. This study demonstrates the utility of non-destructive methods for long-term seed viability monitoring, and shows that seed viability is not affected by population density which varies widely among the sampled *I. webberi* populations.

Keywords: *Ivesia webberi*, seed banking, seed viability, multispectral imaging, seed x-ray imagery

INTRODUCTION

Target 8 of the United Nations' Convention on Biological Diversity (CBD) Global strategy for plant conservation (GSPC) recommends *ex situ* conservation of at least 75% of plant species categorized as threatened nationally or globally, by 2020 (CBD 2019), as an insurance against extirpation and extinction of plant species in their natural habitats due to anthropogenic environmental changes (Meyer et al. 2014). Collecting and banking seeds is the oldest and most common *ex situ* conservation strategy for species management and global food security (Díez et al. 2018; Potter et al. 2017; Liu et al. 2018). There are thousands of local, national and international seed banks (Food and Agriculture Organization [FAO] 2010; Hay and Probert 2013).

However, the success of seed banking conservation programs depends upon the stored seeds staying viable over the long term (Gairola et al. 2019). The majority of crop species can tolerate seed desiccation without losing their viability (Roberts 1973), and as a result, these seeds can be stored for longer periods (Tweddle et al. 2003; Hay and Probert 2013), in contrast to seeds with recalcitrant behavior that lose their viability with time and desiccation. However, most of the threatened plants, the focus of the GSPC target 8, are wild plant species for which knowledge of their seed storage behavior is limited (Probert et al. 2009; Hay and Probert 2013; Wyse and Dickie 2017). An estimated 36% of threatened species on the IUCN Red List may have recalcitrant seed behavior, in which case seed banking will not be effective for their conservation (Wyse et al. 2018; Wyse and Dickie 2018). Therefore, an understanding of the seed storage behavior of threatened species is crucial for effective seed banking (Meyer et al. 2014).

Long-term study and monitoring of seed storage behavior requires repeated use of seed viability tests, but these are most commonly destructive methods, especially seedling emergence and tetrazolium tests. Using such methods that reduce the number of stored seeds over time is clearly prohibitive for rare species of conservation concern. As a result, there is an increased interest in and use of non-destructive methods for assessing seed viability in both seed research and industry (Baek et al. 2019). Non-destructive seed testing methods, such as the seed x-ray and multispectral imaging, reveal seed properties that are used as proxies for seed viability. For example, seed x-rays can be used to visualize seed development, morphology, anatomy and potential pest and pathogenic damage from which inferences are drawn about seed viability and behavior (Gagliardi and Marcos-Filho 2011; Costa et al. 2014). More recently, multispectral imaging has been used to assess seed health, moisture level (Baek et al. 2019), seed purity (Vrešak et al. 2016), fruit maturity, and detect pest damage (Boelt et al. 2018). The spectral regions covered in the multispectral imaging included the visible (380-780 nm) and near-infrared (780-2500 nm) regions (Huang et al. 2015; Boelt et al. 2018), producing a huge volume of data for several variables describing the testa chemical and spectral properties. However, large portions of the electromagnetic spectrum are likely to be redundant with respect to seed viability indicators, resulting in needlessly large computer storage demands and processing times (Baek et al. 2019). It would therefore be useful to employ variable selection methods to identify those spectral variables that contain the most important information for improving performance of seed viability detection models (Chen et al. 2014).

Ivesia webberi A. Gray is a federally threatened perennial forb, with a narrow distribution along the eastern foothills of the northern Sierra Nevada and northwestern margin of the Great Basin Desert (Figure 1). The species regenerates annually from a dormant root caudex in late winter or early spring but seed recruitment has also been observed (Witham 2000; Bergstrom 2009).

This study investigates whether (a) viability of *I. webberi* seeds remains stable or decreases with storage time, (b) there is significant inter-annual and inter-population variability in seed viability across multiple populations of varying abundance, (c) seed size is correlated with seed viability, and (d) multispectral imaging and x-ray imagery, both non-destructive methods, accurately predict viability of *I. webberi* seeds. Knowledge of seed behavior in *I. webberi* is unknown and therefore results of this study can be used to support management and conservation of this rare species.

MATERIALS AND METHODS

Experiment 1: The effect of storage time on the viability of *I. webberi* seeds

Seed collection and processing

Seeds were collected from the United States Fish and Wildlife service (USFWS) designated unit 5 *I. webberi* population (Figure 1), in August of 2017, 2018 and 2019 when matured seeds were ready for abscission. The collections were processed to remove empty seeds, and the remaining healthy seeds were stored under cool, dry conditions, in coin envelopes.

Seed viability test

A tetrazolium test (hereafter, TZ test) was used to evaluate seed viability following protocols described in Nurse and DiTommaso (2005), Gosling et al. (2009) and França-Neto and Krzyzanowski (2019), and was conducted at the Idaho State seed laboratory, Boise Idaho.

Data collection and analysis

We fitted a logistic regression model to test the effect of storage duration on viability of *I. webberi* seeds. Viability of individual seeds collected between 2017 and 2019 (0 = non-viable, and 1 = viable) was modeled as a function of storage time, treated as a categorical variable with three levels: 0, 1 and 2 years in storage. A Tukey's HSD test was used to perform post-hoc pairwise comparisons (Abdi and Williams 2010). Data analysis for this and all subsequent analyses were conducted using R (R Core Team 2019).

Experiment 2: Population-level difference in the viability of *I. webberi* seeds

Seed collection, processing, and viability test

Seeds were collected from 11 *I. webberi* populations of varying population sizes and geographical distances to each other (Figure 1, Table 1), in August of 2017 and 2018. Depending on the population size, between 50 and 100 seeds were collected from each population, cleaned and sorted as described in experiment 1. The remaining healthy seeds were stored under cool, dry conditions, for eight months in coin envelopes, followed by seed viability testing to investigate inter-annual variability in seed viability across the 11 populations. Seed viability was assessed using a TZ test following the protocol described in experiment 1.

Data collection and analysis

Seed sample size for the 2017 collection ranged from 25 to 33, and from the 2018 collection, ranged from 26 to 45 seeds, due to the limitation on the numbers of seeds that could be collected from a threatened plant species, and large number of empty seeds (Table 1). We fitted separate logistic regression models and Tukey's HSD post-hoc multiple comparisons for seeds collected in 2017 and 2018 to determine if seed viability was different among the 11 sampled populations. We also fitted a generalized linear mixed model for both years combined to investigate inter-annual variability in seed viability, using years and populations as fixed and random effects respectively.

Experiment 3: Is there a relationship between seed size and seed viability?

Seed collection and processing

A total of 441 healthy seeds collected in 2018, and used in part for experiment 2, were also used for this experiment (see Table 1).

Seed size estimation

Seed size was estimated as the product of seed length and width. Seed length and width were estimated from vertical and horizontal dimensions respectively for images of each seed. The dimensions of each seed were measured using the videometerLab 3 instrument (Videometer A/S, Hørsholm, Denmark) at Skyway Analytics LLC, Longmont, Colorado [https://getskywayanalytics.com/], as part of the multispectral imaging.

Data collection and analysis

To investigate a statistical relationship between seed size and viability, we conducted a logistic regression with 70% of the data and used the remaining 30% for model evaluation.

Experiment 4: Can the non-destructive x-ray and multispectral imaging data accurately discriminate between viable and non-viable seeds?

Seed collection and processing

Seed lots used for experiment 3 were also used for this experiment. In total, we used 441 seeds for this experiment (Table 1).

Non-destructive seed imaging

The x-ray imaging was conducted at the United States Forestry Service (USFS) seed extractory, Bend, Oregon, following methods described in Gomes et al. (2016). The x-ray images were captured using a digital Kubtec medical imaging Xpert 40 specimen radiography system, for each individual seed, exposed to a radiation intensity of 26 kV for 1.2 seconds.

Multispectral imaging was conducted at Skyway Analytics LLC, Longmont, Colorado [https://getskywayanalytics.com/]. Digital images were captured with a VideometerLab 3 instrument (Videometer A/S, Hørsholm, Denmark) for each seed in a petri dish (90 mm) with dish cover removed to avoid reflection during image capturing

(Halkjaer Olesen et al. 2011; Su and Sun 2018). The multispectral images of 1280 x 960 pixels were captured at 26 different spectral bands from ultraviolet to near-infrared wavelengths ranging from 375 nm for multicolorMean0 to 970 nm for multicolorMean18. In addition, standard features related to seed size, shape, orientation and color were also collected. In total, 42 variables were obtained from the multispectral imaging describing the seed dimensions and testa reflectance properties.

Seed viability test

We used the TZ test results for seed viability to represent true seed viability in our statistical analyses. TZ test is recognized by the Association of Official Seed Analysts and the International Seed Testing Association as a high precision and accuracy test of seed vigor (França-Neto and Krzyzanowski 2019).

Data collection and analysis

The seed x-ray images were scored based on whether the seeds were “filled” or not. Filled seeds were assumed to have matured embryos, hence viable and scored 1, while unfilled seeds were considered non-viable, and scored 0. The 42 variables from the multispectral imaging and x-ray imagery scores were used as predictors, while seed viability from the TZ test was used as the binary response variable. The 43 variables were reduced to 21 following a feature selection run that uses a wrapper algorithm designed for Random Forest, implemented in the *Boruta* R package (Kursa and Rudnicki 2010). The 21 predictor variables were further reduced to three variables using the backward stepwise recursive feature elimination algorithm in the *caret* R package (Kuhn 2019). These three uncorrelated selected variables – seed x-ray imagery, seed width and seed spectral reflectance at 690 nm (multicolor mean 13) - were used to build a final predictive model of seed viability.

We fitted a random forest classification model (*ntree* = 500, *mtry* = 2) to the three selected variables using the *party* package (Hothorn et al. 2006) with supporting utility functions written by KTS. Variable importance was assessed as the loss of predictive accuracy (Gini statistic) when random permutations of each predictor variable were performed for randomly drawn samples (Cutler et al. 2007). Partial dependence plots were used to illustrate the relationship between each of the three predictors and seed viability (Friedman 2001). We used a 10-fold cross validation to assess overall predictive performance (Cutler et al. 2007), using the area under the receiver operating characteristic curve (AUC; *ROCR* package in R; Sing et al. 2005) as the primary performance metric (Fielding and Bell 1997).

RESULTS

The effect of storage time on the viability of *I. webberi* seeds

The viability of *I. webberi* seeds decreased with storage time. Seeds collected and stored for three months had 86% viability, while seeds stored for one and two years had 53% and 34% viability, respectively. A post-hoc pairwise test showed significant pairwise differences in seed viability between samples stored for three months and those stored for one and two years ($z = -3.33$, $p < 0.01$, $z = -4.91$, $p < 0.01$).

Inter-annual and population-level differences in the viability of *I. webberi* seeds

The results demonstrate significant inter-annual variability ($p < 0.01$) in the viability of *I. webberi* seeds between the two years. Viability was lower in seeds collected in 2017 than those collected in 2018. For example, only three populations had ≥50% seed viability in 2017 collections, in contrast to nine populations in 2018 (Table 1). In most of the populations, seed viability was inconsistent from 2017 to 2018 (Table 1).

Seed viability varied across the 11 sampled populations for both 2017 and 2018 collections, but this variation was significantly different for only a few of the populations. For the 2017 collections, the Tukey HSD post-hoc tests resulted in only three significant pairwise differences between units 3 and 11, 3 and 14, and 5 and 14 (adjusted $p < 0.005$). For the 2018 collections, post-hoc tests support a significant pairwise difference only between units 7 and 8 (adjusted $p < 0.005$).

Is there a relationship between seed size and seed viability?

A large number of the viable *I. webberi* seeds were relatively small (Figure 2, Supplemental Table 1), but the logistic regression model showed that seed size had no significant effect on the viability of the seeds ($z = -1.57$, $p > 0.12$).

Could the seed testa spectral properties and x-ray imagery reliably predict viability of *I. webberi* seeds?

Of the 441 individual seeds used for the TZ test, 260 were categorized as viable, while the remaining 181 were non-viable. The combination of x-ray imagery, seed width, and spectral properties reliably predicted seed viability (AUC > 0.8). Simple t-tests for viable and nonviable seeds conducted between mean values for seed x-ray, seed area and MCM 13 were significantly different at $p < 0.01$ (Supplemental Table 1). The Random Forest model produced high predictive performance (accuracy: 0.8163, specificity: 0.9308, sensitivity: 0.6519, kappa: 0.6056); seed x-ray imagery had the highest variable importance, followed by seed width and MCM 13, with 16% and 13% of the predictive power of the x-ray imagery respectively. Univariate partial dependence plots showed that the probability of *I. webberi* seed viability increases with decreasing seed testa spectral reflectance at 690 nm (Figure 3a), filled seeds in the x-ray imagery (Figure 3b) and smaller seed width (Figure 3c). The AUC values for the training and cross-validated data were 0.91 and 0.81 respectively.

DISCUSSION

Our data showed that viability of *I. webberi* seeds is reduced as storage time increases. This suggests that *I. webberi* seeds are non-dormant at abscission, and that this species has a potentially recalcitrant storage behavior, which is common in many perennial plant species (Baldos et al. 2014; Duncan et al. 2019), including Great Basin Desert perennial species (Allen and Nowak 2008). Furthermore, seeds that have recalcitrant storage behavior are likely to form a transient seed bank *in situ* (Guo et al. 1998; Tweddle et al. 2003; Tonetti et al. 2015), an ecological feature that has been observed for *I. webberi*. In this study, viability loss could be attributed to seed aging from light exposure (Ellis et al. 2008; Schwember and Bradford 2011), which mimics what could be expected in natural habitats. However, further studies are needed to investigate the tolerance of *I. webberi* seeds to desiccation which are required for long-term storage. Furthermore, long-term seed viability monitoring under low temperature storage is needed to reliably assess *I. webberi* seed storage behavior.

This study showed significant temporal variability in the viability of *I. webberi* seeds, which suggest the impacts of climatic and environmental conditions and genetic factors not accounted for in this study on seed viability. This agrees with previous studies that have reported significant interannual variability in seed viability for many species across different ecosystems, which was attributed to differing reproduction strategy, pollinator abundance, climatic factors (Morgan 2001; Giménez-Benavides et al. 2005; Stuble et al. 2017; Daskalakou et al. 2017; Barga et al. 2017).

Despite the wide-ranging differences in population size, seed viability was not significantly different across the sampled *I. webberi* populations. Therefore, seed viability does not appear to be negatively affected by small population size in *I. webberi*. The EHJ population with the lowest density consistently had higher seed viability in the two years of sampling. This is contrary to previous studies that have reported significantly higher seed viability for larger population sizes (e.g., Morgan 2001), but studies also show that environmental conditions can impact seed viability regardless of population size (Morgan 2001). Moreover, some studies have shown that small populations of species that exhibit a mixed breeding strategy could still produce a high number of viable seeds (Mayer et al. 1996; Baldwin and Schoen 2019) by delaying selfing till end of the flowering season when chances of cross-pollination have become reduced (Kalisz and Vogler 2003; Hildesheim et al. 2019). Therefore, such species could produce seeds from different breeding strategies possibly with varying recruitment potentials. Therefore, it may be necessary to investigate the viability of seeds produced from possible xenogamous, geitonogamous and autogamous pollination.

This study also showed that non-destructive seed viability tests can replace the more widely used tests that result in the loss of seed viability, an important finding especially for rare and endangered plant species. The seed x-ray imaging, which distinguishes between filled and empty seeds, contributed the most to the random forest model, despite previously reported limitations of its use for seeds with small size or round shape (Bruggink and van Duijn 2017). This suggests that filled, well-developed and undamaged *I. webberi* seeds could be used as a proxy for viability and consequently, higher germination potential (Costa et al. 2014). Many studies have also reported the accuracy of seed x-ray images for predicting seed viability (e.g., Costa et al. 2014; Alencar et al. 2016; Gomes et al. 2016; Kim et al. 2017). Riebkes et al. (2015) found no significant accuracy difference in estimating seed viability from seedling emergence, TZ test, and seed x-ray images for three studied species. Moreover, exposure to radiation from the seed x-ray test was reported to have minimal effect on seed health and germination (Bino et al. 1993; Young et al. 2007).

Of the 42 variables obtained from the multispectral imaging, MCM 13 was the only selected spectral variable from the variable selection runs. Viable seeds had significantly lower MCM 13 spectral values than the non-viable seeds (Supplemental Table 1, Figure 3a), suggesting the usefulness of this spectral feature for classification and separation of viable from non-viable seeds. At the 690 nm wavelength for MCM 13, non-viable seeds have stronger fluorescent intensity which is associated with higher chlorophyll *a* content (Cerovic et al. 1999; Li et al. 2019) and oxidation, both of which have been linked to seed germination biochemical processes (El-Maarouf-Bouteau et al. 2013), reduced tolerance to abiotic stress, and reduced germination potential (Dell'Aquila 2009; Smolikova et al. 2011; Boelt et al. 2018; Li et al. 2019).

Non-destructive seed x-ray imaging and multispectral imaging at 690 nm wavelength compare well with the destructive seedling emergence and TZ tests, and therefore justify their use for seed vigor testing, which can reduce the cumulative long-term seed loss for threatened plant species that produce limited numbers of healthy seeds. Though not documented, we observed that the majority of *I. webberi* seed collections in the two years across the sampled populations were empty seeds, which underscores the need for long-term preservation of the few, viable seeds. Furthermore, the recalcitrant seed storage behavior highlights the challenges of seed banking for *I. webberi*, and recommends more focus on *in situ* management for this species. However, given the increase in disturbance and invasion by alien weeds across the Great Basin, seed banking has become an inevitable conservation strategy. This study showed little inter-population variability in seed viability. Therefore, collections for seed banking could be made from few selected populations with consistently higher viability, as guided by the population genetic structure of this species, to capture genetic diversity in the stored viable seeds. However, further studies are recommended to estimate the viability of seeds produced from different breeding strategies, and to evaluate storage behavior under long-term seed banking.

REFERENCES

Abdi H, Williams LJ (2010) *Tukey's Honestly Significant Difference (HSD) test*. Encyclopedia of Research Design, Sage, Thousand Oaks, CA., pp. 1-5.

Alencar NE, dos Reis Figueira A, dos Santos HO, Duarte PSG (2016) Image analysis and physiological quality assessment of seeds produced in pumpkin plants infected with the squash mosaic virus (SqMV). Res J Seed Sci 9: 14-21. 10.3923/rjss.2016.14.21

Allen EA, Nowak RS (2008) Effect of pinyon-juniper tree cover on the soil seed bank. Range Ecol Mgt 61: 63–73. https://doi.org/10.2111/07-007R2.1

Baek I, Kusumaningrum D, Kandpal LM, Lohumi S, Mo C, Kim MS, Cho B-K (2019) Rapid measurement of soybean seed viability using kernel-based multispectral image analysis. Sensors 19(2): 271. https://doi.org/10.3390/s19020271

Baldos OC, DeFrank J, Kramer M, Sakamoto GS (2014) Storage humidity and temperature affect dormancy loss and viability of Tanglehead (*Heteropogon contortus*) seeds. HortSci 49(10): 1328-1334. https://doi.org/10.21273/HORTSCI.49.10.1328

Baldwin SJ, Schoen DJ (2019) Inbreeding depression is difficult to purge in self-incompatible populations of *Leavenworthia alabamica*. New Phytol 224: 1330–1338. https://doi.org/10.1111/nph.15963

Barga S, Dilts TE, Leger EA (2017). Climate variability affects the germination strategies exhibited by arid land plants. Oecologia 185: 437-452. https://doi.org/10.1007/s00442-017-3958-5

Bino RJ, Aartse JW, Burg WJ van der. (1993) Non-destructive x-ray analysis of *Arabidopsis* embryo mutants. Seed Sci Res 3(3): 167-170. https://doi.org/10.1017/S0960258500001744

Boelt B, Shrestha S, Salimi Z, Jørgensen JR, Nicolaisen M, Carstensen JM (2018) Multispectral imaging – a new tool in seed quality assessment? Seed Sci Res 28: 222–228. https://doi.org/10.1017/S0960258518000235

Bruggink H, van Duijn B. (2017) X-ray based seed analysis. Seed Test Int 153: 45-50.

Cerovic ZG, Samson G, Morales F, Tremblay N, Moya I. (1999) Ultraviolet-induced fluorescence for plant monitoring: Present state and prospects. Agronomie 19(7): 543–578. https://doi.org/10.1051/agro:19990701

Chen Z, Tang M-L, Shi N-Z (2014) New robust variable selection methods for linear regression models. Scand J Stat 41(3): 725-741. https://doi.org/10.1111/sjos.12057

Convention on Biological Diversity [CBD] (2019) Global strategy for plant conservation: targets 2011–2020 [Internet]. [Cited 30 Dec 2019]. Available from: http://www.cbd.int/gspc/targets.shtml

Costa DS, Kodde J, Groot SPC (2014) Chlorophyll fluorescence and X-ray analyses to characterise and improve paddy rice seed quality. Seed Sci Tech 42: 449-453. https://doi.org/10.15258/sst.2014.42.3.11

Cutler DR, Edwards TC, Beard KH, Cutler A, Hess KT (2007) Random forests for classification in ecology. *Ecology* 88: 2783-2792. https://doi.org/10.1890/07-0539.1

Daskalakou E, Koutsovoulou K, Mavroeidi L, Tsiamitas C, Kafali E, Radaiou P, Ganatsas P, Thanos C (2018) Interannual variability of germination and cone/seed morphometric characteristics in the endemic Grecian fir (*Abies cephalonica*) over an 8-year-long study. Seed Sci Res 28: 24-33. https://doi.org/10.1017/S0960258517000290

Dell'Aquila A (2009) Development of novel techniques in conditioning, testing and sorting seed physiological quality. Seed Sci Tech 37: 608–624. https://doi.org/10.15258/sst.2009.37.3.10

Díez MJ, De la Rosa L, Martín I, Guasch L, Cartea ME, Mallor C, Casals J, Simó J, Rivera A, Anastasio G, Prohens J, Soler S, Blanca J, Valcárcel JV, Casañas F (2018) Plant genebanks: present situation and proposals for their improvement: the case of the Spanish network. Front Plant Sci 9: 1794. https://doi.org/10.3389/fpls.2018.01794

Duncan C, Schultz N, Lewandrowski W, Good MK, Coo S (2019) Lower dormancy with rapid germination is an important strategy for seeds in an arid zone with unpredictable rainfall. PLoS ONE 14(9): e0218421. https://doi.org/10.1371/journal.pone.0218421

Ellis RH, Hong TD, Roberts EH (2008) Seed moisture content, storage, viability and vigor. Seed Sci Res 1: 275–279. https://doi.org/10.1017/S0960258500001008

El-Maarouf-Bouteau H, Meimoun P, Job C, Job D, Bailly C (2013) Role of protein and mRNA oxidation in seed dormancy and germination. Front Plant Sci 4: 77. https://doi.org/10.3389/fpls.2013.00077

Fielding AH, Bell JF (1997) A review of methods for the assessment of prediction errors in conservation presence/absence models. Env Cons 24 (1): 38-49. https://doi.org/10.1017/S0376892997000088

Food and Agriculture Organization of the United Nations [FAO] (2010) *The Second Report on the state of the world's plant genetic resources for food and agriculture*. Commission on genetic resources for food and agriculture, Food and Agriculture Organization of the United Nations, Rome.

França-Neto J de Barros, Krzyzanowski FC (2019) Tetrazolium: an important test for physiological seed quality evaluation. J Seed Sci 41(3): 359-366. https://dx.doi.org/10.1590/2317-1545v41n3223104

Friedman JH (2001) Greedy function approximation: A gradient boosting machine. Ann Stat 29 (5): 1189-1232. https://doi.org/10.1214/aos/1013203451

Gagliardi B, Marcos-Filho J (2011) Relationship between germination and bell pepper seed structure assessed by the X-ray test. Scient Agricol 68 (4): 411-416. https://doi.org/10.1590/S0103-90162011000400004

Gairola S, Shabana HA, Mahmoud T, El-Keblawy A, Santo A (2019) Evaluating germinability of eight desert halophytes under long-term seed storage: Implications for conservation. Plant Div 41(4): 229–236. https://doi.org/10.1016/j.pld.2019.07.002

Giménez-Benavides L, Escudero A, Pérez-García F (2005) Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability. Ecol Res 20(4): 433-444. https://doi.org/10.1007/s11284-005-0059-4

Gomes KBP, Matos JMM, Martins IS, de Carvalho Cristo Martins R (2016) X-ray test to evaluate the physiological potential of *Platypodium elegans* seeds. Res J Seed Sci 9: 29-35. 10.3923/rjss.2016.29.35

Guo Q, Rundel PW, Goodall DW (1998) Horizontal and vertical distribution of desert seed banks: patterns, causes, and implications. J Arid Env 38: 465-478. https://doi.org/10.1006/jare.1997.0353

Halkjaer Olesen M, Carstensen JM, Boelt B (2011) Multispectral imaging as a potential tool for seed health testing of spinach (*Spinacia oleracea* L.). Seed Sci Tech 39: 140-150. https://doi.org/10.15258/sst.2011.39.1.12

Hay FR, Probert RJ (2013) Advances in seed conservation of wild plant species: a review of recent research. Cons Physiol 1: 1–11. https://doi.org/10.1093/conphys/cot030

Hildesheim LS, Opedal ØH, Armbruster WS, Pélabon C (2019) Fitness costs of delayed pollination in a mixed-mating plant. Ann Bot 124(5): 869–881. https://doi.org/10.1093/aob/mcz141

Hothorn T, Buehlmann P, Dudoit S, Molinaro A, Van Der Laan M (2006) Survival Ensembles. Biostatistics 7(3): 355--373. https://doi.org/10.1093/biostatistics/kxj011

Huang M, Wang QG, Zhu QB, Qin JW, Huang G (2015) Review of seed quality and safety tests using optical sensing technologies. Seed Sci Tech 43: 337–366. https://doi.org/10.15258/sst.2015.43.3.16

Kalisz S, Vogler DW (2003) Benefits of autonomous selfing under unpredictable pollinator environments. Ecology 84: 2928–2942. https://doi.org/10.1890/02-0519

Kim JH, Kim KO, Lee AK, Roh MS, Suh JK (2017) Germination of Corylopsis seeds evaluated by X-ray imaging and cold stratification. Hort Sci 44: 105–111. https://doi.org/10.17221/194/2015-HORTSCI

Kursa MB, Rudnicki WR (2010) Feature Selection with the Boruta Package. J Stat Soft 36(11): 1-13. URL http://www.jstatsoft.org/v36/i11/

Li Y, Sun J, Wu X, Chen Q, Lu B, Dai C (2019) Detection of viability of soybean seed based on fluorescence hyperspectra and CARS-SVM-AdaBoost model. J Food Process Preserv 43(12): e14238. https://doi.org/10.1111/jfpp.14238

Liu U, Breman E, Cossu TA, Kenney S (2018) The conservation value of germplasm stored at the Millennium Seed Bank, Royal Botanic Gardens, Kew, UK. Biodiv Cons 27: 1347–1386. https://doi.org/10.1007/s10531-018-1497-y

Mayer SS, Charlesworth D, Meyers B (1996) Inbreeding depression in four populations of *Collinsia heterophylla* Nutt. (Scrophulariaceae). Evolution 50(2): 879-891. https://doi.org/10.1111/j.1558-5646.1996.tb03896.x

Meyer E, Jensen N, Fraga N (2014) Seed banking California's rare plants. *California Fish and Game* 100(1): 79-85

Morgan JW (2001) Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. Cons Biol 13(2): 266-273. https://doi.org/10.1046/j.1523-1739.1999.013002266.x

Potter KM, Jetton RM, Bower A, Jacobs DF, Man G, Hipkins VD, Westwood M (2017) Banking on the future: progress, challenges and opportunities for the genetic conservation of forest trees. New For 48(2): 153-180. https://doi.org/10.1007/s11056-017-9582-8

Probert RJ, Daws MI, Hay FR (2009) Ecological correlates of ex situ seed longevity: a comparative study on 195 species. Ann Bot 104 (1): 57–69. https://doi.org/10.1093/aob/mcp082

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Riebkes JL, Barak RS, Kramer AT (2015) Evaluating seed viability in prairie forbs: a test of three methods. Native Plants 16(2): 96-106

Roberts EH (1973) Predicting the storage life of seeds. Seed Sci Tech. 1: 499-514.

Schwember AR, Bradford KJ (2011) Oxygen interacts with priming, moisture content and temperature to affect the longevity of lettuce and onion seeds. Seed Sci Res 21: 175–185. https://doi.org/10.1017/S0960258511000080

Sing T, Sander O, Beerenwinkel N, Lengauer T (2005) ROCR: visualizing classifier performance in R. *Bioinformatics* 21 (20): 7881. <URL: http://rocr.bioinf.mpi-sb.mpg.de>.

Smolikova GN, Laman NA, Boriskevich OV (2011) Role of chlorophylls and carotenoids in seed tolerance to abiotic stressors. Rus J Plant Physiol 58(6): 965–973. https://doi.org/10.1134/S1021443711060161

Stuble KL, Fick SE, Young TP (2017) Every restoration is unique: testing year effects and site effects as drivers of initial restoration trajectories. J Appl Ecol 54: 1051-1057. https://doi.org/10.1111/1365-2664.12861

Su W-H, Sun D-W (2018) Multispectral imaging for plant food quality analysis and visualization. Comp Rev Food Sci Food Saf 17(1): 220-239. https://doi.org/10.1111/1541-4337.12317

Tonetti OAO, Faria JMR, José AC, Oliveira TGS, Martins JC (2015) Seed survival of the tropical tree *Cryptocarya aschersoniana* (Lauraceae): consequences of habitat disturbance. Austral Ecol. 41(3): 248-254. https://doi.org/10.1111/aec.12305

Tweddle JC, Dickie JB, Baskin CC, Baskin JM (2003) Ecological aspects of seed desiccation sensitivity. J Ecol 91(2): 294-304. https://doi.org/10.1046/j.1365-2745.2003.00760.x

United States Fish and Wildlife Services [USFWS] (2014). *Species Report for Ivesia webberi (Webber's ivesia).* Nevada Fish and Wildlife Office, Reno, 49 pp.

Vrešak M, Halkjaer Olesen M, Gislum R, Bavec F, Ravn Jørgensen J (2016) The use of image-spectroscopy technology as a diagnostic method for seed health testing and variety identification. PLoS ONE 11(3): e0152011. https://doi.org/10.1371/journal.pone.0152011

Wyse SV, Dickie JB (2017) Predicting the global incidence of seed desiccation sensitivity. J Ecol 105(4): 1082-1093. https://doi.org/10.1111/1365-2745.12725

Wyse SV, Dickie JB (2018) Taxonomic affinity, habitat and seed mass strongly predict seed desiccation response: a boosted regression trees analysis based on 17, 539 species. Ann Bot 121(1): 71–83. https://doi.org/10.1093/aob/mcx128

Young LW, Parham C, Zhong Z, Chapman D, Reaney MJT (2007) Non-destructive diffraction enhanced imaging of seeds. J Exp Bot 58(10): 2513-2523. https://doi.org/10.1093/jxb/erm116

Table 1. Location, properties and tetrazolium mean seed viability of the seed collections from 11 *Ivesia webberi* population sites in the western Great Basin Desert, United States

Unit[a]	**Site location**	**County and State**	**Site area (m^2)**[b]	**Elevation (m)**[c]	**Abundance estimate**[d]	**Sample size (2017)**	**Mean ± SE viability (2017)**	**Sample size (2018)**	**Mean ± SE viability (2018)**
2	Near Constantia	Lassen CA	7,700	1435.97	100-999	31	0.20 ± 0.07	40	0.00 ± 0.00
3	East of Hallelujah Junction	Lassen CA	1,400	1561.68	115-130	31	0.68 ± 0.09	39	0.62 ± 0.08
5	Dog Valley Meadows	Sierra CA	289,700	1834.70	100,000	25	0.64 ± 0.10	45	0.53 ± 0.08
6	White Lake Overlook	Sierra CA	54,900	1715.15	10,000	30	0.47 ± 0.09	45	0.64 ± 0.07
7	Mules Ear flat	Sierra CA	1,400	1680.01	<100	27	0.33 ± 0.09	35	0.83 ± 0.06

8	Ivesia flat	Washoe NV	3,000	1775.63	100,000	27	0.44 ± 0.10	26	0.46 ± 0.10
11	Hungry Valley	Washoe NV	600	1599.01	2,120	33	0.15 ± 0.06	38	0.63 ± 0.08
12	Black Springs	Washoe NV	25,500	1730.17	>500-1000	31	0.52 ± 0.09	45	0.69 ± 0.07
13	Raleigh Heights	Washoe NV	38,600	1602.60	<100,000-4,000,000	30	0.23 ± 0.08	44	0.66 ± 0.07
14	Dutch Louie flat	Washoe NV	5,500	1922.55	600,000-693,795	30	0.07 ± 0.05	41	0.68 ± 0.07
16	Dante Mine Road	Douglas NV	2,300	1894.81	3,179-36,500	30	0.23 ± 0.08	43	0.70 ± 0.07

---

[a] USFWS unit assignment for the *I. webberi* populations (see USFWS 2014); [b] Site size was calculated from USFWS (2014); [c] Elevation was extracted from 30 m digital elevation model of (USGS 2016) in ArcMap; [d] Abundance estimate for each population was sourced from USFWS (2014).

FIGURE CAPTIONS

Figure 1. Global distribution of *Ivesia webberi* populations. Unit numbers follow the USFWS designations, circles represent the geographic center of extant, mapped occurrences, and circles with same color indicate USFWS-designated subpopulations of the same population. Asterisk on unit 17 indicates it is a new proposed unit, as it was recently discovered.

Figure 2. Box plot showing the relationship between seed area and viability for *Ivesia webberi*. Viability was determined using the tetrazolium test.

Figure 3 (a-c). Univariate plots depicting seed viability for each of the three predictor variables computed from a random forest model for non-destructive *Ivesia webberi* seed viability classification


120°0'0"W
40°0'0"N
39°0'0"N
Unit 1
Unit 2
Unit 3
Unit 4
Unit 5
Unit 6
Unit 7
Unit 8
Unit 9
Unit 10
Unit 11
Unit 12
Unit 13
Unit 14
Unit 15
Unit 16
Unit 17*
Reno
Carson City
Lake Tahoe
Nevada
California
CALIFORNIA
NEVADA
WASATCH RANG
an Francisco
SIERRA NEVADA
0 5 10 20 30 40 Kilometers
1 cm = 7 km
N

Figure 1.

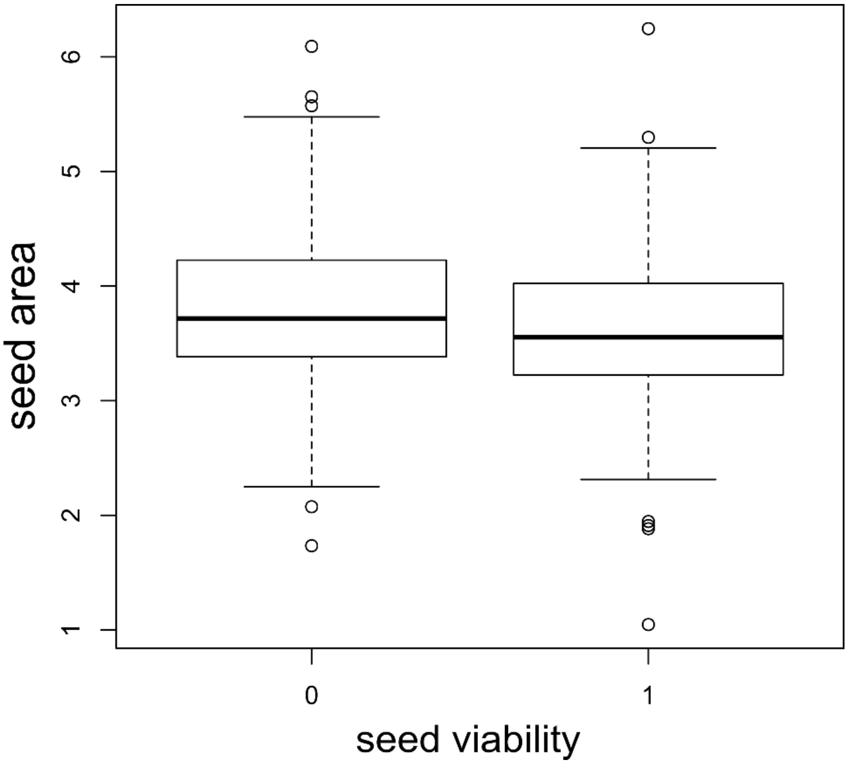


Figure 2.

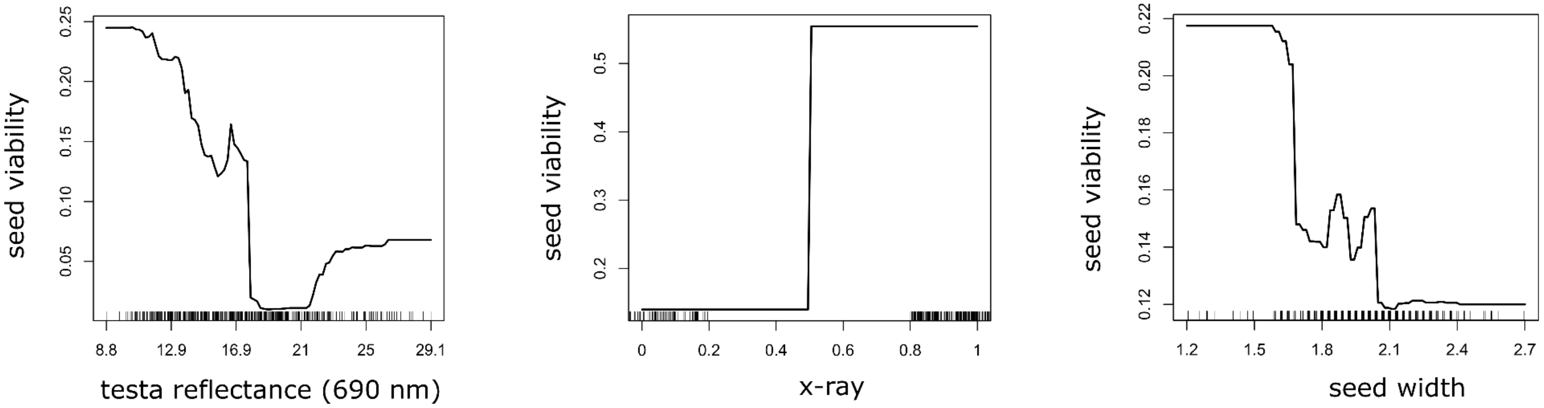

seed viability
0.05
0.10
0.15
0.20
0.25
8.8
12.9
16.9
21
25
29.1
testa reflectance (690 nm)
seed viability
0.2
0.3
0.4
0.5
0
0.2
0.4
0.6
0.8
1
x-ray
seed viability
0.12
0.14
0.16
0.18
0.20
0.22
1.2
1.5
1.8
2.1
2.4
2.7
seed width

Figure 3 (a-c).

ACKNOWLEDGEMENT: The authors are grateful to Thomas Dilts for help with making the map of *Ivesia webberi* distribution, and to Ms. Tara de Queiroz for her review of the manuscript.

Funding: This study was funded by a gift to the University of Nevada, Reno Foundation by StoneGate: A Heinz Ranch Land Company, LLC Development

Availability of data and R codes: All data and R codes used for this study are uploaded in the Knowledge Network for Biocomplexity: doi:10.5063/F1Q52N09.

SUPPLEMENTAL INFORMATION

Supplemental Table 1. Seed x-ray imagery and multispectral reflectance variables used to construct random forest tree model on *Ivesia webberi* seed viability

Predictors	**Viable seeds**		**Nonviable seeds**		**T-test**	**Predictor description**
	Mean±SD	**Range**	**Mean±SD**	**Range**		
Seed area	3.62±0.65	1.05 – 6.25	3.79±0.74	1.73 – 6.09	$p < 0.001$	Computed in mm^2 from both vertical and horizontal dimensions of seed image
Seed x-ray	0.97±0.18	0.00 – 1.00	0.53±0.50	0.00 – 1.00	$p < 0.001$	Binary score of 0 and 1 for unfilled and filled seeds respectively, based on likelihood of presence of seed embryo
Multicolor mean 13	16.50±4.30	8.84 – 29.09	18.09±3.95	9.65 – 27.75	$p < 0.001$	Seed testa spectral reflectance value obtained using 690 nm wavelength

SYNTHESIS AND CONCLUSION

Israel T. Borokini

Program in Ecology, Evolution and Conservation Biology, Department of Biology, University of Nevada, Reno

This five-chapter dissertation has increased scientific knowledge on *Ivesia webberi*, a federally threatened perennial forb in the Great Basin Desert. These integrated studies have revealed (1) an ecological niche driven by climatic and topographic variables, (2) that the soil seed bank is dominated by invasive plant species, which reduced the floristic similarity with the aboveground vegetative communities in sites harboring *I. webberi*, (3) low genetic diversity, reduced gene flow, increased genetic differentiation among the sampled populations, as well as significant spatial genetic structure, (4) that *I. webberi*, like other *Ivesia* taxa, are diploid with 28 chromosomes and the genome size has potentially adaptive significance, and (5) that the viability of *I. webberi* seeds can exhibit significant temporal variation, and can be reliably monitored using nondestructive methods. Furthermore, this dissertation also resulted in the discovery of several new locations of the species and expanded the distribution range by 65 km. These studies also confirmed that *I. webberi* undergoes both vegetative and sexual reproduction, which underscores the importance of regeneration from its transient soil seed bank on maintaining genetic diversity.

Biodiversity is inherently multifaceted, covering taxonomic, functional, phylogenetic, genetic, landscape, and many other elements of variability of life on the Earth; therefore, it is best understood through integrated, multi-empirical, and interdisciplinary studies (Stevens and Gavilanez, 2015; Naeem et al., 2016). This multi-empirical dissertation covered the hierarchical and integrated nature of biodiversity to inform the conservation of a federally threatened perennial forb species and its habitat in the Great Basin Desert. For example, the geographical prediction map from the niche modeling of *I. webberi* was integrated into the landscape genetics study to test the effect of isolation by landscape resistance on gene flow and genetic structure. The ecological predictor variables used for the niche modeling were also used to test the isolation by environment hypothesis on gene flow. Ground truthing of the geographical prediction maps and iterative niche modeling resulted in the discovery of several novel locations that may contain important genetic information that can improve the landscape genetics study. The floristic community diversity data obtained from Borokini et al. (2021) was used to test genetic-species diversity relationship. The use of microsatellite loci for the landscape genetic study was informed by the genome size study that confirmed that *I. webberi* is a diploid species. Findings from the intraspecific genome size variation and landscape genetics studies are complementary and show a latitudinal gradient in genome size and genetic diversity among *I. webberi* populations, since both molecular functional traits are influenced by the same evolutionary processes. Literature has shown that the extinction vortex for threatened species involves synergistic effects of ecological, demographic, and genetic processes (Blomqvist et al., 2010; Fagan and Holmes, 2012). Therefore, integrated studies can support a reliable assessment of the extinction risks for *I. webberi* and other imperiled species.

Conservation implications for *Ivesia webberi*

Lack of mobility in plants limits their response to environmental changes to either adaptation or extinction (Corlett, 2016; Panetta et al., 2018). The ability of plant species to adapt to environmental changes is strongly tied to their genetic diversity (Frankel and Soule, 1981; Barrett and Schluter, 2008; Hughes et al., 2008). I have shown in this dissertation that *I. webberi* has a relatively low genetic diversity and among-population gene flow, which was attributed to isolation by distance and ecological dissimilarity among the patches. Low genetic diversity and gene flow among the populations may increase the frequency of mating with closely related individuals and result in fitness costs (Keller and Waller, 2002). Higher genetic differentiation among the populations may indicate local adaptations of these populations to the prevailing ecological conditions in their sites, but this can increase their extirpation risks when these ecological conditions change in the future. Furthermore, a significant genetic diversity relationship with ecological dissimilarity among the sites was also observed, which is indicative of an isolation by environment (Sexton et al., 2014). Field observations reveal varying levels and timing of regeneration and flowering in *I. webberi* populations. For example, populations in the lower elevations tend to regenerate and flower earlier than those in higher elevations. This can result in phenological mismatch that reduce the chances of pollen-based gene flow between populations in the higher and lower elevations (Wadgymar and Weis, 2017; Slatyer et al. 2020).

Maintaining the overall genetic diversity in a plant species requires cross pollination, fertilization, seed production, and new recruitments from seed germination, as well as genetic admixture among populations (Hamrick et al., 1992; Kolbe et al., 2007; Rius and Darling, 2014). However, there are many stochastic risks associated with these fecundity stages, particularly seedling mortality due to physiological constraints and competition from invasive weeds that are prevalent in the sites harboring *I. webberi* (Chambers et al., 2007; Lander et al., 2019). In a pilot study, *I. webberi* seedlings did not survive beyond the two seed-leaf stage under greenhouse conditions (Daniel Harmon pers. comm.). The dominance of *Bromus tectorum* and other invasive plant species in the soil seed bank of sites harboring *I. webberi*, as observed in this dissertation, suggest increased risk of seedling mortality due to competition (Porté et al., 2011; Dickson et al., 2012). Moreover, invasive plant species are linked with altered fire regimes in the Great Basin Desert. Frequent wildfires destroy the soil seed bank of native species and further reduce their potential to add new individuals into their populations (Pyšek et al., 2012; Morris and Rowe, 2014). Fewer recruitments into the populations can reduce *I. webberi* genetic diversity and hence their evolutionary potentials to adapt to current and future environmental changes. However, successful recruitments have been observed in many populations where *I. webberi* successfully colonized bare soil microsites and decommissioned trails and minor roads.

Findings from this dissertation suggest that *I. webberi* habitat protection and management, not seed banking, is a more effective conservation strategy. *I. webberi* seeds lose their viability with storage time, which suggests that they have a recalcitrant storage behavior. This finding also complements the results in Chapter 3 that indicate that *I. webberi* seeds have a transient seed bank. Moreover, *I. webberi* and many perennial native plant species in the Great Basin Desert invest more in vegetative regeneration and often produce empty seeds (Allen and Nowak, 2008). However, cryopreservation technology may be considered as an effective *ex situ* conservation strategy (Pence et al., 2020). An understanding of the seed biology of the Great Basin native plants is critical to seed banking and may inform conservation strategies used for the U.S. Bureau of Land Management (BLM) Seeds of Success (SOS) program (Haidet and Olwell, 2015). Where seed banking was used, this dissertation shows that non-destructive testing methods, like x-ray imagery and multispectral imaging, can be used to monitor seed viability, provide a more reliable estimate of temporal trends in seed viability loss, and also prevent the cumulative reduction of the stored germplasm used for periodic testing. The viability of *I. webberi* seeds did not vary significantly across the sampled populations, indicating that seed collections for banking can be done in any of the populations which is necessary to capture local adaptations across the species range for optimal restoration projects (Baughman et al., 2019).

This dissertation has also revealed a significant positive relationship between genome size and seed size in *I. webberi* and 31 sampled *Ivesia* taxa. Furthermore, we also observed an inverse significant relationship between genome size and actual evapotranspiration. For the desert-dwelling *Ivesia*, these findings indicate that relatively small genome size may have adaptive significance. Furthermore, we determined that *I. webberi* is a diploid species with 28 chromosomes, a cytological condition that has been observed for many of the taxa in *Ivesia*. Diploids, unlike polyploids, have limited evolutionary mechanisms to reduce the fitness cost of inbreeding (Lowry and Lester 2006, Guggisberg et al. 2006), therefore the suspected disjunctions and lack of gene flow among the populations of the taxa in *Ivesia* due to their restriction to the edaphic and mountain sky islands in the desert ecosystems of the western North America may have significant implications for their persistence and survival.

The iterative niche modeling identified the role of topographic and climatic predictor variables in the ecological niche of *I. webberi*. Annual mean precipitation, representing water availability which is the most limiting factor for desert-dwelling species, and actual evapotranspiration (AET), describing water-energy balance, are important climatic variables that influence the ecological niche of *I. webberi*. The model-based field sampling resulted in the discovery of new locations and extended the northern distribution range of the species by 65 km. The ecological properties in the new locations expanded the species' climatic niche breadth and increased scientific understanding of *I. webberi* species-environment relationships. The geographical projection of *I. webberi* niche also identified predicted suitable sites for further field surveys that could potentially result in the discovery of additional novel locations. Predicted suitable sites where *I. webberi* was not discovered could be reserved for potential future translocations and assisted migrations.

Recommendations for the conservation of *Ivesia webberi*

Habitat loss and fragmentation remain the greatest threats to global biodiversity (Figueiredo et al., 2019; Lughadha et al., 2020), therefore, the protection of *Ivesia webberi* habitats is critical to its persistence. For example, *I. webberi* maintains a transient seed bank which is replenished annually from seed abscission from the matured stands. Therefore, habitat protection is necessary to sustain the matured stands and protect their seed banks. This species is located in different sites with a wide range of land ownership and management. The sites located within the National Forests are relatively well protected with minimal human activities. However, *I. webberi* sites on private properties and public lands under the Bureau of Land Management and the State of California jurisdictions allow livestock grazing which may have facilitated the colonization of cheat grass in these sites (Knapp, 1996). We recommend monitoring the impacts of livestock grazing especially in the Hallelujah Junction Wildlife Area where five *I. webberi* patches are located. Similar measures of monitoring the impacts of grazing should be carried out on the BLM land where the most recent northernmost location was recently discovered. The occurrence of seven *I. webberi* locations on private properties demonstrates the need for a stronger private-public partnership in the management of imperiled species. Additionally, predicted suitable sites where *I. webberi* is currently not detected should also be protected. Those predicted suitable habitats could act as connectivity corridors for gene flow among populations, potential sites for future translocation and human assisted translocations.

Habitat protection is not enough to ensure the persistence of an imperiled species. The ecological quality of these habitats is also important to support viable populations. For *I. webberi*, research and management efforts to reduce the abundance and impacts of invasive plant species should be intensified. Most of the known sites are heavily impacted by *B. tectorum* and *Taeniatherum caput-medusae* monocultures (USFWS, 2014). Field observations suggest that the invasive species may not outcompete established *I. webberi* stands. However, taller growth and dense stands of *B. tectorum* and other invasive alien weeds can shield pollinators away from *I. webberi* individuals (Goodell and Parker, 2017), reduce conspecific pollen transfer and deposition (Bjerknes et al., 2007), check localized seed dispersal (Vanier and Walker, 1999), and outcompete nascent seedlings (Humphrey and Schupp, 2004; DiVittorio et al., 2007). This dissertation showed that the soil seed bank of most of the *I. webberi* sites are dominated by invasive plant species. Furthermore, these invasive plants have facilitated an increase in wildfire frequency in the Great Basin Desert, which destroys the soil seed bank of many native plants (Humphrey and Schupp, 2001; Esque et al. 2010). However, historical records show that the majority of the *I. webberi* sites have experienced wildfires in the last 40 years but the species persisted in those locations. Since cheatgrass fueled fires are mostly surface fires, vegetative structures buried deep in the argillic horizon may withstand surface fires and contribute significantly to post-fire vegetative recovery (Humphrey and Schupp, 2001; Klimesˇová and Klimeš, 2007).

Ivesia webberi does not exist in isolation, but in complex ecological interactions with other species and abiotic environmental factors in the Great Basin Desert ecosystem. This includes native plant species such as *Balsamorhiza hookeri, Artemisia arbuscula*, and *Antennaria dimorpha* that have been observed in almost all known populations (USFWS, 2014), as well as floral visitors, which are potential pollinators. However, it remains unclear how these native plant species interact with *I. webberi*. Field observations show that *B. hookeri* may share similar a niche with *I. webberi*. Though *B. hookeri* is a taller herbaceous species belonging to the Asteraceae, it regenerates, flowers, and senesces at the same time as *I. webberi*. Also, both *I. webberi* and *B. hookeri* produce yellow flowers and an achene fruit type, however *B. hookeri* flowers are larger and produce scents that potentially attract pollinators. Therefore, *B. hookeri* could potentially be a “magnet species” by providing pollinators for *I. webberi*, which have smaller flowers (Molina-Montenegro et al., 2008). Investigations on a possible *I. webberi-B. hookeri* association provide an excellent opportunity to advance scientific knowledge on ecological interactions in the desert ecosystem, which can also inform the conservation of the vegetative communities.

Future studies

This dissertation has advanced scientific knowledge of *Ivesia webberi* and vegetative communities in which it is located, through studies on the landscape genetic studies, ecological niche modeling, soil seed bank characterization, and genome size variation in *Ivesia*. *I. webberi* offers a great opportunity to contribute to scientific understanding on species-environment relationships, adaptive genome evolution, species adaptation and persistence in arid environments, and the application of this information to design effective conservation programs. These studies can also elucidate the impact of the geological history of western United States on the evolution of its extant taxa. Specifically, these studies can be extrapolated to identify important functional traits that contribute to ecosystem functioning and vegetative community resilience to environmental change.

An ongoing independent study that focuses on species abundance estimation and modeling will provide a reliable estimate of *I. webberi* density in the known locations. Species reports show wide ranging patch sizes and abundance estimates ranging from less than a hundred to millions of *I. webberi* stands in the 16 originally known locations (USFWS, 2014). The varying abundance of *I. webberi* in these sites could be indicative of differing habitat quality in these sites, which may not be captured using binary response variables typical of ecological niche models, therefore a comparison of the niche models with abundance models should increase the understanding of species-environment relationships and habitat qualities for *I. webberi*. This study used hierarchical distance sampling to estimate the abundance and density of *I. webberi* in the

16 originally known locations. The resulting abundance estimates would be integrated into species abundance models using the same ecological predictor variables in the niche models; thus, the predictions of the ecological niche models can be compared with abundance models (Dallas and Hastings, 2017; Yu et al., 2020). Furthermore, the sampling strategies used in this study is replicable and can be used for long-term monitoring of the *I. webberi* populations and population viability analysis. Population viability analysis and models would be a great way to quantify new recruitment from seedlings, determine interannual variability in seedling and vegetative regeneration, and the associated environmental predictors. Reliable abundance estimates can also permit empirical investigation on the relationship among census size, genetic diversity, and effective population size in *I. webberi* (Knaepkens et al., 2004; Leimu et al., 2006; Frankham et al., 2010).

Microsatellite markers may be sufficient to answer questions related to population genetic diversity and structure, admixture, gene flow, kinship, parentage, mating system, taxonomic fingerprinting, genome mapping, and population history (Parker et al., 1998; Luikart et al., 2003). However, these neutral markers do not have adequate genetic signatures to identify loci that are associated with local adaptation or under selection hence understand the adaptive genetic potential of each population to persist under environmental pressures (Barbosa et al., 2018; Silva et al., 2020). As a result, genome-wide association studies (GWAS) are gradually replacing the use of microsatellite markers to assess genetic diversity (Benestan et al., 2016; Carreras et al., 2020). Also, studies show that findings from microsatellite-based population genetic studies do not correlate with genome-based analysis (Holderegger et al., 2006; Väli et al., 2008). As a desert-dwelling species, neutral markers may not be enough to understand adaptive potentials of *I. webberi* to environmental selective pressures. Moreover, *I. webberi* samples from the recently-discovered locations from the model-guided field surveys were inevitably excluded from the population genetic studies. Therefore, genome-based analysis may provide additional information on the evolutionary dynamics in *I. webberi* and allows for the inclusion of samples from the new locations.

Current genetic analysis suggests that both seed dispersal and pollen-based gene flow are common among the sampled *I. webberi* populations and underscore the importance of pollinators and dispersal vectors. This is complementary to field observations that identified five *I. webberi* flower visitors, including Common blue butterfly (*Plebejus* sp.), Halictid bees (*Lasioglossum* sp.), Cuckoo wasp, Common ground ant (*Camponotus* sp.), and Horsefly (*Tabanus laticeps*). However, empirical studies may be required to determine if these flower visitors are effective pollinators for *I. webberi*, their foraging and movement patterns, landscape effects on their movement patterns, as well as an ecological network of their mutualism with *I. webberi*. Previous studies have stressed the importance of pollen-based gene flow in plants (Sork et al., 1999; Auffret et al., 2017). Therefore, effective conservation should not be limited to the management of *I. webberi*, but also include protection of known pollinators.

Ivesia webberi and the systems in which it is located offer excellent opportunities to investigate phenological shifts in response to climate change. Studies have shown a significant association between changes in spring temperature and phenology shifts among native plant species in the Northern hemisphere (Thompson and Gilbert, 2014).

An association between spring temperature and plant regeneration is stronger in the Great Basin Desert where the timing of snowmelt, influenced by spring temperatures, determines soil moisture content and regeneration of dormant vegetative structures of *I. webberi* and other native perennials (USFWS, 2014). Therefore, climatic fluctuations in winter precipitation and spring temperatures which are very frequent in the Great Basin Desert have been associated with phenological shifts in the regeneration of native plant species (Tang et al., 2015; Snyder et al., 2016). When winter precipitation is less severe, *I. webberi* regenerates earlier in mid-winter, while regeneration is delayed till early spring in heavy winter years. Furthermore, variation in regeneration up to four weeks has been observed among *I. webberi* populations; populations in the lower elevation regenerate earlier. Elevational gradients in phenological variation represent the effect of climatic difference among *I. webberi* sites. Additionally, delay in regeneration and flowering was observed for *I. webberi* stands under tree shades within a population, underscoring the effect of shade (McKinney and Goodell, 2011; Heberling et al., 2019; Baker et al., 2019). These field observations suggest that microhabitat conditions and ecological dissimilarity may influence phenological velocity among *I. webberi* populations which could be further exacerbated by climate change (Augspurger et al., 2005; Cornelius et al., 2013; Rafferty et al., 2020). Phenological mismatches could result in assortative mating and restrict gene flow to populations with similar habitat conditions, thus deepening the genetic divergence among *I. webberi* populations (Aitken et al., 2008; Frank and Weis, 2009; Wadgymar and Weis, 2017, but see Cortés et al., 2014). Phenological plasticity could also be related to the intraspecific genome size variation, which can further advance scientific knowledge on genome size evolution (Ren et al., 2020). Long-term phenological monitoring may be beneficial in predicting *I. webberi* response to climate change, among population gene flow patterns, and evolutionary dynamics. In the context of ecological interactions, phenological synchrony between *I. webberi* and *Balsamorhiza hookeri* is worth investigating due to similarity in functional traits, regeneration, and flowering.

REFERENCES

Aitken S.N., Yeaman S., Holliday J.A., Wang T., and Curtis-McLane S. (2008). Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* 1(1): 95-111. https://doi.org/10.1111/j.1752-4571.2007.00013.x

Allen E.A., and Nowak R.S. (2008). Effect of pinyon-juniper tree cover on the soil seed bank. *Rangeland Ecology and Management* 61: 63–73. https://doi.org/10.2111/07-007R2.1

Auffret, A. G., Rico, Y., Bullock, J. M., Hooftman, D. A., Pakeman, R. J., Soons, M. B., Suárez-Esteban, A., Traveset, A., Wagner, H. H., & Cousins, S. A. (2017). Plant functional connectivity-integrating landscape structure and effective dispersal. *Journal of Ecology* 105(6): 1648-1656. http://dx.doi.org/10.1111/1365-2745.12742

Augspurger C.K., Cheeseman J.M., and Salk C.F. (2005). Light gains and physiological capacity of understorey woody plants during phenological avoidance of canopy shade. *Functional Ecology* 19(4): 537-546. https://doi.org/10.1111/j.1365-2435.2005.01027.x

Baker B.H., Sultan S.E., Lopez-Ichikawa M., and Waterman R. (2019). Transgenerational effects of parental light environment on progeny competitive performance and lifetime fitness. *Philosophical transactions of the Royal Society of London Series B, Biological Sciences* 374(1768): 20180182. https://doi.org/10.1098/rstb.2018.0182

Barbosa S., Mestre F., White T.A., Paupério J., Alves P.C., and Searle J.B. (2018). Integrative approaches to guide conservation decisions: using genomics to define conservation units and functional corridors. *Molecular Ecolog*y 27:3452–3465. https://doi.org/10.1111/mec.14806

Barrett R.D.H., and Schluter D. (2008). Adaptation from standing genetic variation. *Trends in Ecology and Evolution* 23(1): 38-44. https://doi.org/10.1016/j.tree.2007.09.008

Baughman O.W., Agneray A.C., Forister M.L., Kilkenny F.F., Espeland E.K., Fiegener R., Horning M.E., Johnson R.C., Kaye T.N., Ott J., St. Clair J.B., and Leger E.A. (2019). Strong patterns of intraspecific variation and local adaptation in Great Basin plants revealed through a review of 75 years of experiments. *Ecology and Evolution* 9(11): 6259–6275. https://doi.org/10.1002/ece3.5200

Benestan L., Ferchaud A.-L., Hohenlohe P.A., Garner B.A., Naylor G.J.P., Baums I.B., Schwartz M.K., Kelley J.L., and Luikart G. (2016). Conservation genomics of natural and managed populations: Building a conceptual and practical framework. *Molecular Ecology* 25(13): 2967–2977. https://doi.org/10.1111/mec.13647

Bertness M.D., and Callaway R.M. (1994). Positive interactions in communities. *Trends in Ecology and Evolution* 9(5): 191–193. https://doi.org/10.1016/0169-5347(94)90088-4

Bjerknes A.-L., Totland Ø., Hegland S.J., and Nielsen A. (2007). Do alien plant invasions really affect pollination success in native plant species? *Biological Conservation* 138(1–2): 1-12. https://doi.org/10.1016/j.biocon.2007.04.015.

Blomqvist D., Pauliny A., Larsson M., and Lars-Åke F. (2010). Trapped in the extinction vortex? Strong genetic effects in a declining vertebrate population. *BMC Evolutionary Biology* 10: 33. https://doi.org/10.1186/1471-2148-10-33

Carreras C., García-Cisneros A., Wangensteen O.S., Ordóñez V., Palacín C., Pascual M., and Turon X. (2020). East is East and West is West: Population genomics and hierarchical analyses reveal genetic structure and adaptation footprints in the keystone species *Paracentrotus lividus* (Echinoidea). *Diversity and Distributions* 26(3): 382-398. https://doi.org/10.1111/ddi.13016

Chambers, J. C. (2008). Climate Change and the Great Basin. In: Chambers J.C., Devoe N., & Evenden A. (eds.). *Collaborative management and research in the Great Basin - examining the issues and developing a framework for action*. Gen. Tech. Rep. RMRS-GTR-204. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. p. 29-32

Chambers, J. C., Roundy, B. A., Blank, R. R., Meyer, S. E., & Whittaker, A. (2007). What makes Great Basin sagebrush ecosystems invasible by *Bromus tectorum*? *Ecological Monographs* **77**(1): 117-145. https://doi.org/10.1890/05-1991

Corlett R.T. (2016). Plant diversity in a changing world: Status, trends, and conservation needs. *Plant Diversity* 38(1): 10– 16. https://doi.org/10.1016/j.pld.2016.01.001

Cornelius C, Estrella N., Franz H., and Menzel A. (2013). Linking altitudinal gradients and temperature responses of plant phenology in the Bavarian Alps. *Plant Biology* 15(s1): 57-69. https://doi.org/10.1111/j.1438-8677.2012.00577.x

Cortés A., Waeber S., Lexer C., Sedlacek J., Wheeler J.A., van Kleunen M., Bossdorf O., Hoch G., Rixen C., Wipf S., and Karrenberg S. (2014). Small-scale patterns in snowmelt timing affect gene flow and the distribution of genetic diversity in the alpine dwarf shrub *Salix herbacea*. *Heredity* 113: 233–239. https://doi.org/10.1038/hdy.2014.19

Dallas T.A., and Hastings A. (2017). Habitat suitability estimated by niche models is largely unrelated to species abundance. *Global Ecology and Biogeography* 27(12): 1448-1456. https://doi.org/10.1111/geb.12820

Dickson T.L., Hopwood J.L., and Wilsey B.J. (2012). Do priority effects benefit invasive plants more than native plants? An experiment with six grassland species. *Biological Invasions* 14: 2617–2624. https://doi.org/10.1007/s10530-012-0257-2

DiVittorio C.T., Corbin J.D., and D'Antonio C.M. (2007). Spatial and temporal patterns of seed dispersal: an important determinant of grassland invasion. *Ecological Applications* 17(2): 311-316. https://doi.org/10.1890/06-0610

Esque T.C., Young J.A., and Tracy C.R. (2010). Short-term effects of experimental fires on a Mojave Desert seed bank. *Journal of Arid Environments* 74(10): 1302-1308. https://doi.org/10.1016/j.jaridenv.2010.04.011

Fagan W.F, and Holmes E.E. (2006). Quantifying the extinction vortex. *Ecology Letters* 9(1): 51-60. https://doi.org/10.1111/j.1461-0248.2005.00845.x

Figueiredo L., Krauss J., Steffan-Dewenter I., and Sarmento Cabral J. (2019). Understanding extinction debts: Spatio–temporal scales, mechanisms and a roadmap for future research. *Ecography* 42(12): 1973– 1990. https://doi.org/10.1111/ecog.04740

Frank S.J., and Weis A.E. (2009). Climate change alters reproductive isolation and potential gene flow in an annual plant. *Ecological Applications* 2(4): 481-488. https://doi.org/10.1111/j.1752-4571.2009.00073.x

Frankel O.H., and Soule M.E. (1981). *Conservation and evolution*. Cambridge University Press, Cambridge, England. 327 pp

Frankham R., Ballou J., and Briscoe D. (2010). *Introduction to Conservation Genetics* (2nd ed.). Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511809002

Gasparin, E., Faria, J.M.R., José, A.C., Tonetti O.A.O., de Melo R.A., and Hilhorst H.W.M. (2020). Viability of recalcitrant *Araucaria angustifolia* seeds in storage and in a soil seed bank. *Journal of Forestry Research* 31: 2413–2422. https://doi.org/10.1007/s11676-019-01001-z

Gómez J.M. (2004) Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution* 58(1):71–80. https://doi.org/10.1554/02-617

Goodell K., and Parker I.M. (2017). Invasion of a dominant floral resource: effects on the floral community and pollination of native plants. *Ecology* 98(1): 57–69. https://doi.org/10.1002/ecy.1639

Guggisberg A., Mansion G., Kelso S., and Conti E. (2006). Evolution of biogeographic patterns, ploidy levels, and breeding systems in a diploid–polyploid species complex of *Primula*. *New Phytologist* 171(3): 617–632. https://doi.org/10.1111/j.1469-8137.2006.01722.x

Haidet M., and Olwell P. (2015). Seeds of success: A national seed banking program working to achieve long-term conservation goals. *Natural Areas Journal* 35(1): 165–173. https://doi.org/10.3375/043.035.0118

Hamrick J.L., Godt M.J.W., and Sherman-Broyles S.L. (1992). Factors influencing levels of genetic diversity in woody plant species. *New Forests* 6: 95–124. https://doi.org/10.1007/BF00120641

Heberling J.M., McDonough MacKenzie C., Fridley J.D., Kalisz S., and Primack R.B. (2019). Phenological mismatch with trees reduces wildflower carbon budgets. *Ecology Letters* 22(4): 616-623. https://doi.org/10.1111/ele.13224

Holderegger R., Kamm U., and Gugerli F. (2006). Adaptive vs. neutral genetic diversity: implications for landscape genetics. *Landscape Ecology* 21: 797–807. https://doi.org/10.1007/s10980-005-5245-9

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008) Ecological consequences of genetic diversity. *Ecology Letters* 11(6): 609– 623. https://doi.org/10.1111/j.1461-0248.2008.01179.x

Humphrey, L. D., & Schupp, E. W. (2001). Seed banks of *Bromus tectorum*-dominated communities in the Great Basin. *Western North American Naturalist* 61: 85–92.

Humphrey, L. D., & Schupp, E. W. (2004). Competition as a barrier to establishment of a native perennial grass (*Elymus elymoides*) in alien annual grass (*Bromus tectorum*) communities. *Journal of Arid Environments* 58: 405-422. https://doi.org/10.1016/j.jaridenv.2003.11.008

Keller L.F., and Waller D.M. (2002). Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17(5): 230-241. https://doi.org/10.1016/S0169-5347(02)02489-8

Klimesˇová J., and Klimeš L. (2007). Bud banks and their role in vegetative regeneration – A literature review and proposal for simple classification and assessment. *Perspectives in Plant Ecology, Evolution and Systematics* 8(3): 115-129. https://doi.org/10.1016/j.ppees.2006.10.002.

Knaepkens G., Bervoets L., Verheyen E., and Eens M. (2004). Relationship between population size and genetic diversity in endangered populations of the European bullhead (*Cottus gobio*): implications for conservation. *Biological Conservation* 115(3): 403-410. https://doi.org/10.1016/S0006-3207(03)00156-3.

Knapp P.A. (1996). Cheatgrass (*Bromus tectorum* L.) dominance in the Great Basin Desert: History, persistence, and influences to human activities. *Global Environmental Change* 6(1): 37-52. https://doi.org/10.1016/0959-3780(95)00112-3

Kolbe J.J., Glor R.E., Rodriguez Schettino L., Chamizo Lara A.R., Larson A., and Losos J.B. (2007). Multiple sources, admixture, and genetic variation in introduced Anolis lizard populations. *Conservation Biology* 21(6): 1612– 1625. https://doi.org/10.1111/j.1523-1739.2007.00826.x

Lander T.A., Harris S.A., Cremona P.J., and Boshier D.H. (2019). Impact of habitat loss and fragmentation on reproduction, dispersal and species persistence for an endangered Chilean tree. *Conservation Genetics* 20: 973–985. https://doi.org/10.1007/s10592-019-01187-z

Leimu R., Mutikainen P., Koricheva J., and Fischer M. (2006). How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* 94(5): 942-952. https://doi.org/10.1111/j.1365-2745.2006.01150.x

Lowry E., and Lester S.E. (2006). The biogeography of plant reproduction: potential determinants of species' range sizes. *Journal of Biogeography* 33(11): 1975–1982. https://doi.org/10.1111/j.1365-2699.2006.01562.x

Lughadha E.N., Bachman S.P., Leão T.C.C., Forest F., Halley J.M., Moat J., Acedo C. Bacon K.L., Brewer R.F.A., Gâteblé G., Gonçalves S.C., Govaerts R., Hollingsworth P.M., Krisai-Greilhuber I., de Lirio E.J., Moore P.G.P., Negrão R., Onana J.M., Rajaovelona L.R., Razanajatovo H., Reich P.B., Richards S.L., Rivers M.C., Cooper A., Iganci J., Lewis G.P., Smidt E.C., Antonelli A., Mueller G.M., and Walker B.E. (2020). Extinction risk and threats to plants and fungi. *New Phytologist* 2(5): 389-408. https://doi.org/10.1002/ppp3.10146

Luikart G., England P.R., Tallmon D., Jordan S., and Taberlet P. (2003). The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics* 4: 981–994 (2003). https://doi.org/10.1038/nrg1226

Maestre F.T., Bautista S., and Cortina J. (2003). Positive, negative, and net effects in grass–shrub interactions in Mediterranean semiarid grasslands. *Ecology* 84(12): 3186-3197 https://doi.org/10.1890/02-0635

McKinney A.M., and Goodell K. (2011). Plant–pollinator interactions between an invasive and native plant vary between sites with different flowering phenology. *Plant Ecology* 212: 1025–1035. https://doi.org/10.1007/s11258-010-9882-y

Molina-Montenegro M.A., Badano E.I., and Cavieres L.A. (2008). Positive interactions among plant species for pollinator service: assessing the 'magnet species' concept with invasive species. *Oikos* 117(12): 1833-1839. https://doi.org/10.1111/j.0030-1299.2008.16896.x

Morris L.R., and Rowe R.J. (2014). Historical land use and altered habitats in the Great Basin. *Journal of Mammalogy* 95(6): 1144–1156. https://doi.org/10.1644/13-MAMM-S-169

Naeem S., Prager C., Weeks B., Varga A., Flynn Dan F.B., Griffin K., Muscarella R., Palmer M., Wood S., and Schuster W. (2016). Biodiversity as a multidimensional construct: a review, framework and case study of herbivory's impact on plant biodiversity. *Proceedings of the Royal Society B Biological Sciences* 283(1844): 20153005. http://doi.org/10.1098/rspb.2015.3005

Panetta A.M., Stanton M.L., and Harte J. (2018). Climate warming drives local extinction: Evidence from observation and experimentation. *Science Advances* 4(2): eaaq1819. doi: 10.1126/sciadv.aaq1819

Pence V.C., Ballesteros D., Walters C., Reed B.M., Philpott M., Dixon K.W., Pritchard H.W., Culley T.M., and Vanhove A.-C. (2020). Cryobiotechnologies: Tools for expanding long-term *ex situ* conservation to all plant species. *Biological Conservation* 250: 108736. https://doi.org/10.1016/j.biocon.2020.108736.

Porté A.J., Lamarque L.J., Lortie C.J., Michalet R., and Delzon S. (2011). Invasive *Acer negundo* outperforms native species in non-limiting resource environments due to its higher phenotypic plasticity. *BMC Ecology* 11: 28. https://doi.org/10.1186/1472-6785-11-28

Pyšek P., Jarošík V., Hulme P.E., Pergl J., Hejda M., Schaffner U., and Vilà M. (2012). A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Global Change Biology* 18: 1725-1737. https://doi.org/10.1111/j.1365-2486.2011.02636.x

Rafferty N.E., Diez J.M., and Bertelsen C.D. (2020). Changing climate drives divergent and nonlinear shifts in flowering phenology across elevations. *Current Biology* 30(3): 432-441.e3, https://doi.org/10.1016/j.cub.2019.11.071.

Ren L., Guo X., Liu S., Yu T., Guo W., Wang R., Ye S., Lambertini C., Brix H., and Eller F. (2020). Intraspecific variation in *Phragmites australis*: Clinal adaption of functional traits and phenotypic plasticity vary with latitude of origin. *Journal of Ecology* 108(6): 2531-2543. https://doi.org/10.1111/1365-2745.13401

Rius M., and Darling J.A. (2014). How important is intraspecific genetic admixture to the success of colonising populations? *Trends in Ecology and Evolution* 29(4): 233-242. https://doi.org/10.1016/j.tree.2014.02.003.

Sexton J.P., Hangartner S.B., and Hoffmann A.A. (2014). Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution* 68: 1–15. https://doi.org/10.1111/evo.12258

Silva A.R., Resende-Moreira L.C., Carvalho C.S., Lanes E.C.M., Ortiz-Vera M.P., Viana P.L., and Jaffé R. (2020). Range-wide neutral and adaptive genetic structure of an endemic herb from Amazonian Savannas. *AoB PLANTS* 12(1): plaa003. https://doi.org/10.1093/aobpla/plaa003

Slatyer R.A., Schoville S.D., Nufio C.R., and Buckley L.B. (2020). Do different rates of gene flow underlie variation in phenotypic and phenological clines in a montane grasshopper community? *Ecology and Evolution* 10(2): 980-997. https://doi.org/10.1002/ece3.5961

Snyder K.A., Evers L., Chambers J.C., Dunham J., Bradford J.B., and Loik M.E. (2019). Effects of changing climate on the hydrological cycle in cold desert ecosystems of the Great Basin and Columbia Plateau. *Rangeland Ecology & Management* 72(1): 1-12. https://doi.org/10.1016/j.rama.2018.07.007.

Snyder K.A., Wehan B.L., Filippa G., Huntington J.L., Stringham T.K., and Snyder D.K. (2016). Extracting plant phenology metrics in a Great Basin watershed: methods and considerations for quantifying phenophases in a cold desert. *Sensors (Basel, Switzerland)* 16(11): 1948. https://doi.org/10.3390/s16111948

Soliveres S., Eldridge D.J., Maestre F.T., Bowker M.A., Tighe M., and Escudero A. (2011). Microhabitat amelioration and reduced competition among understorey plants as drivers of facilitation across environmental gradients: Towards a unifying framework. *Perspectives in Plant Ecology, Evolution and Systematics* 13(4): 247-258. https://doi.org/10.1016/j.ppees.2011.06.001.

Sork V.L., Nason J., Campbell D.R., and Fernandez J.F. (1999). Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology and Evolution* 14(6): 219-224. https://doi.org/10.1016/S0169-5347(98)01585-7.

Stevens R.D., and Gavilanez M.M. (2015). Dimensionality of community structure: phylogenetic, morphological and functional perspectives along biodiversity and environmental gradients. *Ecography* 38(9): 861–875. https://doi.org/10.1111/ecog.00847

Tang G., Arnone III J.A., Verburg P.S.J., Jasoni R.L., and Sun L. (2015). Trends and climatic sensitivities of vegetation phenology in semiarid and arid ecosystems in the US Great Basin during 1982–2011. *Biogeosciences* 12(23): 6985–6997. https://doi.org/10.5194/bg-12-6985-2015

Thompson K., and Gilbert F. (2014). Phenological synchrony between a plant and a specialised herbivore. *Basic and Applied Ecology* 15(4): 353-361. https://doi.org/10.1016/j.baae.2014.05.003

Väli Ü, Einarsson A., Waits L, and Ellegren H. (2008). To what extent do microsatellite markers reflect genome-wide genetic diversity in natural populations? *Molecular Ecology* 17(17): 3808-3817. https://doi.org/10.1111/j.1365-294X.2008.03876.x

Vanier C.H., and Walker L.R. (1999). Impact of a non-native plant on seed dispersal of a native. *Madroño* 46(1): pp. 46-48.

Wadgymar S.M., and Weis A.E. (2017). Phenological mismatch and the effectiveness of assisted gene flow. *Conservation Biology* 31(3): 547–558. https://doi.org/10.1111/cobi.12877

Yu H., Cooper A.R., and Infante D.M. (2020). Improving species distribution model predictive accuracy using species abundance: Application with boosted regression trees. *Ecological Modelling* 432: 109202. https://doi.org/10.1016/j.ecolmodel.2020.109202.