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Eco-Evolutionary Dynamics of Invasion in the Exotic Grass *Bromus rubens* L.

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by

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ABSTRACT OF THE DISSERTATION

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A minority of exotic plant species undergo differentiation in vigor following introduction, leading to an explosion in population sizes and aggressive range expansion. Investigations into the mechanisms that determine successful invasion historically emphasized phenotypic traits in hopes of identifying ecological predictors and subsequent control mechanisms. Yet, it is now recognized that post-introductory evolution of invasiveness is common in many systems, frustrating efforts to identify ecological predictors. This suggests that evolutionary mechanisms ought to be given increased consideration. But this does not mean that regional differences in ecological interactions are unimportant. Many investigations demonstrate that invasive plant species experience facilitation in introduced relative to native range soils. My objective was to integrate these two promising fields of study in order to obtain a more holistic view of the mechanisms underlying invasion. Here I utilized seed and soils from native and introduced regions of the locally abundant grass species *Bromus rubens* L. (Pavlick and Anderson 2007, = *B.*

madritensis ssp. rubens, Fortune et al. 2008), also known as Red brome. *B. rubens* is a winter annual common in the Mediterranean (native range) and Southwestern United States (introduced range). I examined the complexities of potential evolutionary and ecological factors leading to the invasion success of this species by concentrating on 1) patterns and promoters of regional differentiation, 2) the impacts of differentiation on competitive ability, and 3) the contribution of multiple ecological factors to plant-soil interactions. I found that introduced populations showed a strong signal for diversifying selection toward more aggressive growth. In a competitive environment introduced genotypes demonstrated greater reproductive fitness relative to native genotypes, regardless of competitor's genotypic or region of origin. Finally, a plant-soil interaction growth assay suggested that increased resource availability coupled with decreased interactions with both antagonistic and beneficial soil fungi in introduced soils contributed to the invasion success in *B. rubens*. Together these patterns indicate that the occurrence of post-introductory evolution is of major importance to the development of invasive characters and increased competitive ability, and that ecological interactions among hosts and respective soil communities greatly contributed to the dynamics observed in this system.

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Introduction

Exotic plant species are experiencing increased translocated to novel environments (Vitousek et al. 1997, Mack et al. 2000). Of those species that become naturalized, approximately a tenth of a percent experience an explosion in population size, followed by proliferation and aggressive range expansion (Williamson 1996). Species that express such traits in non-native ranges are defined as *invasive* (Richardson et al. 2011). The process of invasion is characterized by a naturalization-invasion continuum in which non-native plants must overcome various abiotic and biotic barriers (Richardson et al. 2000). Interest in the mechanisms that facilitate passage through such barriers extend well beyond Darwin's *Origins* (1859). Since that time investigations into invasive plant biology increased dramatically, prompting the development of at least 29 non-mutually exclusive hypotheses to explain mechanisms underlying invasion (Catford et al. 2009). Such a large number of hypotheses underscore the difficulties in understanding biological invasions. The reason for this discrepancy can partially be attributed to a general aboveground bias (Bever et al. 2010). Recent work demonstrates that plant-soil interactions are important factors determining plant community composition (Allen 1991, van der Heijden et al. 1998, Bever 2003), and thus directly influences species invasiveness and community invasibility (Stampe and Daehler 2003, Seastedt and Pysek 2011, Bever et al 2012).

Hypotheses that attempt to explain the processes of plant invasion all sought an answer to the same question; Why are species that have no evolutionary history in their

novel environments able to establish and display competitive superiority over species that have had a long evolutionary history in said environment (Sax and Brown 2000)? The idea is paradoxical in terms of early evolutionary and ecological thought. Based on Darwin's (1859) ideas of the struggle for existence, these species would be required to at least be good enough to withstand the antagonistic interactions that structure the composition of evolved communities (Reznick 2010). However, being good enough is not the same as displaying the competitive superiority commonly observed in invasive species. Darwin and others observed these phenomena, but did not explicitly posit hypotheses (Chew 2011). Rather, these observations were used to exemplify the importance of antagonistic interactions as regulatory mechanisms and selectional forces.

Although invasive species did not go unnoticed throughout the early 20th century (Tansley 1935, Egler 1942, Baker 1948), it was not until the 1950's that the field of invasion ecology truly began to take form. The publication of *The Ecology of Invasions by Animals and Plants* (1958), by Charles Elton, is generally considered the birth of the field (Parker 2000). In this work, Elton proposed hypotheses, explanations and predictions about the underlying mechanisms of invasion. The most well-known and influential of these were the diversity-invasibility (later repackaged, and referred to from here on, as the 'biotic resistance hypothesis' [Levine et al. 2004]) and enemy release hypotheses (Keane and Crawley 2002).

In the formation of the biotic resistance hypothesis (BRH), Elton largely drew upon the concepts of the niche (Grinnel 1917, Hutchinson 1957) and the principle of competitive exclusion (Gause 1934). Essentially, species with similar resource

requirements experience intense competition resulting in exclusion of one or the other. Therefore, a communities' ability to resist invasion is expected to be proportional to its diversity (Kennedy et al. 2002). This concept is similar to those proposed in the 1960's and 1970's pertaining to the contribution of resource-based competition (May and MacArthur 1972) and limiting similarity (MacArthur and Levins 1967) to resultant community structure and stability (MacArthur 1970, May 1972). As with most hypotheses of invasion ecology, patterns of biotic resistance against invasion remain unresolved and often scale dependent (Lonsdale 1999, Stohlgren et al. 1999, Shea and Chesson 2002).

The enemy release hypothesis (ERH) is among the most widely accepted and yet under tested hypotheses of invasion ecology. This is possibly due to its parsimonious, intuitive nature (Colautti et al. 2004). The ERH posits that upon introduction to a novel environment, plant species experience decreased population regulation by coevolved enemies, resulting in rapid increases in distribution and abundance (Keane and Crawley 2002). The hypothesis, therefore, fits in with classic concepts of antagonism, predation and top-down regulation as significant evolutionary and ecological forces (Hairston et al. 1960, Paine 1969, Gurevitch et al. 1992). The conceptual simplicity of the ERH may be detrimental to its applicability however. While meta-analyses do reveal that invasive species tend to experience decreased antagonism (Mitchell and Powers 2003), the act of transportation itself is expected to decrease the occurrence of coevolved antagonists (Colautti et al. 2004). Therefore, some degree of enemy release is expected in all introduction events, but the significance of such release events may be context dependent

(Inderjit et al. 2005).

It may indeed be that release from antagonists allows for increased resource use resulting in increased growth and reproduction. But given what we know about the naturalization-invasion continuum – especially the long lag phase prior to the expression of invasive behavior – there must be other variables acting besides the lack of these ecological interactions. A corollary to the ERH may explain this discrepancy. The increased competitive ability hypothesis (Blossey and Nötzold 1995) posits that invasiveness is the product of post-introductory selection on reallocation of resources away from once useful, defensive traits toward increased growth and reproduction. Thus, differences in selective environments may be a better explanatory variable for invasion success, rather than a change to one in particular set of ecological interactions.

The biotic resistance and enemy release hypotheses remain the most commonly addressed hypotheses of invasion ecology. However, there are a wide variety of possible mechanisms that can lead to invasion success (Catford et al. 2009). Conceptually, invasion can proceed if non-native species have greater resource acquisition rates, or have lower maintenance requirements relative to native species (i.e. they display lower equilibrium resource levels [Monod 1950, Tilman 1977, 1982, Shea and Chesson 2002]). Thus, hypotheses pertaining to resource acquisition propose that non-native species succeed due to inherent 'invasive' characters (Baker 1965, Williamson and Fitter 1996), responsiveness to disturbance (Sher and Hyatt 1999) and increased resource availability (Davis et al. 2000), novel associations (e.g. novel weapons, Callaway and Ridenour 2004; enhanced mutualisms, Reinhart and Callaway 2006) or due to the sheer number of

individuals introduced (Lonsdale 1999, Simberloff 2009). As noted above, all of these factors may contribute to invasion success, but these ecological explanations do not align with observed lag phases experienced by populations during the naturalization-invasion continuum. In contrast to these and other hypotheses, a promising direction in invasive plant biology may be to investigate post-introductory evolution via adaptation and/or hybridization as explanatory variables for the promotion of invasive habits (Blossey and Nötzold 1995, Ellstrand and Schierenbeck 2000).

In addition to the lack of incorporation of evolutionary mechanisms into a field previously dominated by ecological theory, the problem of context dependency and non-predictability common in invasion ecology literature is likely due to single hypothesis driven research (Catford et al. 2009, Seastedt and Pyšek 2011). However, as noted earlier, these hypotheses are not mutually exclusive. Therefore, a more integrated approach is required to reduce this context dependency. Plant-soil interaction based studies may serve to ameliorate these issues for the following reasons: 1) the inherent complexity of abiotic and biotic components of soil is amenable to testing multiple hypotheses simultaneously and 2) the emergent patterns from seminal plant-soil interaction studies are generally consistent (Callaway and Rout 2011). Such consistency suggests that plant-soil interactions may be a general underlying mechanism of plant invasion that as of yet has remained lacking in the field of invasion ecology.

The objective of this dissertation was to explore potential interactions between ecological and evolutionary forces that led to the promotion of invasion success in a locally dominant invasive grass species, *Bromus rubens* L. (Pavlick and Anderson 2007, = *B.*

madritensis ssp. rubens, Fortune et al. 2008). My approach included multiple common garden studies and analytic techniques rarely utilized in the study of invasive plant ecology. Multiple scales of observation – from a portion of the chloroplast genome to host-soil community interactions – were undertaken to ascertain a more holistic view of the factors underlying phenotypic divergence among native (ancestral) and introduced (descendent) populations in this species. Overall, I hypothesized that interactions between post-introductory selection and beneficial host-soil associations helped to produce the noxious invader *B. rubens*.

In chapter one, I investigated the potential for post-introductory evolution as an explanation for the invasive behavior observed in the introduced range of *B. rubens*. By quantifying both molecular diversity and trait expression among multiple populations originating from respective regions a large degree of phenotypic divergence was found between closely related, yet regionally isolated, populations, as well as resource availability acting as a selective agent to drive observed changes. In my second chapter, I explored the implications of these observed differences with the use of an intraspecific competition experiment. Results from this chapter suggest that regional variation in selective environment promoted increased competitive abilities within introduced populations of both close and distant relation to an ancestral population identified in chapter 1.

In chapter three I moved beyond the role of regional differentiation in molecular and quantitative traits, which led to the expression of increased competitive abilities observed in chapter 2, to explore how regional differences in plant-soil interactions may

explain the invasion success of *B. rubens*. Populations of close and distant relation were grown in the presence and absence of soil microbial communities cultured by each respective population to investigate which soil components promoted invasion in this system. My results demonstrated that individuals in the introduced range experienced increased resource availability, as well as decreased interactions with both antagonistic and beneficial soil fungi. Together, this multifaceted approach highlights the complexity of successful invasion and suggests that increased emphasis on post-introductory dynamics may provide a more comprehensive view of the factors leading to successful invasion of exotic plant species.

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Phenotypic trade-offs promote invasive traits in the exotic grass *Bromus rubens* L.

Abstract

Invasive plant species tend to exhibit more vigorous growth habits in introduced relative to native ranges. Classic hypotheses propose that release from inhibitory biotic factors provide immediate benefit and drive these observed invasive habits. In contrast, recent hypotheses propose that post-introductory evolutionary change is the causal mechanism underlying divergent growth patterns. If selection for these habits did occur in introduced ranges, then these genotypes should display increased growth rate and altered allocation patterns relative to native genotypes. I utilized polymorphic molecular markers along with morphological, physiological and climatic traits among seven native and seven introduced populations of *Bromus rubens* to test for the existence of post-introductory trait change, and investigate whether regional variation in climate could drive any observed divergence in phenotypic trait expression. I found that despite large losses of molecular variation in introduced relative to native populations, introduced populations tended to display more aggressive growth. A trade-off in growth strategies among regional populations appeared to be the explanation for this observed difference; native populations tended to invest more in light harvesting organs and photosynthetic activity, while introduced populations displayed traits indicative of more aggressive behavior. Further analyses revealed that newly translocated populations from native to introduced ranges experienced strong selection pressure on traits associated with invasiveness. Finally, variation in light availability was found to be the primary selective agent driving

phenotypic trade-offs from leafy-high photosynthetically active native populations to taller-faster growing introduced populations. Together, these results suggest that variation in abiotic resources hold the capacity to drive once benign, native populations toward more aggressive invaders.

Introduction

Comparative growth assays demonstrate that native populations tend to display traits that promote persistence while introduced populations display traits that promote rapid growth (Felker-Quinn et al. 2013). Investigations into the mechanisms that determine successful invasion historically involved emphasis on phenotypic traits in hopes of identifying ecological predictors and subsequent control mechanisms (Pyšek et al. 1995, Williamson and Fitter 1996). While no consistency in trait expression was identified, many traits which appeared to facilitate invasion were observed, including: increased physiological activities, faster growth rates, increased height and greater reproduction (Baker 1965, Pyšek et al. 1995, Rejmánek and Richardson 1996, Williamson and Fitter 1996, Leishman et al. 2007). Divergence between regionally isolated native and introduced populations may reflect a shift from weakly competitive-stress tolerating populations toward ruderal or competitive growth strategies as they change from 'native' to 'invasive' (Universal Adaptive Strategy Theory [UAST]; Grime 1977, Pyšek et al. 1995, Grime and Pierce 2012). Many non-mutually exclusive mechanisms propose explanations for observed shifts in trait expression (reviewed in Catford et al. 2009). Common biotic explanations posit that 'invasive behavior' (i.e. expression of traits listed above) occurs when populations are released from coevolved predators and competitors, properties which promoted conservative, stress tolerating behavior in the native range (Elton 1958, Blossey and Nötzold 1995, Keane and Crawley 2002, Colautti et al. 2004). Abiotic explanations are equally as plausible, though not as thoroughly investigated (Colautti et al. 2008). For example, increased resource availability decreases competitive intensity over small time-

scales, promoting ‘luxury consumption’ that leads to rapid growth in introduced ranges (Davis et al. 2000). How these regional differences in climate act as selective agents over longer time-scales to drive formally benign, stress tolerating populations to ruderals or aggressive competitors remains unclear.

Large-scale investigations into phenotypic trait relationships amongst a diverse array of plant species (regardless of ‘native’ or ‘invasive’ status) consistently found trade-offs among traits associated with persistence and rapid growth. Wright et al. (2004) found that differences in growth strategies among plants could be explained by variation in leaf-level physiological activities and construction costs. Specifically, they identified what became known as the leaf economics spectrum (LES). Faster growing individuals displayed greater photosynthetic activity and light harvesting abilities (i.e. greater SLA, LAR and LMR), while slower growing individuals showed the opposite pattern. These coupled traits could be scaled up to a single ‘fast-slow’ growth continuum at the whole plant-level (Reich 2014). Leishman et al. (2007) applied this idea and demonstrated that ‘exotic invasive’ and ‘native’ populations differ in that invasive populations are further toward the fast growth end of the continuum. These concepts provide a predictive framework in which physiological variation among regional populations act as the mechanism to promote morphological divergence and a subsequent shift along the LES and the ‘fast-slow’ growth continuum (Wright et al. 2004, Shipley et al. 2006, Leishman et al. 2007, Reich 2014). With the exception of work by DeWalt et al. (2004) and Leishman et al. (2007), demonstrable examples of this shift remain poorly documented.

Evolutionary changes in growth strategy are often revealed by divergence in quantitative traits between native and introduced populations. Plant genotypes with traits correlated to increased fitness (e.g. elevated growth, biomass production and reproductive capacity) are expected to increase their frequency as a result of selection (Blair and Wolfe 2004, Bossdorf et al. 2005, Handley 2008, Franks et al. 2008, Preite et al. 2015). However, in addition to novel selection pressures, neutral processes (e.g. genetic drift during following bottlenecks) and historical events (e.g. founder effects, non-random filtering of genotypes and/or hybridization of previously isolated genotypes) during the invasion process also hold the capacity to promote divergence (Ellstrand and Schierenbeck 2000, Sakai et al. 2001, Lee 2002, Novak and Mack 2005, Novak 2007, Dlugosch and Parker 2007, Keller and Taylor 2008). These are not mutually exclusive hypotheses. Determining which forces promote post-introductory shifts requires a multi-faceted approach. Growth assays focused on morphological traits are the most commonly utilized (e.g. Li et al. 1998, Olsson and Ågren 2002, Bossdorf et al. 2004, Dlugosch and Parker 2008a), but some investigations have also included correlated physiological traits (e.g. DeWalt et al. 2004, Leishman et al. 2007). By providing mechanistic explanations for morphological differentiation, investigations that quantify both morphological and leaf-level physiological activities are preferred due to their increased explanatory power (LES; Wright et al. 2004). While these data reveal the end result of regional divergence, the inclusion of molecular information can reveal what evolutionary forces acted to alter physiological activities, leading to subsequent differences in observed growth and morphology. Given the current interest in post-introductory evolution (reviewed in

Ellstrand and Schierenbeck 2000, Sakai et al. 2001, Bossdorf et al. 2005, Felker-Quinn 2011, 2013), it is surprising that many investigations do not employ molecular data and analytical methods capable of identifying potential evolutionary forces. Analytical techniques that incorporate both morphological and neutral sequence data (i.e. Φ_{RT} /Q_{RT} comparisons) reveal whether stochastic vs. directed forces promoted behaviors observed at present, while selection analyses reveal which traits are actively being selected upon and what factors are acting as selective agents. Therefore, to acquire a comprehensive understanding of post-introductory change in growth strategy, investigations should quantify morphological, physiological and molecular data while utilizing applicable analytical techniques.

Attention to potential contributions of novel abiotic conditions in the promotion of post-introductory change is increasing in the literature (Olsson and Ågren 2002, Colautti et al. 2008, Colautti and Barrett 2013, Preite et al. 2015). Genetically based variation in phenotypic traits among isolated populations (regardless of native or invasive status) of a species often correlate with climatic variables associated with latitude (Colautti et al. 2008, Moles et al. 2009, Preite et al. 2015). Such correlations between trait expression and climatic gradients suggest that abiotic interactions act as a selective agents. All else being equal, latitudinal differences in photoperiod and irradiance have the potential to engender predictable adaptive change (Lambers et al. 2008). Populations adapted to low irradiance, for example, could invest more in light harvesting organs (i.e. increased leaf numbers and area), while populations adapted to high levels of irradiance would be expected to show the opposite pattern, allowing reallocation of resources towards increased growth and

reproduction (DeWalt et al. 2004, Lambers et al. 2008, Moles et al. 2009). Given these patterns it is easy to imagine a scenario in which a benign 'native' population was translocated in the past from high (low light environment) to low latitude (high light environment) where formerly adaptive traits were selected against and subsequently replaced by traits commonly observed in 'invasive' populations.

Bromus rubens L. (Pavlick and Anderson 2007, = *B. madritensis ssp. rubens*, Fortune et al. 2008), also known as Red brome or Foxtail chess, is a winter annual grass common in the Mediterranean (native range) and Southwestern United States (introduced range). Individuals germinate promptly after precipitation events, display rapid growth rates through the winter growing season, with reproduction and senescence in early spring (Heady 1977, DeFalco et al. 2007). Individuals are primarily autogamous with high reproductive output (Sales 1993, 1994). The earliest recorded observation of *B. rubens* within the introduced range dates to before 1880 (Watson 1880). Since that time *B. rubens* has become one of the most aggressive invasive plant species in the Mediterranean climatic regions of California, often forming near-monospecific stands that decrease native biodiversity (Salo 2005, Minnich 2008). However, in its native Mediterranean range it occurs only sparsely or patchily in disturbed areas (Jackson and Roy 1989, Allen and Allen *personal observation*). Such disparity in distribution and abundance made *B. rubens* a model candidate to observe mechanisms promoting behavioral divergence among native and introduced populations.

Here I utilized polymorphic molecular markers along with morphological, physiological and climatic traits among seven native and seven introduced populations of

B. rubens to investigate whether regional variation in climate could drive post-introductory shifts along the LES continuum. Since *B. rubens* is a tetraploid (Sales 1994), nuclear markers were not used to avoid a preponderance of heterozygous nucleotide sites (Fortune et al. 2008, Avise 2009). For this reason single copy, non-coding sequences within chloroplast DNA (cpDNA) provide greater quality data and were used. A common garden approach was used to quantify divergence in growth strategies among regional populations. Finally, Φ_{RT}/Q_{RT} comparisons were coupled with correlational and selection analyses to determine which evolutionary forces historically acted on introduced populations, and which traits were under pressure to promote more aggressive growth in these populations. Using these data I sought to answer the following questions: 1) Do regional populations display growth strategies indicative of post-introductory evolution of invasive characters?; 2) What evolutionary forces (e.g. drift vs. selection) promoted divergence among native and invasive populations?; and 3) How do regional differences in climate act as selective agents on traits commonly found in invasive populations?

Materials and Methods

Seed collections

Recognizing that many patterns of plant invasion mirror historical commercial interactions among regions, this information can be used to identify which regions possess the highest probability of harboring 'ancestral' populations (Novak et al. 1993). With this in mind, and the results of a Mediterranean-wide survey of *B. rubens* morphology (Allen and Allen *unpublished data*), native seed collections were limited to Spain.

Seed collections were made after senescence throughout 2011-2012. At least 60 individuals, spaced >1m apart, were sampled in 7 populations from each respective range. Locations of seed collection from within the native range (Spain) included: El Pardo (Madrid Province), Aranjuez (Madrid Province), Los Cuadros (Murcia Province), Murcia University campus (Murcia Province), Cartagena (Murcia Province), Quentar (Granada Province) and Granada (Granada Province) (Table 1.1). The locations of seed collection from within the introduced range (California) included: Big Bear (San Bernardino Co.), Joshua Tree (Riverside Co.), Riverside (Riverside Co.), Perris (Riverside Co.), Irvine (Orange Co.), Temecula (Riverside Co.) and El Rosario (Estado de Baja California, Mexico) (Table 1.1).

Molecular variation

Seeds from 20 randomly chosen individuals per population were germinated, transplanted into pots of UC Soil Mix III (75% fine quartz sand, 25% ground peat moss; Padgett and Allen 1999) and grown in a greenhouse on the University of California, Riverside (UCR) campus. After 30 days of growth ~ 50-100mg of leaf tissue was harvested from each individual, transferred to ice, and stored at -80°C. A DNeasy Plant Mini Kit (Qiagen, USA) was used to isolate DNA from leaf tissue as directed, with the addition of elongating the lytic step for 15 minutes at 65°C instead of 10, and eluting twice with 50µl elution buffer in the final elution step (following Ridley et al. 2008). DNA quality was quantified by spectrophotometry using a Nanodrop ND-1000 (Thermo Fisher Scientific, USA).

Polymerase chain reaction (PCR) was carried out using a PTC-100 Programmable Thermal Controller (MJ Research, USA) in a 25µl volume containing the following: 1µl of template DNA (5-20ng), 2.5µl 10x Taq buffer (New England Biolabs [NEB], USA), 2.5µl 2mM solution $MgCl_2$, 1µl 10mM solution dNTP mix (NEB), 0.5µl 10mM solution forward primer, 0.5µl 10mM solution reverse primer and 0.5µl Taq polymerase (NEB). Primers [*trn*L5'(UAA)F and *trn*F(GAA)] were those used by Shaw et al. (2005). The *trn*L-*trn*F intergenic region is a reliable single-copy region of the chloroplast genome useful in high- to low-level taxonomic studies (Shaw et al. 2005, Shaw et al. 2007). PCR conditions were the following: initial denaturation at 80°C for 5 minutes; 30 cycles of denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute and extension at 72°C for 2 minutes; final extension at 72°C for 5 minutes. Replicate PCR reactions were carried out for each individual to allow for quality control via gel electrophoresis.

All PCR products were cleaned with ExoSAP-IT (USB, USA). Sequencing was carried out on an ABI 3730xl using the BigDye Terminator Sequencing Kit (Applied Biosystems, USA) at the Institute for Integrative Genome Biology core instrumentation facility at UCR. Each PCR product was subjected to 2 sequencing reactions, one in the forward and another in the reverse direction. Consensus sequences for each population have GenBank accession numbers from MG657033 to MG657046.

Molecular analyses

Forward and reverse reads were combined into single contiguous sequences (contigs) and edited using Sequencher 5.1 (Gene Codes Corporation, USA). Resulting

contigs were then aligned using the program BioEdit (Hall 1999). Standard population genetic indices were calculated for each population and region using GenAlEx v6.5 (Peakall and Smouse 2006) and the *Poppr* 2.0.2 package (Kamvar et al. 2014) within R version v3.2.0 (R Core Team 2015). Population genetic indices included the following: number of haplotypes identified (NH), percent polymorphic nucleotide sites (%P), allelic richness (NA) and an index of genetic differentiation for haploid data, Φ_{PT} [with Φ being an analogue of F; Meirmans (2006)]. Given the ancestral (native) and descendent (introduced) relationship among regions, I used a Wilcoxon signed-rank test to determine whether mean genetic indices differed among regions. Due to the autogamous reproductive strategy commonly observed in this species, all indices and analyses (including those below) were repeated assuming a clonal relationship among similar haplotypes within populations. This was done by omitting replicate haplotypes within populations prior to analyses. Interpretations of results from the smaller, 'clonal' data set did not differ from the full data set. Therefore, results of molecular analyses discussed are based on the full data set.

Hierarchical analyses of molecular variance (AMOVA) were conducted to examine molecular diversity and test for differentiation among populations, regions and genetic clusters. Genetic structure was further investigated using the Bayesian clustering program STRUCTURE 2.3.4 (Pritchard et al. 2000). This program allows for the inference of genetic structure and admixture among populations by probabilistically assigning haplotypes to clusters without using geographic information of individuals. STRUCTURE was run using the admixture model and correlated allele frequencies with 100,000 MCMC

repetitions and a 50,000 burn-in period. The number of potential populations or clusters (*K*) was set from 1 to 17, with each *K* independently replicated 15 times. The optimal number of clusters (*K*) was determined following methods described by Evanno et al. (2005) and carried out in Structure Harvester v0.6.94 (Earl and vonHoldt 2012). For the optimal *K*, an individual's cluster assignment coefficient (*q*) to each genetic cluster was averaged across all replicate runs using the program CLUMPP (Jakobsson and Rosenberg 2007) and visualized using STRUCTURE PLOT (Ramasamy et al. 2014).

Quantitative trait variation

To compare variation in plant vigor among native and introduced populations I conducted a common garden under environmentally controlled conditions. Seeds from each population were randomly chosen, sterilized with 10% sodium hypochlorite and germinated on moistened filter paper under ambient laboratory conditions. No significant differences in timing or percentage of germination among populations was observed. Sixty seedlings per population (total N = 840 plants) were transplanted to 656ml pots (Stuewe & Sons, Inc., Tangent, Oregon) containing UC Soil Mix III and transferred to a greenhouse on the UCR campus (light levels ranged from 700 to 1200 μmol m^{-2} s^{-1}). Pots were randomly relocated weekly to reduce 'bench effects' throughout the growth period. Pots received equal amounts of water as needed throughout the experiment.

The objective of this experiment was to quantify and compare maximal growth under optimal conditions (i.e. plant vigor) among native and introduced populations. Growth duration was limited to a 50-day period since plant physiological activities tend to

slow at older, more reproductive ages. In a separate study (chapter 2), I found that *B. rubens* has a life span of between 75-140 days under greenhouse conditions (*unpublished data*). Shoot height (cm) was recorded on the 10^{th} day after transplant and at harvest (50^{th} day) in order to estimate total relative shoot growth rates (RGR_{SHOOT}). RGR_{SHOOT} for each individual was calculated as: $RGR = (\ln H_2 - \ln H_1)/(t_2 - t_1)$ where H_2 and H_1 represent observed height following establishment (H_1) and just prior to harvest (H_2), and t_2 and t_1 represent the respective dates these measurements were made. At harvest shoot height, leaf number and leaf area were recorded. A subset of 10 individuals per population were randomly selected for leaf area measurements on a LI-COR 3100 leaf area meter (LI-COR, Lincoln, Neb., USA), N = 140. Plants were divided into leaves, stems and roots, and dried at 70°C until constant mass. These data were used to derive variables representative of physiological activity and allocation patterns, including total plant mass (biomass [g]), shoot mass ratio (SMR, shoot mass per total plant mass [$g\ g^{-1}$]), leaf mass ratio (LMR, leaf mass per total plant mass [$g\ g^{-1}$]), and leaf area ratio (LAR, total leaf area per whole plant mass [$cm^2\ g^{-1}$]). Allocation to reproductive organs was not measured. I was able to use biomass as a reliable index of reproductive fitness, as biomass of *B. rubens* was correlated with seeds production ($r = 0.82$, $p < 0.05$) (Huxman et al. 1999).

Prior to harvest all plants were transferred to the laboratory. Following dark induction (30 minutes at 10 μmol $m^{-2}\ s^{-1}$), photosynthetic performance was measured on one fully expanded leaf per plant for five plants per population (total leaves sampled = 70) via chlorophyll fluorescence. This was done using a photosynthesis yield analyzer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany). From constructed light response curves

maximum electron transport rate (ETR_{MAX}) and yield of photosystem II (Φ_{PSII}) at the highest light level were calculated. ETR_{MAX} is a metric of photosynthetic capacity. Specifically, it measures electron flow from photosystem II to photosystem I, and therefore serves as a rapid screen for photosynthetic activity. Φ_{PSII} measures light conversion to energy per photon received, reflecting the efficiency of the photosynthetic process.

Local climatic data for each population was extracted from IWMI Online Climate Service Model (World Water and Climate Atlas; International Water Management Institute, Colombo, Sri Lanka). Data utilized were percent daily sunshine hours (SUN), mean daily temperature (MDT), mean monthly rainfall (MRF) and moisture availability (MAI).

Quantitative trait analyses

Generalized mixed-effects models were performed using the *lmer*() function within the package *lme4* version 1.1-6 (Bates et al. 2015) in R (R Core Team 2015) for all quantitative traits. Region of origin (native vs. introduced) was treated as a fixed effect and populations within region were considered random. The denominator degrees of freedom were calculated using the Satterthwaite approximation for F-tests of fixed effects. If post-introductory phenotypic differentiation toward more aggressive growth occurred in populations sampled (i.e. increased biomass, shoot height, RGR_{SHOOT}, SMR and photosynthetic activity; and decreased allocation to light harvesting organs such as No. leaves, LMR and LAR), then significant differences among all eight traits considered would be expected.

To assess adaptation to environmental conditions simple correlation analyses were performed between population trait means and latitude of origin (LAT), SUN, MDT, MRF and MAI using the function *corr.test* in R-package *psych* version 1.3.10 (Revelle 2013). Significant correlations between phenotypic and environmental traits would suggest some degree of local adaptation.

Potential maternal effects

Seeds were selected at random from each population, mass was recorded in groups of 10 seeds, and divided by that number to derive 60 (N = 600 seeds sampled per population) data points of average seed mass for each respective population. These data points were then randomly assigned to each focal plant within each respective population. This allowed for the avoidance of spurious interpretation as a result of maternal effects.

To determine the effect of average seed mass on observed traits of interest I performed ANCOVAs using type-III sums of squares. Separate analyses were run for each of the eight phenotypic traits of interest with the following model; region of origin and the interaction of region x average seed mass as independent variables, and trait of interest as dependent. A significant interaction term would have indicated maternal influence on observed traits. No significant interaction terms were detected.

Φ_{RT} /Q_{RT} Comparisons

In theory, differentiation of neutral molecular markers (Φ_{RT}) would be most influenced by the force of genetic drift, while trait differentiation (Q_{RT}) should be more

influenced by natural selection (Whitlock 2008). The following formula, with variance estimates obtained from generalized mixed-effects models (see section on *Quantitative trait variation* above), was used to calculate Q_{RT} values for pairwise comparisons among regions (native vs. introduced): Q_{RT} = variance among groups/ (variance among groups + variance within groups). The estimators of Φ_{RT} and Q_{RT} were compared based on their 95% confidence intervals (95% CIs). The magnitude of difference between Φ_{RT}/Q_{RT} indicates the level of selection on populations for morphological traits considered (Merilä and Crnokrak 2001, O'Hara and Merilä 2005, Whitlock 2008). Theory predicts that when $Q = \Phi$ trait differentiation occurred neutrally, when $Q < \Phi$ stabilizing selection acted on phenotypic traits, and when $Q > \Phi$ disruptive selection was in action (Merilä and Crnokrak 2001, Scheepens et al. 2010).

Selection Analyses

The magnitude of selection acting on traits of interest were estimated for each region and population using the methods outlined by Lande and Arnold (1983) and Mitchell-Olds and Shaw (1987). Since biomass was used to derive the index of reproductive fitness, this trait was not included in this analysis. Prior to univariate and multiple regressions, traits were first standardized to a mean of zero and variance of one.

Fitness indices were calculated by dividing individual biomass by the grand mean of biomass among all regions and populations (Lande and Arnold 1983). Selection differentials (S) were calculated as the covariance between reproductive fitness and each standardized trait. Here S represents direct linear selection on traits as well as any indirect

effects from selection on any correlated traits. Standardized linear selection gradients (β) were calculated as partial regression coefficients from multiple regressions of relative fitness on all traits. Thus, β is a measure of the effect of each trait on relative fitness, holding all other traits fixed. In theory adaptive evolution should be slowed by negative values of S. Negative values of β indicate a decelerating relationship between trait values and fitness. Positive values of β indicate accelerating selection where a unit change in the trait is associated with a greater fitness increase for more extreme trait values.

Significance of linear selection gradients was determined from the results of original regressions of traits on S and β, respectively. When variables did not meet the assumptions of regression analysis, the significance of selection gradients were determined using 95% CIs estimated by 10,000 bootstrap replicates of the original data. To identify potential abiotic selective agents ANCOVAs were performed with each respective abiotic variable as a covariate and phenotypic trait as a fixed effect. Significant interactions between traits and abiotic variables would indicate that the pattern of selection was at least partially due to the environmental covariate (i.e. selective agent). Regressions and ANCOVA models were performed using the *car* package in R.

Results

Sequence variation

Sequencing reactions of the *trn*L-*trn*F region resulted in reads of 770 bps in length. Of these aligned base pairs, 48 were variable sites (6.2%). Observed variation among

sequences was primarily the result of single-nucleotide polymorphisms. However, one insertion/deletion (7bp) segment was also present.

Genetic diversity

Indices of genetic diversity were consistently lower and more homogenous within introduced relative to native populations of *B. rubens* (Table 1.1). Allelic richness ranged from 1.02 to 1.21 for introduced population vs. 1.04 to 1.33 for native populations (x^2 = 2.18, p = 0.14). Mean observed polymorphic nucleotide sites followed a similar pattern; introduced populations ranged from 0.02 to 0.21 vs. 0.04 to 0.33 in native populations (x^2 = 1.81, p = 0.18). The native range also harbored greater overall NH (28 ranging from 3-8 per population vs. 13 ranging from 2-9 in the introduced range; x^2 = 0.95, p = 0.33), despite the largest level NH belonging to an introduced population (El Rosario with 9 NH). This pattern may be the product of greater sequencing success among native vs. introduced range individuals, but is unlikely given that sample sizes among regions only differed by 4 individuals (Table 1.1).

Haplotype distribution and population structure

Thirty four unique haplotypes were identified. Haplotype E was the most common (accounting for 35% of individuals sampled), followed by haplotype G (27% of individuals sampled). Eight of the haplotypes detected (including E and G) were shared by two or more populations among regions. The remaining 26 haplotypes were singletons or doubletons particular to individual populations. These were grouped into general native (N = 20) or

introduced (N = 6) haplotype categories for visualization and were not included in the inference of distributional patterns (Figure 1.1).

Analyses of molecular variance (AMOVA) supported the qualitative patterns revealed from haplotype distributions (Table 1.2). When populations were grouped by continent of origin (native and introduced) the majority (51%) of variation was explained as regional differences (Φ_{RT} = 0.51, p = 0.001), with populations within region (est. of variation = 22%, Φ_{PR} = 0.46, p = 0.001) and within population variation (est. of variation = 26%, Φ_{PT} = 0.74, p = 0.001) explaining the remainder. Further subdivision of populations into regions of origin (central and southern native populations, and introduced populations) increased the proportion of variation explained by region by 31% (est. of variation = 70%, Φ_{RT} = 0.69, p = 0.001), while variation explained among populations within region was decreased (est. of variation = 4%, Φ_{PR} = 0.14, p = 0.001).

Pairwise comparison of Φ_{PT}-values within and among respective regions allowed for a more detailed view of gene flow and differentiation among populations. Introduced populations demonstrated very little differentiation between most population pairs (Table 1.3). Φ_{PT}-values for populations not showing differentiation ranged from 0 to 0.05 (all p-values > 0.05). However, 3 of the 7 introduced populations sampled did show significant differentiation with at least one other introduced population. The distribution of Φ_{PT}-values within the native range was similar to the introduced. The majority of populations (southern populations: LC, MU, CR, QU and GR) showed no signs of differentiation amongst each other (Φ_{PT}-values ranged from 0 to 0.027, all p-values > 0.05). In contrast, the more northerly populations (EP and AR) showed differentiation from one another and all other

native populations (Φ_{PT}-values ranged from 0.553 to 0.823, all p-values < 0.01). Observed Φ_{PT}-values between populations among regions ranged from 0 to 0.935. Native populations of southern origin showed the greatest degree of differentiation from introduced populations (Φ_{PT}-values ranged from 0.526 to 0.935, all p-values < 0.01), while the two northerly native populations (EP and AR) showed the least (Φ_{PT}-values ranged from 0 to 0.343, respective p-values > 0.05 and p < 0.05).

Consistent with the patterns of haplotype distribution and AMOVA results, Bayesian cluster analyses indicated a large degree of genetic structure in the data set. Based on 15 replicate runs at each *K*, the statistic ΔK (Evanno et al. 2005) indicated that two clusters optimally explained the genetic structure across sampled individuals (Table 1.4, Figure 1.2). Individuals were highly assigned ($q > 0.9$) to either genetic Clusters 1 or 2. A large proportion of central native (80.6%) and introduced populations (90.5%) were assigned to Cluster 1 (the 'invasive' cluster). Of the remaining individuals from these groups, 19.4% of the former and 9.4% of the latter populations were assigned to Cluster 2 (the 'native' cluster). All individuals from southern native populations were assigned to Cluster 2 (making up 83.9% of this Cluster). When considered at a broader level, all but two populations assigned to either genetic Cluster 1 or 2 at $q > 0.8$. These two populations [AR (native) and RI (introduced)] showed evidence of admixture and were also among the most genetically similar based upon Φ_{PT}-values.

Quantitative trait variation

To determine whether ancestral (native) populations possessed the capacity for evolutionary change in phenotypic trait expression, the coefficient of variation for each of the traits of interest was calculated, for each respective population. The relative magnitude of variation in these traits were then compared among native and introduced populations using a Wilcoxon signed-rank test. Results demonstrated that native populations contained greater variability in biomass ($S = 46$, $p = 0.23$), shoot height ($S = 34$, $p = 0.01$), No. leaves ($S = 34$, $p = 0.01$), RGR_{SHOOT} ($S = 46$, $p = 0.23$), SMR ($S = 47$, $p = 0.27$) and LMR ($S = 46$, $p = 0.23$), while introduced populations contained greater variability in LAR ($S = 55$, $p = 0.40$), ETR_{MAX} ($S = 67$, $p = 0.04$) and Φ_{PSII} ($S = 69$, $p = 0.02$).

Among the 14 populations included in this study, one (Aranjuez - AR) displayed significantly smaller stature and biomass. I therefore omitted this population from tests of divergence in height and biomass production. The remaining traits were either not significantly smaller (No. Leaves), based on physiological activity, or standardized by size (RGR_{SHOOT}) or biomass (SMR, LMR and LAR).

Native populations of *B. rubens* displayed greater growth in the common garden experiment (Table 1.5). Populations from the native range demonstrated greater allocation to leaf production ($F = 11.07$, $p = 0.006$), LMR ($F = 5.86$, $p = 0.03$) and LAR ($F = 25.05$, $p = 0.0003$), as well as photosynthetic activity (ETR_{MAX}, $F = 32.01$, $p = 0.0001$; Φ_{PSII}, $F = 22.6$, $p < 0.0001$), compared to introduced range populations. In contrast, introduced populations were comparatively taller ($F = 8.51$, $p = 0.004$) and tended to have greater biomass and RGR_{SHOOT} (Figure 1.3, 1.4 and 1.5).

Correlations between phenotypic traits

Phenotypic trait correlations exposed a pattern suggestive of physiological trade-offs between leafy-high photosynthetic performing individuals and larger-faster growing individuals. Traits indicative of greater leafiness (No. leaves, LAR and LMR) consistently demonstrated positive relationships with each other (r-values ranged from 0.65-0.79) and with photosynthetic performance (r-values ranged from 0.41-0.79). While traits generally associated with greater size (shoot height, biomass and RGR_{SHOOT}) were positively related to each other (r-values ranged from 0.57-0.82), they were negatively related to photosynthetic performance (r-values ranged from -0.33-0.71). Indeed, traits associated with greater size were all negatively related to leafy traits, particularly leaf production to shoot height ($r = -0.65$, $p = 0.01$).

Correlations of phenotypic traits and climate variables

Latitude of origin and sunshine hours displayed the strongest correlations with phenotypic traits (Table 1.6). Leaf production ($r = 0.55$, $p = 0.04$), LAR ($r = 0.73$, $p = 0.003$), LMR ($r = 0.89$, $p < 0.0001$), ETR_{MAX} ($r = 0.78$, $p = 0.001$) and Φ_{PSII} ($r = 0.76$, $p = 0.002$) all demonstrated significant positive relationships with latitude, whereas shoot height ($r = -0.60$, $p = 0.02$) showed a negative relationship. Relationships with percent sunshine hours showed the opposite pattern. Leaf production ($r = -0.65$, $p = 0.01$), LAR ($r = -0.56$, $p = 0.04$), LMR ($r = -0.79$, $p = 0.0006$), ETR_{MAX} ($r = -0.81$, $p = 0.0005$) and Φ_{PSII} ($r = -0.79$, $p = 0.0007$) all displayed significant negative relationships, while shoot height ($r = 0.71$, $p = 0.005$) and RGR_{SHOOT} ($r = 0.54$, $p = 0.04$) showed significant positive

relationships. Leaf production and shoot height also displayed significant, although contrasting, relationships with mean daily temperature and moisture availability (No. Leaves-MDT: $r = -0.55$, $p = 0.04$; No. leaves-MAI: $r = 0.72$, $p = 0.004$; shoot height-MDT: $r = 0.57$, $p = 0.03$; Shoot height-MAI: $r = -0.63$, $p = 0.02$).

Past selection pressures

Genetic differentiation of the *trn*L-*trn*F cpDNA neutral marker among regions was quite high ($\Phi_{RT} = 0.51$, $p = 0.001$). However, trait differentiation among regions (Q_{RT}) exceeded genetic differentiation (Φ_{RT}) for all measured traits except biomass (Table 1.5).

Differential selection patterns

Phenotypic selection analysis on the full data set revealed strong selection on traits associated with stature, shoot allocation and photosynthetic activity (Table 1.7). Selection across populations and regions favored increased allocation to shoot height ($S = 0.19$, $p < 0.01$; $\beta = 0.25$, $p < 0.01$), No. leaves ($\beta = 0.21$, $p < 0.05$), RGR_{SHOOT} ($S = 0.15$, $p < 0.05$), and decreased investment in SMR ($S = -0.14$, $p < 0.05$), LAR ($S = -0.14$, $p < 0.05$), and Φ_{PSII} ($S = -0.16$, $p < 0.05$) (Figure 1.6). ANCOVAs of phenotypic trait and abiotic variables on the fitness index revealed that the primary selective agents included in this study were percent sunshine hours (SMR x SUN, $F = 5.30$, $p < 0.05$; LMR x SUN, $F = 7.25$, $p < 0.05$; LAR x SUN, $F = 14.65$, $p < 0.01$; Φ_{PSII} x SUN, $F = 10.56$, $p < 0.01$) and latitude of population origin (No. leaves x LAT, $F = 5.49$, $p < 0.05$; SMR x LAT, $F = 9.48$, $p < 0.05$; LMR x LAT, $F = 16.52$, $p < 0.01$; LAR x LAT, $F = 16.43$, $p < 0.01$; Φ_{PSII} x LAT, $F = 14.22$,

$p < 0.01$), although water availability also influenced investment in LAR (LAR x MRF, $F = 13.16$, $p < 0.01$; LAR x MAI, $F = 18.47$, $p < 0.01$).

In comparisons of native and introduced range populations, selection analyses revealed stronger selection on the native populations relative to introduced. Total selection (S) in native populations favored increased allocation to shoot height ($S = 0.24$, $p < 0.05$), and decreased allocation to LAR ($S = -0.26$, $p < 0.05$) and Φ_{PSII} ($S = -0.17$, $p < 0.01$) (Figure 1.6). No ANCOVAs for the native data set were significant (Table 1.6). However, ANCOVAs on the introduced data set were significant for No. Leaves (No. leaves x MDT, $F = 17.50$, $p < 0.05$; No. leaves x MRF, $F = 29.34$, $p < 0.05$; No. leaves x MAI, $F = 37.40$, $p < 0.05$), RGR_{SHOOT} (RGR_{SHOOT} x MDT, $F = 25.38$, $p < 0.05$; RGR_{SHOOT} x MRF, $F = 95.04$, $p < 0.01$; RGR_{SHOOT} x MAI, $F = 138.38$, $p < 0.01$), LMR (LMR x LAT, $F = 15.08$, $p < 0.05$), ETR_{MAX} (ETR_{MAX} x LAT, $F = 20.57$, $p < 0.05$), and Φ_{PSII} (Φ_{PSII} x LAT, $F = 29.47$, $p < 0.05$).

Discussion

To investigate whether regional variation in climate could drive post-introductory shifts along the LES/fast-slow continuum toward more aggressive growth I provide data from polymorphic markers, quantitative and climate traits among native and introduced populations of the invasive grass *B. rubens.* With these data I addressed the following questions: 1) Do regional populations display growth strategies indicative of post-introductory evolution of invasive characters?; 2) What evolutionary forces (i.e. stochastic vs. directed) promoted divergence among native and invasive populations?; and 3) How

did regional differences in climate act as selective agents on traits commonly found in invasive populations? Resultant patterns from the common garden experiment revealed a trade-off in growth strategies among regional populations; native populations tended to invest more in light harvesting organs (number of leaves, LMR and LAR) and photosynthetic activity, while introduced populations displayed traits commonly associated with increased aggressive behavior (greater biomass, shoot height and RGR_{SHOOT}). Φ_{RT}/Q_{RT} comparisons demonstrated that these shifts were the primary result of directed relative to stochastic forces. Selection analyses further demonstrated that newly translocated populations from native to introduced ranges experienced strong selective pressure on traits associated with increased stature. Finally, analytical methods utilized identified variation in light availability as the primary selective agent driving observed phenotypic divergence.

Genetic patterns underlying post-introductory change

The results of molecular analyses were consistent with investigations demonstrating large losses in genetic diversity due to bottleneck events or founder effects during introduction (Novak and Mack 2005, Dlugosch and Parker 2008). Many unique haplotypes belonging to both native and introduced populations were identified. Of those haplotypes, two revealed patterns of gene flow and the genetic source(s) of introduced populations sampled. The most abundant of these haplotypes (haplotype E) was found in all populations from the introduced range and two native populations (Aranjuez and El Pardo) from central Spain, but was not found in any other native population sampled.

Haplotype G was less common. It was found in all populations from southern but not central Spain. Importantly, it was also found in one introduced population (Riverside). These data indicated at least two interesting patterns. First, central Spain was the primary genetic source for the introduced populations sampled. Second, at least two introduction events occurred (at least one from central and the other from southern Spain).

The potential for colonizing populations to establish and eventually display growth strategies commonly observed in invasive populations can be determined by early patterns of introduction (Novak 1993). Other investigations demonstrated similar decreases in molecular diversity within introduced populations (Novak and Mack 2005, Dlugosch and Parker 2008, reviewed in Dlugosch and Parker 2008b). It is possible that lower diversity due to bottlenecks or founder effects could terminate the invasion process due to drift (Sakai et al. 2001). However, decreased diversity does not always equal invasion failure. Non-random filtering (environmental filtering) of colonizing genotypes might appear like a random bottleneck event, but may reflect founder effects of colonists 'preadapted' or post-introductory selection on a small number of colonizing individuals which expressed beneficial traits (Baker 1965). When this is the case, common garden studies should show little differentiation among source and introduced populations (i.e. no post-introductory change is necessary). Indeed, pair-wise Φ_{PT} comparisons and STRUCTURE analyses provided no evidence of molecular differentiation among six of seven introduced populations and the one 'source' population (El Pardo). The prevalence of GC1 genotypes found within all populations sampled in the introduced range and those originating from central Spain, indicated some degree of environmental filtering of colonizing genotypes

upon introduction. However, it also appears that while decreased molecular diversity following introduction is common, the lasting effects of these events may be ephemeral in nature (Dlugosch and Parker 2008b). Multiple introductions from previously isolated populations within the native range can increase diversity in established populations of genetically depauperate makeup (Novak et al. 1993). When this occurs admixture within the introduced range should increase genetic diversity, thus increasing phenotypic variation upon which selection can act. One introduced population in this study showed signs of admixture among reproductively isolated populations from the native range. Contrary to predictions (Ellstrand and Schierenbeck 2000, Sakai et al. 2001, Dlugosch and Parker 2008b), admixture did not increase diversity or promote greater growth performance. However, the use of non-coding regions within the chloroplast genome limited my ability to make accurate conclusions on this matter.

Introduced populations and those originating from southern Spain displayed similarly higher levels of fitness (based upon the index used) relative to 'source' populations from central Spain. Comparisons among regional populations assigned to the same genetic clusters ($GC1_{NAT}$ [EP] vs. $GC1_{INT}$ [all introduced populations, excluding RI]; and $GC_{NAT\text{-}ADMIX}$ [AR] vs. $GC_{INT\text{-}ADMIX}$ [RI]) revealed a large amount of within cluster divergence in traits correlated with greater fitness, regardless of cluster assignment (GC1 and GC_{ADMIX}). As demonstrated in previous work (reviewed in Bossdorf et al. 2005, Felker-Quinn et al. 2013), introduced and native populations also showed divergence in allocation patterns. Introduced populations displayed greater biomass accumulation, shoot height and RGR_{SHOOT}, while native populations displayed greater allocation to light

harvesting organs (greater number of leaves, LMR and LAR) and photosynthetic activities (greater ETR_{MAX} and Φ_{PSII}). The patterns observed within introduced populations suggest that post-introductory divergence in growth strategies occurred sometime in the past. However, the observed lack of molecular diversity in introduced populations could not explain these patterns. I infer that sufficient variation in phenotypic expression was likely present upon which selection could act.

Trade-offs, trait-environmental correlations and adaptation

As expected under the LES, strong correlations among traits were identified that together formed a continuum from fast to slow growth strategies (Wright et al. 2004, Leishman et al. 2007). However, traits associated with these strategies were not in accordance with those identified by Wright et al. (2004). Unlike the LES, I found that the leafy-high photosynthetic growth strategy of native populations did not equate to faster growth or increases to the fitness index. In contrast, introduced populations invested little in photosynthetic activity and light harvesting organs, instead allocating resources away from leaf production towards other traits correlated with increased reproductive fitness. This surprising pattern may be explained by two non-mutually exclusive mechanisms. One, the overall larger size of leaves in introduced populations may increase CO_2 diffusion pathways (Parkhurst 1994). Two, the larger leaf size may also dilute nitrogen concentrations (Reich et al. 1998). In either case photosynthetic activity would be limited, thus lowering the cost of protein maintenance and allowing for the redistribution of C and N to non-photosynthetic leaf components (Hikosaka et al. 1998).

In addition to the phenotypic trait relationships found in this and other studies (Wright et al. 2004, Leishman et al. 2007), correlations between the index of reproductive fitness and traits typically found in introduced populations were revealed. Shoot height and RGR_{SHOOT} were consistently positively correlated with the reproductive fitness index across regions, while investment in light harvesting organs and photosynthetic activities were all negatively related to this index of fitness. These patterns suggest that individuals that display relatively greater height and growth rates should outcompete co-occurring genotypes, increasing the taller-faster growing genotypes in subsequent generations, regardless of population origin.

To determine which factors influenced the observed shift in growth strategies among regional populations, correlation analyses were performed on historical climate data from each population's site of origin with phenotypic trait data. Correlations with latitude of origin were negatively related to the fitness index (biomass) across the full data set and native range (i.e. increased latitude caused decreases to the fitness index). These patterns were consistent with similar investigations that found decreased height and biomass accumulation with increased latitude of origin and/or elevation (Turesson 1925, Li et al. 1998, Olsson and Ågren 2002, Moles et al. 2009, Scheepens and Stöcklin 2013, Preite et al. 2015). Furthermore, local irradiance received was positively related to biomass, shoot height and RGR_{SHOOT}, and negatively related to leaf production, shoot mass, LMR, LAR, ETR_{MAX} and Φ_{PSII}. Although many other abiotic and biotic factors not included in this study may have contributed to these patterns, it appears that latitudinal patterns of

differentiation were the result of variation in irradiance received, and to a lesser extent to variation in moisture availability and mean daily temperature.

Directed vs. stochastic patterns of post-introductory change

No evidence for stochastic trait differentiation among regions was observed. Rather, patterns suggestive of past selection pressures were detected in all Φ_{RT} /Q_{RT} trait comparisons. All but one (biomass) of the included traits displayed much greater phenotypic trait differentiation (Q_{RT}) among regions than molecular differentiation (Φ_{RT}) (i.e. shoot height, No. Leaves, RGR_{SHOOT}, SMR, LMR, LAR, ETR_{MAX} and Φ_{PSII}). These large differences in Q_{RT}-values suggest that post-introductory disruptive selection acted on these traits. When coupled with the negative correlative patterns between light harvesting organs, physiological traits and regional variation in irradiance received, it appears that increased light availability experienced by early colonizing native (ancestral) populations selected against allocation to formerly adaptive traits.

In contrast to all other traits considered, total biomass accumulation showed little to no differentiation among regions. This lack of trait relative to molecular differentiation suggested that stabilizing selection acted on biomass production. This suggests that early colonizing populations of *B. rubens* were subject to environmental filtering events of 'preadapted' genotypes with a range of optimal biomass values.

Magnitudes and agents of selection pressure

Results from selection analyses were in accordance with correlational and Φ_{RT}/Q_{RT} trait comparisons. Overall, plant height experienced the greatest magnitude of positive selection, followed by RGR_{SHOOT}, while allocation to shoot mass (SMR), leaf area (LAR), and Φ_{PSII} were selected against. These data reveal a pattern in which any unit increase in plant height or growth rate, or decrease in shoot allocation or investment in light harvesting organs, would result in increased biomass (the index for reproductive fitness), regardless of population origin. That is, genotypes expressing taller-faster growth strategies should display greater reproductive success, and should therefore experience proportional increases in subsequent generations relative to genotypes expressing a leafy-high photosynthetic growth strategy. These results provide a novel evolutionary mechanism, however ostensible, to explain divergence as expected under the Evolution of Increased Competitive Ability hypothesis (Blossey and Nötzold 1995); traits reflective of an individual's ability to compete for light (height) and soil resources (growth rate) were strongly selected for (Westoby et al. 2002, Moles et al. 2009).

Patterns of selection between introduced and native populations were expected to be divergent. For introduced populations it was assumed that if adaptation had taken place in the past, then these populations should experience little selection pressure in the present. Indeed, introduced populations showed no significant selection pressure on traits associated with the fitness index, suggesting that optimal ranges of trait expression had been achieved sometime in the past. Contrasting patterns were observed in native range populations. It was predicted that if the aggressive growth observed in introduced

populations was the result of post-introductory selection, then newly introduced populations should experience strong selection pressure on traits associated with this aggressiveness. As with the analysis on the full data set, strong selection pressure was observed on increased allocation to plant height and decreased allocation to light harvesting organs (LAR and Φ_{PSII}) in native populations. These results provide evidence that evolutionary divergence among regional populations may be promoted immediately after introduction.

Conclusions

This study provides a novel explanation for post-introductory trait change driven by climatic variability between ancestral (native) and descendant (introduced) populations of the invasive species *B. rubens*. Given the lack of molecular diversity, and the increased expression of traits correlated with aggressive growth and reproductive fitness, I propose that introduced populations were likely the product of environmental filtering. Selection analyses revealed that character state change was overwhelmingly driven by directed selection in most traits considered. By including climatic variables this study was able to infer that differences in irradiance received between native and introduced regions contributed to a shift along an ecological trait continuum toward increased aggressiveness. It was further demonstrated that selection acted within the first generation after colonization to engender change in native populations, and that adaptation could be achieved within the time span of ~130 years in introduced populations. While other, unknown abiotic and biotic factors may have contributed to the patterns observed,

divergence in growth strategies between native and introduced populations of *B. rubens* suggest that variation in abiotic conditions hold the capacity to drive once benign, native populations toward more aggressive invaders.

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Table 1.1. Location of *B. rubens* populations sampled in the native range (Spain) and introduced range (California), number of samples sequenced (N), number of haplotypes identified (NH), and population averages for number of alleles (NA) and percent polymorphic nucleotide sites (%P).

Population	Abbreviation	Latitude	Longitude	N	NH	NA	%P
El Pardo	EP	40.525	-3.778	18	7	1.333	0.333
Aranjuez	AR	40.032	-3.603	13	8	1.250	0.250
Los Cuadros	LC	38.037	-1.090	17	6	1.188	0.188
Murcia	MU	38.012	-1.130	20	5	1.229	0.229
Cartagena	CR	37.605	-0.792	15	6	1.125	0.125
Quentar	QU	37.195	-3.462	20	6	1.188	0.167
Granada	GR	37.184	-3.586	17	3	1.042	0.042
Big Bear	BB	34.291	-116.975	9	2	1.021	0.021
Joshua Tree	JT	34.077	-116.355	19	7	1.104	0.104
Riverside	RI	33.980	-117.306	14	2	1.146	0.146
Perris	PE	33.803	-117.255	20	5	1.208	0.208
Irvine	IR	33.631	-117.556	19	5	1.104	0.104
Temecula	TE	33.569	-117.062	15	5	1.188	0.188
El Rosario	EL	30.059	-115.726	20	9	1.167	0.167

Table 1.2. Results of hierarchical Analyses of Molecular Variance (AMOVA) showing the distribution of genetic variation and degree of reproductive isolation (Φ). Separate models were run to analyze patterns at A) regional scale, B) within the native region and introduced regions and C) among genetic clusters identified by STRUCTURE analyses (K = 2).

A) Native vs. Introduced ranges

AMOVA analysis	*df*	SS	MS	Est. Variation	% of total variation	Φ	p-value
Among regions	1	201.93	201.93	1.6	51	Φ_{RT} 0.51	0.001
Among populations|regions	12	150.46	12.54	0.7	22	Φ_{PR} 0.46	0.001
Within populations	222	180.17	0.81	0.81	26	Φ_{PT} 0.74	0.001
Among all populations	235	532.56					

B) Within Native range vs. Introduced range

AMOVA analysis	*df*	SS	MS	Est. Variation	% of total variation	Φ	p-value
Among regions	2	318.21	159.11	2.21	70	Φ_{RT} 0.69	0.001
Among populations|regions	11	34.18	3.11	0.14	4	Φ_{PR} 0.14	0.001
Within populations	222	180.17	0.81	0.81	26	Φ_{PT} 0.74	0.001
Among all populations	235	532.56					

C) Genetic clusters

AMOVA analysis	*df*	SS	MS	Est. Variation	% of total variation	Φ	p-value
Among regions	1	316.64	316.64	2.83	75	Φ_{RT} 0.75	0.001
Among populations|regions	12	35.75	2.97	0.13	3	Φ_{PR} 0.14	0.001
Within populations	222	180.17	0.81	0.81	22	Φ_{PT} 0.79	0.001
Among all populations	235	532.56					

Table 1.3. Φ_{PT}-values for each population pair. Large Φ_{PT}-values indicate less gene flow (greater genetic isolation, distantly related) between populations, while smaller values indicate more gene flow (lower genetic isolation, closely related). Values in bold indicate significant genetic isolation between population pairs. p-values for each population pair are provided on the off diagonal.

Pairwise Φ_{PT}	El Pardo	Aranjuez	Los Cuadros	Murcia	Cartagena	Quentar	Granada	Big Bear	Joshua Tree	Riverside	Perris	Irvine	Temecula	El Rosario
El Pardo		0.004	0.001	0.001	0.001	0.001	0.001	0.464	0.388	0.001	0.357	0.372	0.411	0.415
Aranjuez	**0.193**		0.001	0.001	0.001	0.001	0.001	0.048	0.001	0.259	0.041	0.002	0.161	0.001
Los Cuadros	**0.788**	**0.553**		0.425	0.423	0.371	0.134	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Murcia	**0.801**	**0.575**	0.000		0.354	0.327	0.150	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Cartagena	**0.795**	**0.552**	0.001	0.005		0.290	0.207	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Quentar	**0.794**	**0.568**	0.000	0.004	0.010		0.136	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Granada	**0.823**	**0.590**	0.033	0.018	0.024	0.027		0.001	0.001	0.001	0.001	0.001	0.001	0.001
Big Bear	0.000	**0.216**	**0.878**	**0.887**	**0.898**	**0.878**	**0.935**		0.038	0.007	0.241	0.356	0.302	0.156
Joshua Tree	0.000	**0.281**	**0.853**	**0.862**	**0.864**	**0.853**	**0.890**	**0.119**		0.001	0.089	0.030	0.049	0.386
Riverside	**0.343**	0.000	**0.526**	**0.548**	**0.529**	**0.542**	**0.574**	**0.388**	**0.441**		0.005	0.001	0.024	0.001
Perris	0.000	**0.148**	**0.783**	**0.795**	**0.792**	**0.787**	**0.822**	0.006	0.046	**0.294**		0.486	0.316	0.127
Irvine	0.001	**0.296**	**0.884**	**0.890**	**0.898**	**0.882**	**0.924**	0.000	**0.082**	**0.460**	0.016		0.127	0.097
Temecula	0.000	0.061	**0.758**	**0.772**	**0.765**	**0.764**	**0.799**	0.017	**0.066**	**0.207**	0.000	0.051		0.150
El Rosario	0.000	**0.260**	**0.835**	**0.845**	**0.844**	**0.837**	**0.870**	0.044	0.000	**0.420**	0.031	0.037	0.037	

Table 1.4. STRUCTURE results showing proportion membership (*q*) of each predefined population in each of k = 2 clusters with site origin data not used.

	Group	
Population	1	2
El Pardo	0.957	0.043
Aranjuez	0.616	0.384
Los Cuadros	0.004	0.996
Murcia	0.004	0.996
Cartagena	0.004	0.996
Quentar	0.004	0.996
Granada	0.004	0.996
Big Bear	0.996	0.004
Joshua Tree	0.996	0.004
Riverside	0.502	0.498
Perris	0.897	0.103
Irvine	0.996	0.004
Temecula	0.864	0.136
El Rosario	0.995	0.005

Table 1.5. Comparative results of analyses to quantify regional differentiation in native and introduced populations of *B. rubens.* Values are means (±SE) for each respective trait and region. Percent differences calculated as introduced range value – native range value are provided for comparative purposes. Quantitative trait differentiation are provided among regions (Q_{RT}) for 9 phenotypic traits. The greater-than less-than symbols indicate the comparison between molecular (Φ_{RT}) and phenotypic differentiation (Q_{RT}). (*) p < 0.10, * p < 0.05, ** p < 0.01, *** p < 0.001.

	Native	Introduced	F/x^2	%difference	Q_{RT}	Q_{RT} - Φ_{RT}
Molecular Traits						
NH	6 (0.59)	5 (0.95)	0.95	-18.18		
NA	1.19 (0.04)	1.13 (0.02)	2.18	-5.07		
%P	0.19 (0.04)	0.13 (0.02)	1.81	-34.57		
Quantitative Traits						
Biomass (g)	0.25 (0.005)	0.27 (0.004)	0.29	5.48	0.23 (0.10 - 0.35)	<
Height (cm)	20.16 (0.23)	24.96 (0.19)	**8.51****	21.28	0.89 (0.87-0.89)	>
No. Leaves	34.39 (0.58)	23.32 (0.33)	**11.07****	-38.39	0.99 (0.996-0.997)	>
RGR (cm day^{-1})	0.024 (0.0004)	0.029 (0.0003)	3.59(*)	19.73	0.73 (0.74-0.79)	>
SMR (g g^{-1})	0.82 (0.004)	0.78 (0.003)	3.67(*)	-5.38	0.77 (0.74-0.79)	>
LMR (g g^{-1})	0.61 (0.003)	0.55 (0.003)	**25.05*****	-10.82	0.96 (0.95-0.97)	>
LAR (cm g^{-1})	0.021 (0.001)	0.019 (0.001)	**5.86***	-12.74	0.84 (0.82-0.86)	>
ETR_{MAX} (µmol m^{-2} s^{-1})	8.77 (0.52)	4.79 (0.40)	**32.01*****	-58.55	0.97 (0.96-0.97	>
ϕ_{PSII} (µmolCO2 µmol photon^{-1})	0.013 (0.001)	0.006 (0.001)	**22.6*****	-78.53	0.95 (0.95-0.96)	>

Φ_{RT} = 0.51 (0.48-0.55)
Values in parentheses are ± SE estimates for all traits except Q_{RT}, these are 95% CI's based on jackknifing over populations.

Table 1.6. Pearson's correlation coefficients (r-values) between phenotypic traits of *B. rubens* and each of the 5 abiotic factors included in this study. Significant values are in bold. (*) $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Abiotic traits				
Phenotypic Traits	SUN	MDT	MRF	MAI	LAT
Shoot height	**0.71****	**0.57***	**0.57***	**-0.63***	**-0.60***
No. Leaves	**-0.65***	**-0.55***	**0.72****	**0.72****	**0.55***
RGR_{SHOOT}	**0.54***	0.29	-0.38	-0.47(*)	-0.45
SMR	**-0.49(*)**	-0.23	0.38	0.44	**0.60***
LMR	**-0.79*****	-0.28	0.49(*)	0.51(*)	**0.89*****
LAR	**-0.56***	0.03	0.14	0.21	**0.73****
ETR_{MAX}	**-0.81*****	-0.05	0.16	0.24	**0.78****
ϕ_{PSII}	**-0.79*****	-0.15	0.25	0.36	**0.76****

Table 1.7. Results of selection analyses on the all data sets for comparison. Selection differentials (S) and gradients (β) for 8 important quantitative traits are shown in the first two columns. F-values for interaction terms of phenotypic trait by 5 potential abiotic selective agents were derived from ANCOVAs. Significant values are in bold. (*) $p < 0.10$, * $p < 0.05$, ** $p < 0.01$.

Data set	Trait	S	β	Trait X SUN	Trait X MDT	Trait X MRF	Trait X MAI	Trait X LAT
Full	Shoot height	**0.19****	**0.25****	1.22	0.01	0.52	1.56	3.49(.)
	No. Leaves	-0.01	**0.21***	0.52	0.39	0.13	0.59	**5.49***
	RGR_{SHOOT}	**0.15***	0.03	2.26	0.46	0.01	0.55	2.24
	SMR	**-0.14***	0.001	**5.30***	0.39	1.99	2.54	**9.48***
	LMR	-0.09	-0.06	**7.25***	0.56	2.87	4.26(.)	**16.52****
	LAR	**-0.14***	-0.04	**14.65****	4.19(.)	**13.16****	**18.47****	**16.43****
	ETR_{MAX}	-0.08	0.10	1.58	0.01	0.10	0.56	4.89(.)
	ϕ_{PSII}	**-0.16***	-0.09	**10.56****	0.01	1.79	4.81(.)	**14.22****
Native	Shoot height	**0.24***	--	0.25	0.11	0.04	0.23	0.03
	No. Leaves	0.06	--	0.05	0.05	0.07	0.13	1.05
	RGR_{SHOOT}	0.23	--	3.03	0.28	0.34	0.30	0.38
	SMR	-0.26(.)	--	0.23	0.003	0.0004	0.09	1.68
	LMR	-0.11(.)	--	0.78	0.56	0.63	0.57	0.76
	LAR	**-0.26***	--	0.01	1.55	2.45	1.88	4.58
	ETR_{MAX}	-0.03	--	7.66(.)	0.05	0.09	0.41	0.0001
	ϕ_{PSII}	**-0.17****	--	1.38	0.45	0.69	0.98	0.34
Introduced	Shoot height	0.06(.)	--	1.29	0.04	0.16	0.11	0.66
	No. Leaves	0.03	--	0.06	**17.50***	**29.34***	**37.40****	4.01
	RGR_{SHOOT}	0.004	--	1.30	**25.38***	**95.04****	**138.38****	2.34
	SMR	0.04	--	6.74(.)	0.71	3.15	2.83	0.13
	LMR	0.07(.)	--	7.25(.)	0.0004	4.69	4.14	**15.08***
	LAR	0.05	--	0.94	0.02	0.36	0.29	0.003
	ETR_{MAX}	-0.004	--	4.71	0.32	1.42	0.94	**20.57***
	ϕ_{PSII}	-0.03	--	0.80	0.03	4.58	3.34	**29.47***

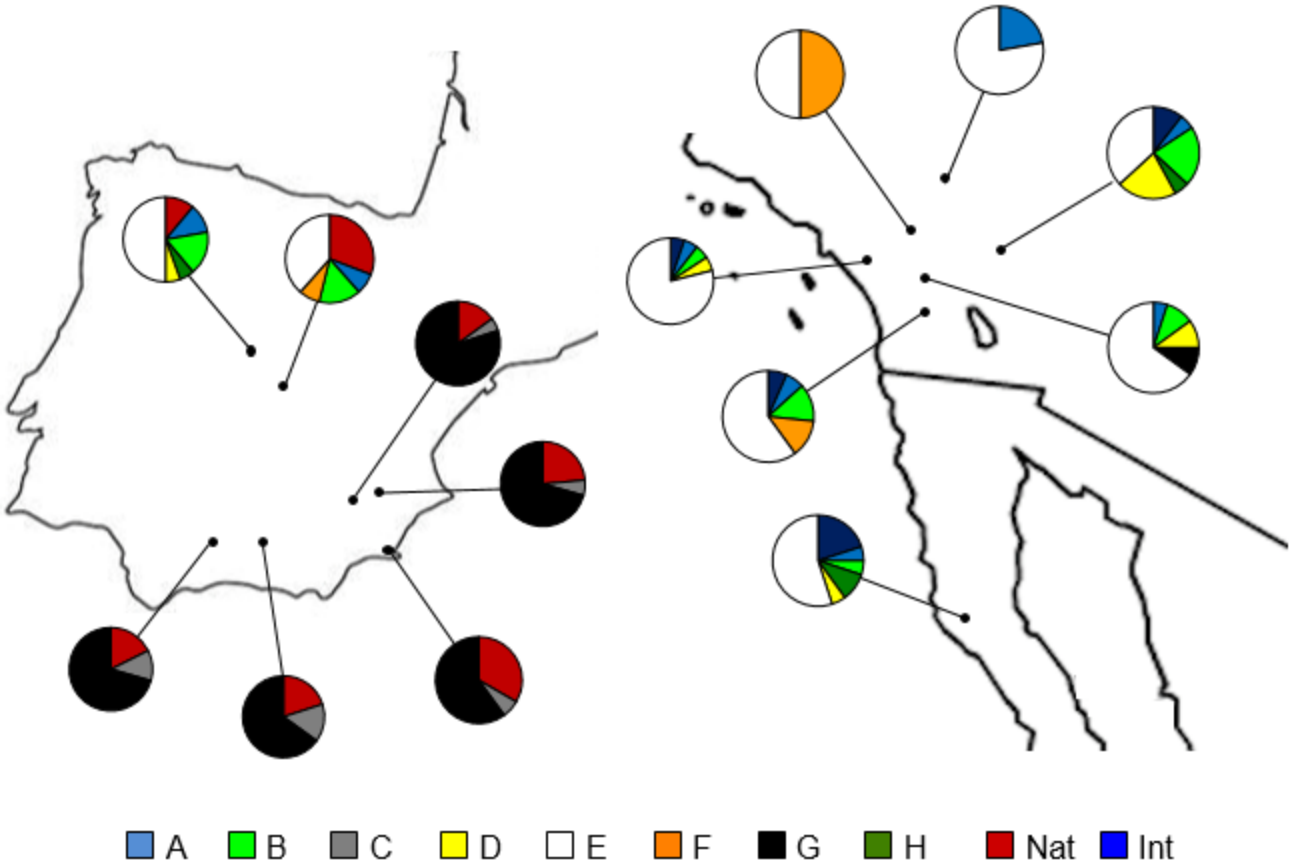

Figure 1.1. Haplotype distribution among populations. Native range haplotypes on the left and Introduced range haplotypes on the right. Each circle represents a single population. Colored portions of circles indicate the proportion of individuals within a population that belong to respective haplotypes. The color code for haplotype designation is provided at the bottom of the figure. Uninformative haplotypes for each region were grouped and identified as Nat (uninformative native haplotypes; red) and Int (uninformative introduced haplotypes; blue).

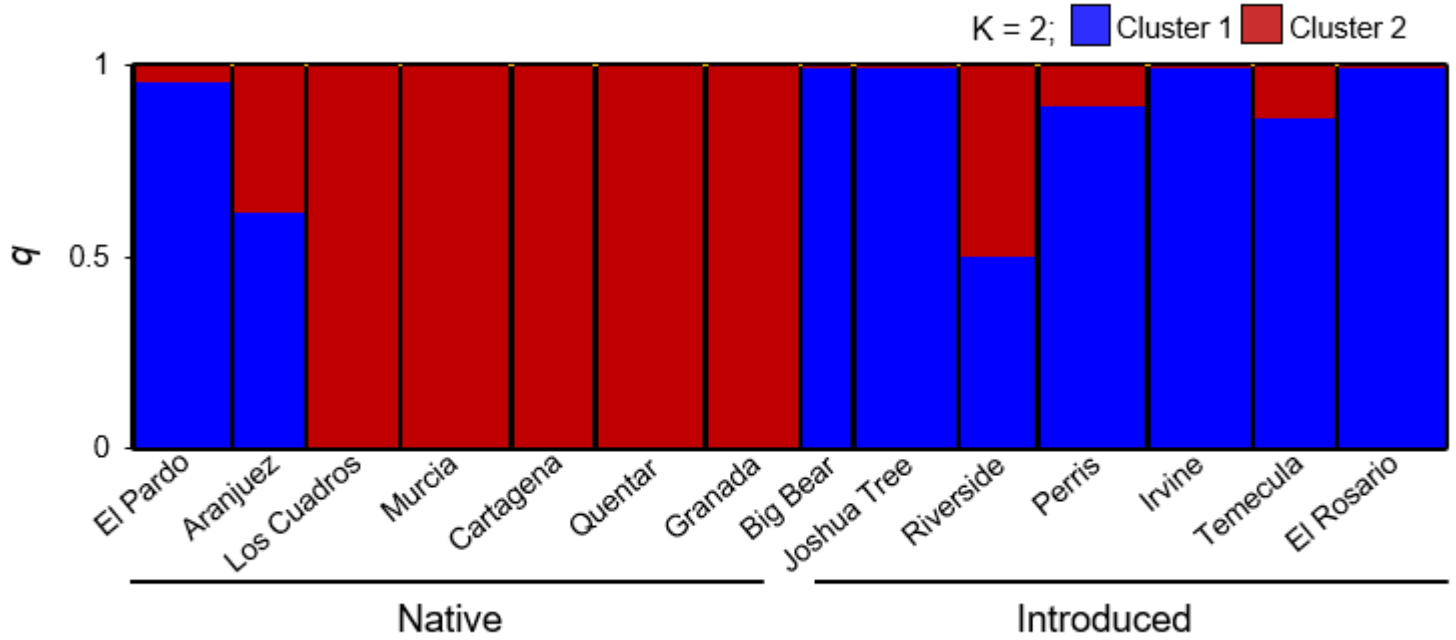

Figure 1.2. Structure graph showing proportional assignment (*q*) of each population to K = 2 genetic clusters. Genetic cluster 1 ('introduced' cluster) is indicated in blue, and genetic cluster 2 ('native' cluster) is indicated in red.

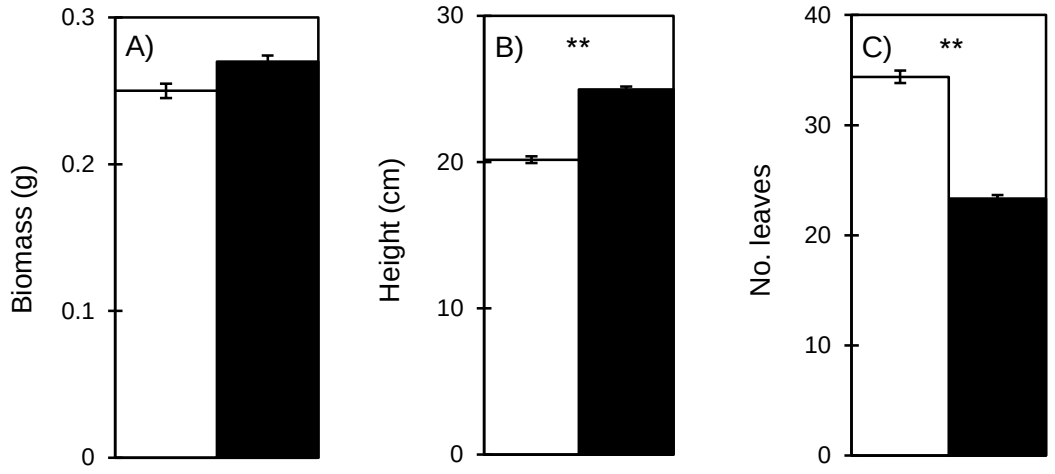

Figure 1.3. Comparison of mean (± SE) growth performance by region of population origin for A) biomass production, B) shoot height and C) no. of leaves produced. Native populations are indicated in white, while introduced populations are indicated in black. Asterisks show the level of significant differentiation derived from GLMM analyses. (*) $p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

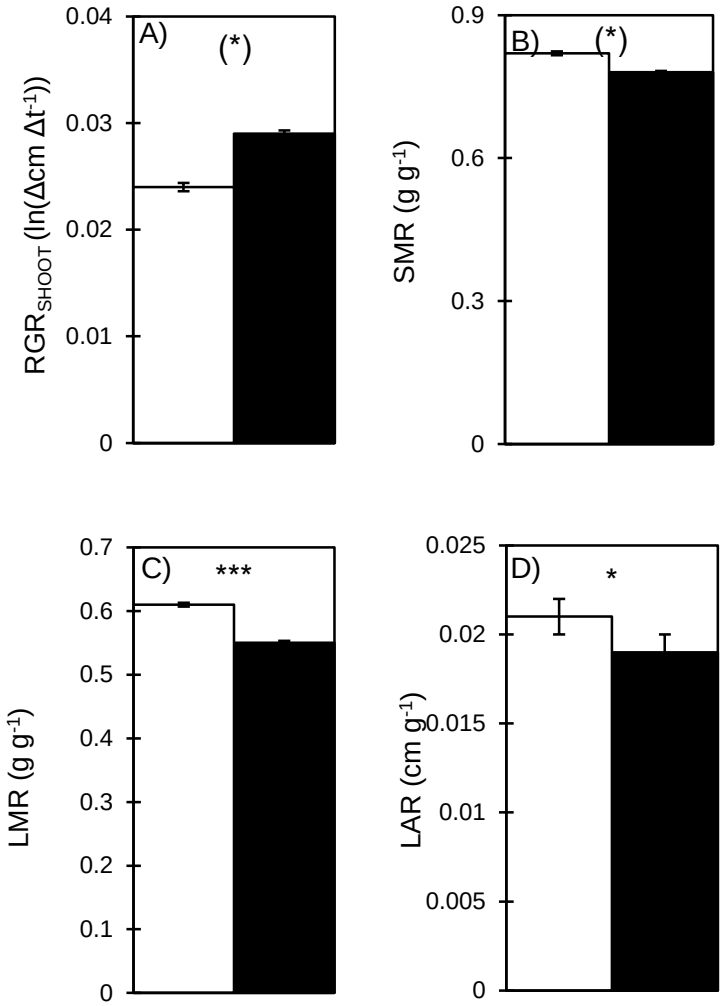

Figure 1.4. Comparison of mean (± SE) biomass allocation by region of population origin for A) RGR_{SHOOT}, B) SMR (= shoot mass ratio), C) LMR (= leaf mass ratio) and D) LAR (= leaf area ratio). Native populations are indicated in white, while introduced populations are indicated in black. Asterisks show the level of significant differentiation derived from GLMM analyses. (*) $p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

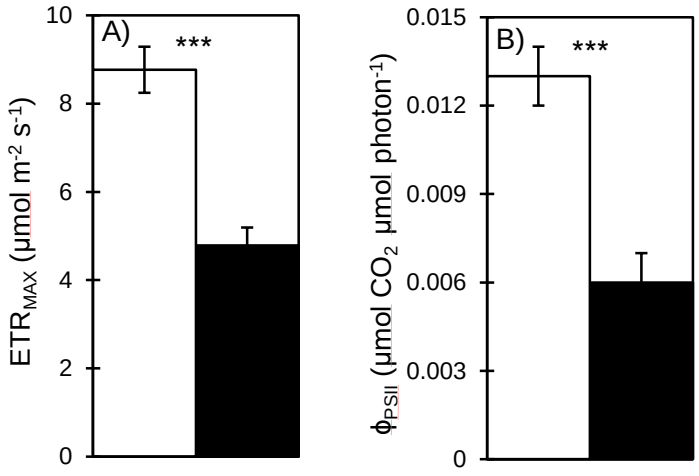

Figure 1.5. Comparison of mean (± SE) photosynthetic activity by region of population origin for A) ETR_{MAX} (= maximum electron transport rate) and B) ϕ_{PSII}. (= yeild of photosystem II). Native populations are indicated in white, while introduced populations are indicated in black. Asterisks show the level of significant differentiation derived from GLMM analyses. ***$p < 0.001$.

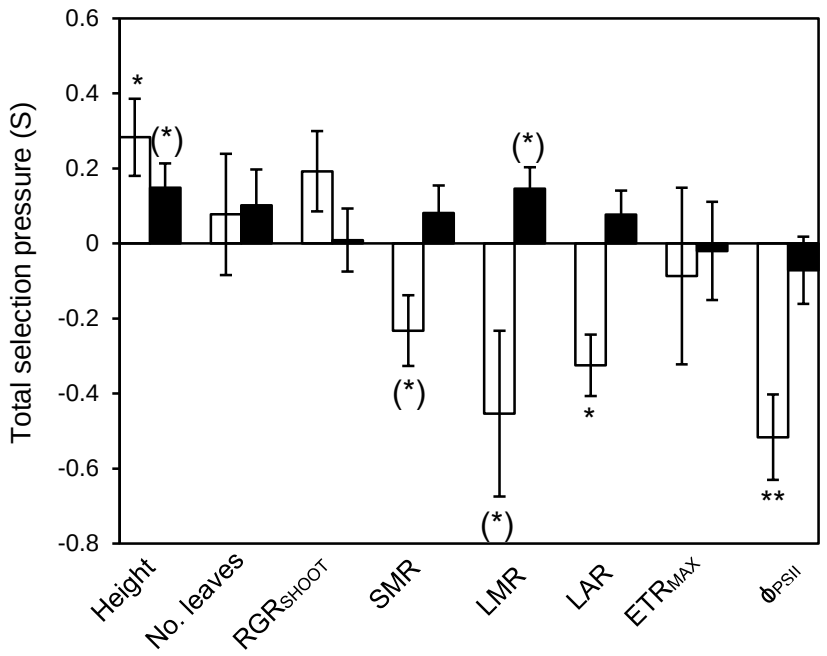

Figure 1.6. Comparison of mean (± SE) total selection pressure (S) on 8 important traits by region of population origin. Native populations are indicated in white, while introduced populations are indicated in black. Asterisks indicate significance derived from original regression analyses. (*) $p < 0.10$, *$p < 0.05$, **$p < 0.01$.

An intraspecific test of the EICA hypothesis reveals both regional and genotypic divergence in the competitive abilities of an exotic grass

Abstract

The evolution of increased competitive ability hypothesis (EICA) posits that traits common to invasive populations are the result of release from herbivorous enemies, with subsequent selection for reallocation of resources away from defense toward increased growth and reproduction. Investigations of the EICA hypothesis used a variety of experimental designs. The vast majority of these approaches were concerned with divergence in herbivory resistance and/or secondary compound production, or did not directly quantify performance in competitive environments. Importantly, molecular genetic data has not been included in any test of the EICA to date. The inclusion of genetic information from experimental populations avoids the problem of determining whether differences in competitive ability between regional populations were the result of post-introductory evolution or unknown variation across the native range. Here I utilized populations of *Bromus rubens* L. with known genetic backgrounds to conduct a competitive, intraspecific common garden in order to compare trait expression among distantly related and closely related genotypes from each respective range of this invasive species. My objective was to determine whether potential differences in phenotypic expression were the product of variation across the native range or post-introductory evolution. Results demonstrated that introduced populations displayed greater vigor relative to native populations for all traits considered when grown singly and in

competition. This increase in overall vigor also seemed to translate into increased competitive effects (i.e. the magnitude of competitive inhibition) of introduced neighbors on both introduced and native target individuals. Taken together these data provide support for the competitive assumption of the EICA, indicate that differentiation can occur at both regional- and population-scales and that increases to overall vigor and competitive effects are the most important factors leading to the dominance of introduced populations in this system.

Introduction

Common garden studies increasingly reveal more vigorous growth in introduced relative to native ranges of invasive plant species (Felker-Quinn et al. 2013). Expressed traits most consistently associated with invasion success (e.g. faster growth strategies and higher fecundity) were historically assumed to be the result of latent preadaptation of 'ideal weeds' or general purpose genotypes whose traits become unmasked upon 'release' from inhibitory interactions (Darwin 1859, Elton 1958, Baker 1965, Keane and Crawley 2002). Yet, it now appears that post-introductory evolutionary dynamics play a dominant role in most invasions (reviewed in Ellstrand and Schierenbeck 2000, Bossdorf et al. 2005, Felker-Quinn et al. 2013). The utilization of selection analyses further reveal that selection acts early and often on traits associated 'invasiveness' (Chapter 1). However, the role molecular differentiation among previously introduced (invasive) and potentially colonizing (native) populations play in the development of invasive characters remains unclear. Of particular interest is whether the expression of increased competitive abilities expected in introduced populations are the product of regional- or population-scale differentiation.

Emphasis on invasive plant population impacts on recipient communities and vice versa previously dominated investigations within invasive plant biology (Elton 1958, Mack et al. 2000, Richardson et al. 2011). This may be the reason for a potential over emphasis on the role of enemy release and biotic resistance in the invasion process (Colautti et al. 2004). The advent of the Evolution of Increased Competitive Ability Hypothesis (EICA; Blossey and Nötzold 1996) captured the attention of many investigators and promoted the integration of evolutionary concepts into a field previously dominated by ecological theory.

The EICA posits that invasiveness is the result of release from herbivorous enemies, with subsequent reallocation of resources away from defense toward increased growth and reproduction. While previous work demonstrated little support for the enemy release aspect of this hypothesis, observed differences in trait expression among regional populations of many invasive species suggested that post-introductory change is common (Felker-Quinn et al. 2013).

To capture the diversity of methods utilized in tests of the EICA I conducted a literature search within the Web of Knowledge data base, with key words "EICA" and "plant". The vast majority of resultant studies were concerned with divergence in herbivory resistance and/or secondary compound production (40 publications), followed by single pot studies (23 publications; 'single pot' studies quantify plant vigor in the absence of herbivores or competitors), intraspecific competition (10 publications) and interspecific competition studies (9 publications). Each of these approaches provided valuable insights into the evolutionary mechanisms leading to increased competitive abilities. Yet, the core of the EICA is concerned with intraspecific differentiation. Therefore, the most parsimonious way to test the competitive aspect of this hypothesis is to compete native (benign) with introduced (invasive) populations of known genetic background. The rationale being that if phenotypic divergence promoted greater competitive ability in introduced genotypes, then these populations should be able to outcompete assumed ancestral populations from the native range (Bossdorf et al. 2005, Hierro et al. 2005). This approach also avoids the disadvantages of previous tests of the EICA for at least four reasons. One, evolutionary divergence in competitive abilities is assumed at the time of

investigation, therefore tests of the EICA do not require a biotic (enemy) element. Two, it avoids the potential bias of using interspecific competitors from either native or introduced ranges. Three, in order to understand the microevolutionary trajectories leading to increased competitive abilities the focus should inherently be on intra- relative to interspecific variation. Four, the inclusion of molecular genetic data avoids the problem of determining whether differences in competitive ability between regional populations are the result of post-introductory evolution or unknown variation across the native range. By designing experiments with both molecular and phenotypic trait data, comparisons between closely related native and introduced genotypes can be conducted to determine if increased competitive ability was the product of translocation of an 'ideal weed' or whether phenotypic differentiation had indeed occurred post-introduction.

A description of the natural history of *Bromus rubens* L. (Pavlick and Anderson 2007, = *B. madritensis ssp. rubens*, Fortune et al. 2008) can be found in chapter 1. In the same chapter I demonstrated evidence for post-introductory evolutionary divergence in traits associated with increased the fitness (biomass, shoot height and relative growth rate) in introduced populations. In addition, correlation and selection analyses were used to identify selective agents driving the observed divergence in these traits. The results suggested that variation in light availability among native and introduced ranges had selected for reduced allocation to light harvesting organs, with reallocation of resources toward increased growth (height). These results provided suggestive evidence for increased competitive ability in introduced populations driven by abiotic differences between

regions. However, the design of the experiment limited my ability to make conclusions on this matter.

The objective of this investigation was to test the competitive assumption of the EICA while taking into account relatedness among populations involved. This is important because most invasive species occupy large areas in their native range, making the identification of 'source' (and/or closely related) genotypes exceedingly difficult (Cox 2004). This can be problematic because without this information it cannot be determined whether differences in phenotypic expression were the product of variation across the native range or post-introductory evolution. Here I selected populations belonging to two distinct genetic clusters that were identified via STRUCTURE analyses in chapter 1. Genetic cluster 1 (referred to as GC1) contained all populations from the introduced range and one population from the native range, while genetic cluster 2 (referred to as GC2) only contained populations from the native range. The populations utilized were the following; El Pardo (native population closely related to most introduced populations sampled, assigned to GC1 at q = 0.957 [here after referred to as $GC1_{NAT}$]), Los Cuadros (native population, assigned to GC2 at q = 0.996 [this can be thought of as the 'native' cluster, here after to referred to as GC2]), Perris (representing the most common genotype found in the introduced range, assigned to GC1 at q = 0.897 [here after referred to as $GC1_{INT}$]) and Riverside (introduced population, showed population-level admixture among respective clusters; assigned to GC1 at q = 0.502, and GC2 at q = 0.498 [here after referred to as GC_{ADMIX}]). By comparing trait expression among distantly related genotypes (GC2, GC1 and GC_{ADMIX}) and closely related genotypes ($GC1_{NAT}$ and $GC1_{INT}$) my objective was

to determine whether potential differences in phenotypic expression were the product of variation across the native range (i.e. $GC1_{INT}$ no different from either $GC1_{NAT}$ or GC2) or post-introductory evolution of increased competitive abilities (i.e. both $GC1_{INT}$ and GC_{ADMIX} different from $GC1_{NAT}$ and GC2).

Here I asked the following questions: 1) Do introduced populations of *B. rubens* display the competitive behavior predicted by the EICA?, and 2) Are observed competitive abilities among native and introduced populations of *B. rubens* the product of regional differentiation, or are competitive outcomes dictated by genetic relatedness among populations (i.e. an 'ideal weed')? If, indeed, the aggressive growth of introduced populations was the product of translocation of an 'ideal weed', then little to no difference between regionally isolated, yet genetically similar populations, would be observed. In this case both populations ($GC1_{NAT}$ and $GC1_{INT}$) would be expected to be competitively dominant. If post-introductory trait change had occurred between regionally isolated, yet genetically similar populations ($GC1_{NAT}$ and $GC1_{INT}$), then only the introduced population ($GC1_{INT}$) would display competitive dominance. And finally, if factors associated with region of population origin (e.g. abiotic factors) promoted post-introductory trait divergence towards increased competitive ability, then both the closely ($GC1_{INT}$) and distantly related (GC_{ADMIX}) populations of introduced origin would display competitive dominance.

Materials and Methods

Seed collections

Seeds of the subset of populations chosen for this experiment were those previously analyzed (Chapter 1).

Experimental design

Bromus rubens is known to be a primarily autogamous species. Therefore, seeds were chosen at random from each of the four populations. All seeds were sterilized with 6% sodium hypochlorite, rinsed in *di*H_2O, and placed on moistened filter paper until germination. One-liter pots (Stuewe & Sons, Ore., USA) were also sterilized with 6% sodium hypochlorite, rinsed in *di*H_2O, dried, and filled to within 5cm of the rim with UC Soil Mix III (75% quartz sand, 25% ground peat moss; Padgett and Allen 1998). Upon germination seeds with similar radicle lengths (~1 cm) were transplanted into respective pots, covered with UC Soil Mix III and gently watered. Pots were randomly arranged on two greenhouse benches at the campus of University of California, Riverside, watered as needed, and re-randomized every third day throughout the course of the experiment. Each population was grown in monoculture at three densities (singly as a control, two plants pot^{-1} and three plants pot^{-1}), and in pairwise mixtures with each other population at two competitive intensities; 1:1 target:neighbor (for a total of two plants pot^{-1}), and 1:2 target:neighbors (for a total of three plants pot^{-1}). Each treatment had 10 replicates yielding a total of 840 plants in 360 pots.

Growth (shoot height) was monitored every 10 days – following an initial 20 day establishment period – until target plants had flowered, produced mature seed and/or senesced (senescence defined as > 70% of plant tissue browned [Novy et al. 2013]). Repeated observations were used to determine maximum shoot height (LH_{MAX}, cm) attained throughout the lifetime of respective focal individuals, as well as the average relative shoot growth rate (RGR_{SHOOT}, cm cm^{-1} d^{-1}). Calculations for RGR_{SHOOT} followed methods outlined in chapter 1.

Target plants were destructively harvested at fruit maturity and/or senescence. Leaves, stems and seeds were separated and all but the latter were dried at 70˚C until constant mass. Total aboveground biomass was calculated as the sum of leaves, stems and seeds. The ability to accurately determine target plant allocation to root biomass was not possible in competitive treatments. Seed viability was assessed by randomly selecting 10 seeds (when available) from each target individual, sterilized as above, placed on moistened filter paper and observed for 14 days. Total reproductive fitness of target individuals was estimated by taking the product of the proportion of viable seeds and the total number of seeds produced (Huxman et al. 1999).

Statistical analyses

Three sets of analyses were performed to evaluate divergence in competitive ability (i.e. plant vigor, target responses and neighbor effects). First, log response ratios were derived for each dependent variable to compare the magnitude of competitive treatments across regions and populations. This was done by taking the natural log of individual target

performance in the presence of competitors over the population mean response to growth singly; relative response to competitive treatment *i*, $\text{Ln}(\text{Trait}_i) = \text{Ln}(\text{Trait}_{comp}/\text{Trait}_{single})$. This simple competition index was beneficial for the following reasons: ease of interpretation (in this context a response ratio describes the fractional decrease in performance when grown in the presence of competitors relative to being grown alone); relativization allows for fair comparisons among differing populations and treatments (e.g. a 50% growth reduction in one population is proportional to other populations regardless of differing raw data values); it describes treatment effect size rather than individual population performance; and response ratios tend to be symmetrical around the 'no-effect' point (where growth in a competitive environment was no different than growth alone) (Brinkman et al. 2010).

The first set of analyses sought to determine the magnitude of competitive effects on individual target growth performance (for each trait by respective region and population of seed origin). Separate one-sample *t*-tests were used to examine whether target response and neighbor effects deviated from a mean of zero for each trait by respective region and population of seed origin. It was expected that if competition did influence performance, then the competition index would be significantly less than zero.

Fixed-factor analysis of variance (ANOVA) models with type-III sums of squares were run at regional and population scales to test for post-introductory divergence in competitive abilities. Models sought to determine the influence of region of target origin (REG), target population | REG, region of competitor (neighbor) origin (NREG), population of competitor origin | NREG and their interactions on the response and

magnitude of competitive effects on target individuals. Population-level models were run separately due to a lack of independence between these variables and NREG. Initial models also included a competitive intensity factor with two levels. No significant differences among intensities were detected in any of the models. Therefore, the intensity factor was removed from subsequent models. Tukey's HSD tests were conducted to determine differences among treatments when significant factors were detected. All models were performed in JMP® Pro 12.0.1 (SAS Institute Inc., Cary, NC, USA, 2015).

Results

Target response to competition

Plants grown in the absence of competition displayed 43% greater reproductive fitness and 52% more biomass than those grown with competitors. Populations from both regions were inhibited by competition, but the magnitude of competitive response was consistently greater in introduced relative to native populations (Table 2.1). The difference between regional performance was primarily due to population-level variation in response to competition (Table 2.2). GC_{ADMIX} was the most responsive for the majority of traits considered, and therefore showed the greatest decreases in trait values. Moderate levels of competitive inhibition were experienced by GC2 individuals (-0.60 [reproductive fitness]). Finally, pairwise comparisons of $GC1_{NAT}$ and $GC1_{INT}$ revealed a large amount of within genetic cluster divergence in competitive response (Figure 2.3). Individuals from $GC1_{NAT}$ experienced greater reductions to reproductive fitness relative to $GC1_{INT}$ individuals, while $GC1_{INT}$ individuals experienced more severe reductions to all remaining traits relative to

$GC1_{NAT}$ individuals. Although the magnitude of competitive response was generally greater in introduced relative to native populations, introduced populations displayed greater overall vigor and remained competitively superior to native populations (Figure 2.2).

The fixed factor ANOVA models on the relative response of target individuals to competition revealed a large amount of divergence among regions (Table 2.2). On average introduced populations displayed greater growth relative to native populations in all traits considered (Figure 2.2). However, introduced populations remained more responsive to competition treatments (i.e. these populations were more negatively influenced by competition) (Figure 2.3). Dissecting the model further I found that region of population origin explained most of the variation in observed divergence in target response for reductions in biomass ($F = 36.61$, $p < 0.001$) and LH_{MAX} ($F = 12.99$, $p < 0.001$), while population within region explained the majority of variation in reproductive fitness ($F = 6.28$, $p < 0.01$) and RGR_{SHOOT} ($F = 8.34$, $p < 0.001$).

Neighbor effect on target performance

Introduced neighbors inflicted a greater magnitude of competitive inhibition on all traits considered relative to native neighbors (Figure 2.1). As with target response to competition, the differences between regional neighbor effects were primarily due to population-level variation in competitive inhibition (Tables 2.1 and 2.2). Of the four populations included, GC2 consistently had the least inhibitory effect on neighbors for reproductive fitness ($\bar{x}$ = -0.35 [± 0.14 SE], t = -2.46, $p < 0.05$) and biomass compared to

the remaining populations, and even showed some degree of facilitation when individuals were grown in the presence GC2 neighbors; LH_{MAX} ($\bar{x}$ = 0.07 [± 0.04 SE], n.s.) and RGR_{SHOOT} ($\bar{x}$ = 0.02 [± 0.06 SE], n.s.). The remaining populations displayed similar competitive effects on target individuals, yet populations originating from the introduced range ($GC1_{INT}$) tended to show greater magnitudes of inhibition for all traits except RGR_{SHOOT}, relative to $GC1_{NAT}$.

The ANOVA models for the relative magnitude of neighbor effects on target individual performance revealed strong regional and genotypic patterns (Table 2.2). When target individuals were grown in the presence of introduced neighbors a greater decrease in allocation to reproductive fitness ($\bar{x}$ magnitude of competitive effect -0.49 [native] vs. -0.73 [introduced]; $F = 4.89$, $p < 0.05$), biomass (-0.43 [native] vs. -0.75 [introduced]; $F = 12.94$, $p < 0.001$) and LH_{MAX} (-0.01 [native] vs. -0.13 [introduced]; $F = 28.16$, $p < 0.001$) was observed, relative to the competitive effects of native populations (Figure 2.1). Target performance was also significantly influenced by the competitive effects of neighbor population within region and the interactive effects between target and neighbor populations. Closer inspection of the data identified the lower competitive ability of GC2 neighbors and the clear pattern of competitive dominance in the remaining populations (the relative magnitude of competitive effects were generally $GC1_{INT} > GC_{ADMIX} > GC1_{NAT}$) as the primary drivers of these patterns (Figure 2.3).

Discussion

I hypothesized that post-introductory divergence in phenotypic trait expression between native and introduced populations promoted increased competitive abilities in introduced populations. To test this I included populations with known backgrounds to ensure any observed differences were the product of post-introductory evolution, not unknown variation from within the native range. By doing so I was able to determine; 1) whether populations of *B. rubens* from the introduced range displayed competitive dominance as predicted by the EICA and 2) the role regional- vs. population-level differentiation played in the divergence of phenotypic trait expression. As predicted, introduced populations displayed greater vigor than native populations for all traits considered when grown singly and in competition. The most important finding was the observed increase in reproductive fitness in introduced relative to native populations, as well as within populations of close genetic relation originating from each respective region (i.e. $GC1_{INT}$ fitness > $GC1_{NAT}$ fitness) regardless of competitive treatment. This increase in overall vigor (described as the degree to which important phenotypic traits are expressed) also translated into increased competitive effects (i.e. the magnitude of competitive inhibition) of introduced neighbors on both introduced and native target individuals. Taken together these data provide support for the competitive assumption of the EICA, indicate that differentiation can occur at both regional- and population-level scales and that increases to overall vigor and competitive effects are the most important factors leading to the dominance of introduced populations in *B. rubens*.

Many investigations of the EICA hypothesis resulted in mixed support (reviewed in Felker-Quinn et al. 2013). Such mixed support suggests that other mechanisms besides the presence or absence of herbivorous enemies are important. Investigations including herbivory treatments found that introduced populations displayed greater vigor than native populations regardless of herbivore presence (Zou et al. 2008), have simultaneously evolved increased tolerance to herbivory and increased competitive ability (Zou et al. 2008, Zheng et al. 2015), that selection acts to promote growth regardless of herbivore presence (Franks et al 2008) and that herbivores can actually select for increased competitive ability (Uesugi et al. 2013). These observations are counter to the predictions of the EICA, and indicate that release from coevolved enemies is not the only selective agent capable of promoting aggressive behavior. For example, in chapter 1 I demonstrated that variation in abiotic environments likely selected for increased expression of traits commonly associated with invasiveness (i.e. increased biomass, height and growth rates). It may be that differences in regional abiotic interactions also hold the capacity to promote increased competitive abilities. If this is the case, the development of increased competitive ability may be dependent upon region of introduction rather than interactions with novel biotic communities.

An alternative explanation for mixed support within the EICA literature may also lie in the experimental approach most commonly utilized. Many studies put more emphasis on divergence in secondary metabolite production and herbivore defense rather than on the result of these or other changes (reviewed in Felker-Quinn et al. 2013). This approach provided information with respect to post-introductory change, but often ignored the very

core of EICA – changes to growth and reproduction with subsequent increases in competitive ability. As pointed out by Shelby et al. (2015), of the studies that did emphasize differences in growth among regional populations of invasive species, most did not directly quantify competition, but rather used growth rate and associated traits reflective of overall vigor as an indirect measure of competitive ability. In addition, only one study (Joshi et al. 2014) identified in my literature survey on EICA research quantified actual reproductive output in competitive environments. This is surprising given that reproductive output is arguably the most important trait leading to the large population sizes observed in invasive plant populations (Baker 1965). Through quantification of growth and reproductive output I demonstrated little difference in growth rate among distantly related native (GC2) and introduced populations ($GC1_{INT}$ and GC_{ADMIX}), but a large degree of divergence in reproductive effort. Thus, the overall focus on growth rate as the best index of competitive ability may be the primary contributor to mixed support reported for the EICA.

Similar to many 'single pot' studies, where individuals are grown singly in the absence of competition (Stastny et al. 2005, Joshi and Vrieling 2005, Guesewell et al. 2006, Handley et al. 2008, Abhilasha and Joshi 2009, Abela-Hofbauerova and Muenzbergova 2011, Flory et al. 2011, Guo et al. 2011, Joshi and Tielboerger 2012, Qin et al. 2013, Turner et al. 2014), results for *B. rubens* showed that introduced individuals displayed greater vigor relative to native individuals for all traits considered. However, the patterns observed in single pot studies may not reflect an individual's performance in competitive environments (Zheng et al. 2015b). For example, Shelby et al. (2016) demonstrated that the growth rates of *Trifolium* populations when grown singly were not positively correlated

with their performance when grown with competitors. In addition, vigor may only be one of many aspects of competitive dominance. As pointed out in a recent study by Joshi et al. (2014), competitive ability is also dictated by an individual's effect on and response to neighboring competitors. Through the use of a simple intraspecific competitive experimental design I revealed that introduced populations expressed greater vigor when grown alone and in competition, but also that introduced populations inflicted greater competitive impacts on neighbor growth relative to distantly related (GC2) and closely related ($GC1_{NAT}$) ancestral populations from the native range. These results provide a partial explanation for community level patterns observed in introduced ranges, and identify post-introductory increases in vigor and competitive inhibition as two key elements of local dominance.

Intense ecological research into the process of successful invasion generated a predictable set of phases that populations experience prior to the expression of invasiveness (Richardson et al. 2000). The most notable and least understood phase is termed the 'lag' phase. It is during this phase that populations of exotic plant species survive and reproduce in their new habitats, but do not yet display the competitive dominance leading to local extirpation of native species. It is suggested that this phase of invasion represents the time necessary for populations to undergo evolutionary change via multiple pathways, ultimately resulting in adaptation to their new environments (Ellstrand and Schierenbeck 2000, Lee 2002). As evidenced by the preponderance of one genotype (GC1) in the introduced range (Chapter 1), environmental filtering likely played a key role in the early establishment of *B. rubens* in the Southwestern region of North America. Yet, as reflected

in the differential performance observed between ancestral ($GC1_{NAT}$) and descendant ($GC1_{INT}$) populations, as well as the dominance displayed by the more distantly related introduced genotype ($GC1_{ADMIX}$) included in this study, post-introductory change toward increased competitive ability did take place sometime in the past. Taken together these patterns suggest that regional differences in selective environments promoted divergence toward more aggressive growth in the *B. rubens* system. The unfortunate implications of these observations are that ecological traits may not be reliable predictors of successful invasion, and that seemingly benign, exotic species may hold the potential to become noxious invaders at some unknown time in the future.

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Table 2.1. Results of *t*-tests on the relative responses of target individuals to competition and neighbor effects on competition. Mean response values (±SE) are provided at both regional and population levels. *t*-ratios are also provided with significance indicated by: (*) p < 0.10, * p < 0.05, ** p < 0.01, *** p < 0.001.

	Reproductive fitness		Biomass		LH_{MAX}		RGR_{SHOOT}	
	Mean (± SE)	*t*-ratio	Mean (± SE)	*t*-ratio	Mean (± SE)	*t*-ratio	Mean (± SE)	*t*-ratio
Target response								
Regions								
Native	-0.59 (0.08)	-7.33***	-0.35 (0.08)	-4.47***	-0.05 (0.03)	-1.97(*)	-0.09 (0.04)	-2.14*
Introduced	-0.66 (0.08)	-8.69***	-0.87 (0.06)	-13.49***	-0.14 (0.02)	-7.15***	-0.05 (0.04)	-1.38
Populations								
GC2	-0.57 (0.12)	-4.56***	-0.27 (0.13)	-2.18*	-0.05 (0.05)	-1.09	-0.05 (0.05)	-1.05
$GC1_{NAT}$	-0.63 (0.09)	-6.79***	-0.43 (0.07)	-5.76***	-0.05 (0.02)	-2.68*	-0.13 (0.07)	-1.91(*)
$GC1_{INT}$	-0.42 (0.13)	-3.31**	-0.75 (0.09)	-7.64***	-0.19 (0.03)	-6.63***	-0.19 (0.05)	-4.11***
GC_{ADMIX}	-0.89 (0.07)	-12.03***	-0.99 (0.08)	-12.21***	-0.08 (0.02)	-3.51***	0.10 (0.05)	2.19*
Neighbor effect								
Regions								
Native	-0.52 (0.08)	-6.36***	-0.48 (0.09)	-5.59***	-0.03 (0.02)	-1.20	-0.05 (0.03)	-1.22
Introduced	-0.72 (0.07)	-9.78***	-0.76 (0.06)	-12.28***	-0.16 (0.02)	-7.89***	-0.08 (0.04)	-2.25*
Populations								
GC2	-0.35 (0.14)	-2.46*	-0.21 (0.15)	-1.43	0.07 (0.04)	1.52	0.02 (0.06)	0.39
$GC1_{NAT}$	-0.66 (0.09)	-7.02***	-0.67 (0.09)	-7.27***	-0.10 (0.02)	-4.19***	-0.09 (0.05)	-2.00*
$GC1_{INT}$	-0.78 (0.11)	-7.05***	-0.81 (0.09)	-9.13***	-0.16 (0.03)	-5.95***	-0.09 (0.04)	-2.09*
GC_{ADMIX}	-0.67 (0.09)	-6.77***	-0.71 (0.09)	-6.78***	-0.16 (0.03)	-5.21***	-0.08 (0.06)	-1.31

Table 2.2. Results of ANOVA models on the relative response of target individuals to competition with regional populations and each respective population. Regional models sought to determine the influence of region of target origin (REG), target population, region of competitor origin (NREG), population of competitor origin and their interactions on the response and magnitude of competitive effects on target individuals of *Bromus rubens*. F-values from original analyses. Least square means (± SE) are provided for comparison of competitive response and magnitude of effect on four quantitative traits. (*) $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Regional models	*df*	Reproductive fitness	Biomass	LH_{MAX}	RGR_{SHOOT}
REG	1	0.58	**36.61*** **	**12.99*** **	0.03
Target population | REG	2	**6.28** **	**3.14***	**5.16** **	**8.34*** **
NREG	1	**4.89***	**12.94*** **	**28.16*** **	1.49
Neighbor population | NREG	2	2.68(*)	**7.29*** **	**10.29*** **	1.99
REG x NREG	1	**4.24***	2.85(*)	**5.63***	0.73
Target pop x NREG	2	**4.82** **	**20.06*** **	**7.23*** **	**3.41***
Neighbor pop x REG	2	**5.23** **	**7.87*** **	**8.97*** **	**3.24***
Population models					
Target population	3	**4.17** **	**12.67*** **	**5.36** **	**5.59** **
Neighbor population	3	2.06	**4.89** **	**8.18*** **	1.25
Target pop x Neighbor pop	9	**3.42*** **	**7.77*** **	**6.38*** **	1.68(*)
Least square means (± SE)					
REG					
Native		-0.57 (± 0.08)	-0.32A (± 0.07)	-0.03A (± 0.02)	-0.05 (± 0.04)
Introduced		-0.65 (± 0.07)	-0.85B (± 0.06)	-0.13B (± 0.02)	-0.06 (± 0.04)
NREG					
Native		-0.49A (± 0.08)	-0.43A (± 0.07)	-0.01A (± 0.02)	-0.02 (± 0.04)
Introduced		-0.73B (± 0.07)	-0.75B (± 0.06)	-0.16B (± 0.02)	-0.08 (± 0.04)
Target response					
GC2		-0.60A (± 0.10)	-0.32A (± 0.09)	-0.06A (± 0.03)	-0.04AB (± 0.05)
$GC1_{NAT}$		-0.54A (± 0.13)	-0.32A (± 0.09)	-0.05A (± 0.03)	-0.07BC (± 0.06)
$GC1_{INT}$		-0.40A (± 0.09)	-0.71B (± 0.08)	-0.19B (± 0.03)	-0.19C (± 0.05)
$GC_{ADMIXED}$		-0.89B (± 0.09)	-1.00C (± 0.08)	-0.08A (± 0.03)	0.09A (± 0.05)
Neighbor effects					
GC2		-0.32 (± 0.12)	-0.19A (± 0.10)	0.04A (± 0.03)	0.06A (± 0.06)
$GC1_{NAT}$		-0.66 (± 0.10)	-0.68B (± 0.08)	-0.10B (± 0.03)	-0.09B (± 0.05)
$GC1_{INT}$		-0.78 (± 0.09)	-0.78B (± 0.08)	-0.16B (± 0.03)	-0.08AB (± 0.05)
$GC_{ADMIXED}$		-0.67 (± 0.10)	-0.72B (± 0.08)	-0.16B (± 0.03)	-0.09AB (± 0.05)

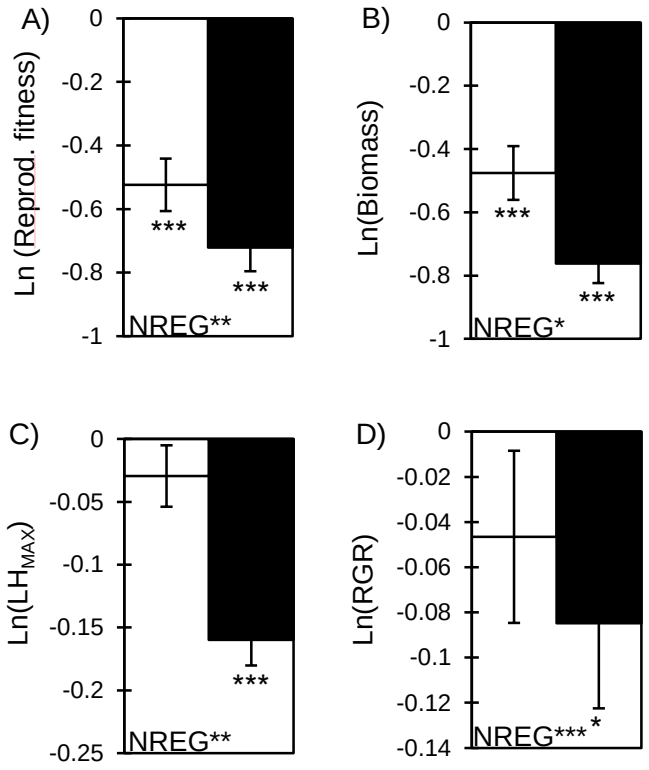

Figure 2.1. Neighbor effects (Mean ± SE) on individual A) reproductive fitness, B) biomass, C) maximum height and D) relative growth rates when grown in the presence of native (white bars) vs. introduced (black bars) genotypes of *Bromus rubens*. Asterisks indicate significant competitive effect based upon one-sample *t*-tests. Asterisks beside 'NREG' (NREG = neighbor region of origin) indicate significance of this terms from fixed-factor ANOVAs. Levels of significance were the following: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

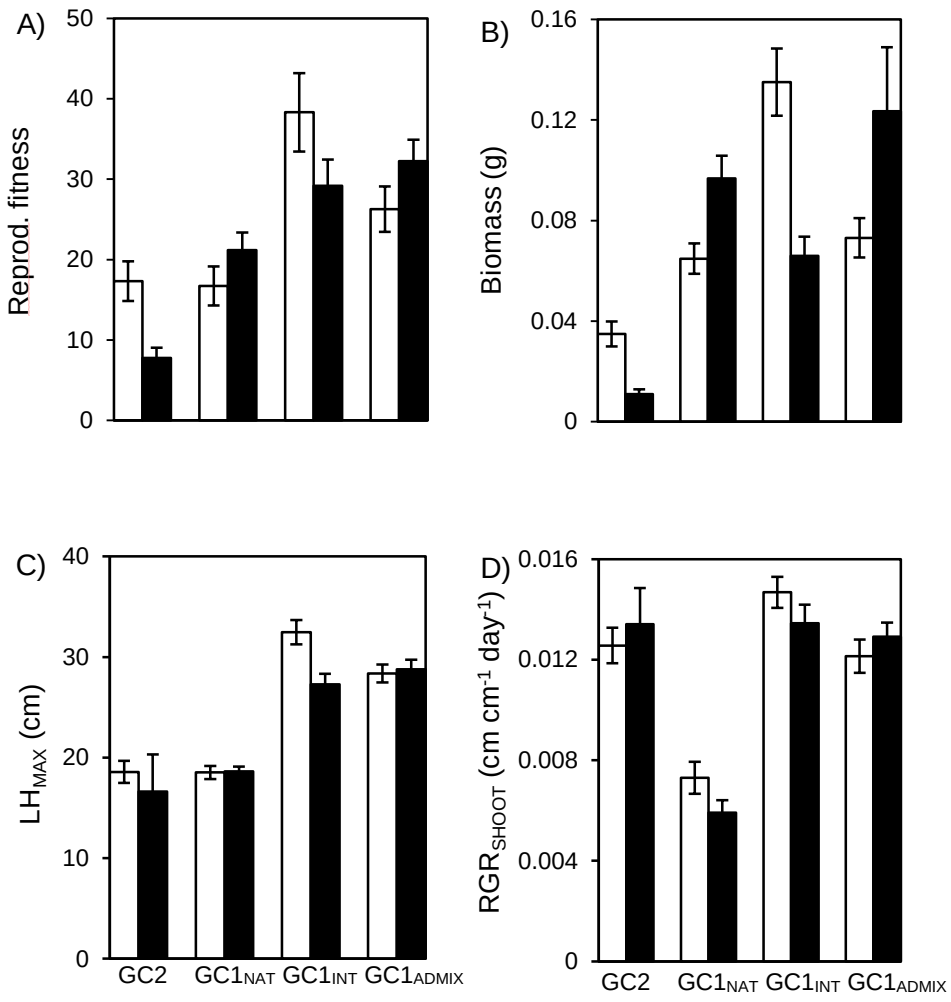

Figure 2.2. Vigor of target individuals A) reproductive fitness, B) biomass, C) maximum height and D) relative growth rates when grown in the presence of native (white bars) and introduced (black bars) genotypes of *B. rubens* for comparative purposes. Populations were the following: GC2 = Los Cuadros, $GC1_{NAT}$ = El Pardo, $GC1_{INT}$ = Perris and GC_{ADMIX} = Riverside.

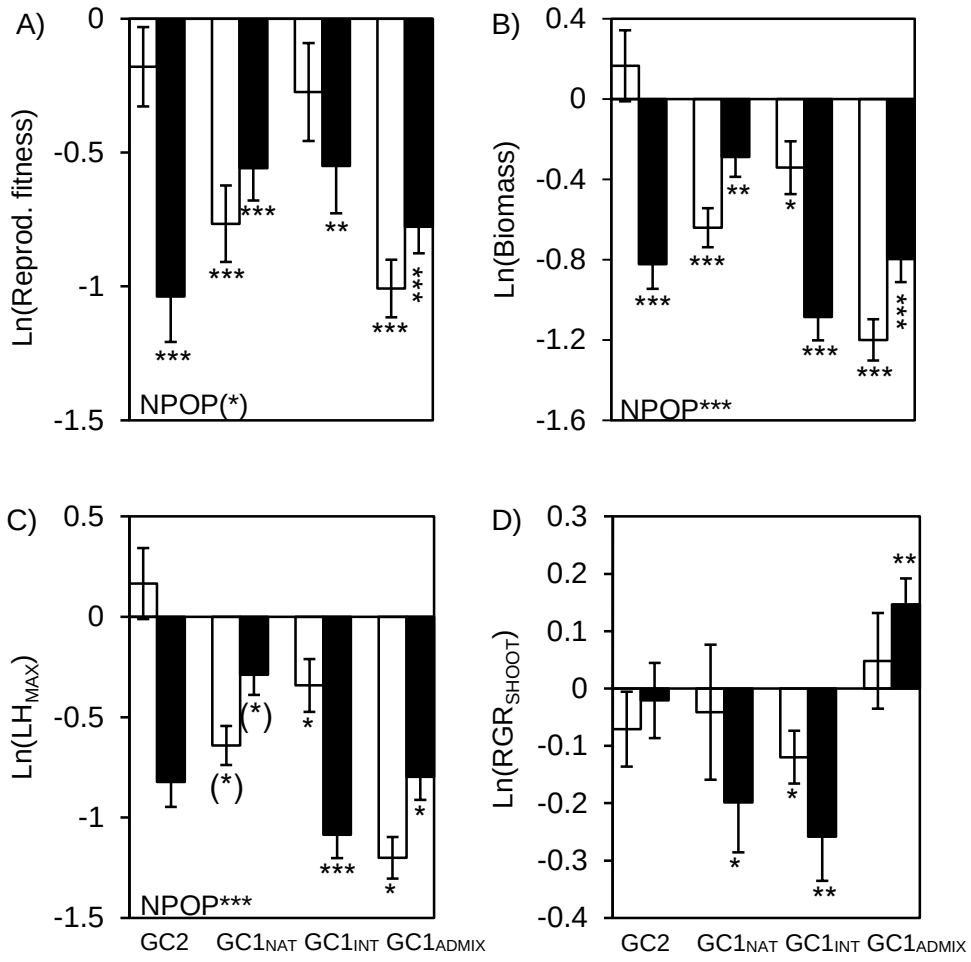

Fig 2.3. Relative response ratios of individual A) reproductive fitness, B) biomass, C) maximum height and D) relative growth rates when grown in the presence of native (white bars) and introduced (black bars) genotypes of *B. rubens*. Populations were the following: GC2 = Los Cuadros, $GC1_{NAT}$ = El Pardo, $GC1_{INT}$ = Perris and GC_{ADMIX} = Riverside. Asterisks above error bars indicate significant competitive effect based upon one-sample *t*-tests. Asterisks beside 'NPOP' (NPOP = neighbor population of origin) indicate significance of this terms from fixed-factor ANOVAs. Levels of significance were the following; (*) $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Plant-soil interactions of *Bromus rubens* L. in introduced versus native soils

Abstract

Biogeographical differences in invasive plant-soil interactions consistently demonstrate more beneficial interactions with introduced relative to native range soils. I tested the idea that biogeographical differences among hosts and soils may explain differential plant growth. I utilized the invasive grass species *Bromus rubens*, building upon previous molecular work on the microbial composition of biogeographical soil communities, to simultaneously test two dominant hypotheses of invasive plant biology, the increased resource availability and the enemy release hypotheses, and a lesser known hypothesis, the enhanced mutualism hypothesis, in order to determine which soil components contributed to invasion success in this system. I used a full-factorial growth experiment in which native and introduced plant populations were grown in each respective soil in the presence and absence of microbiota. Soil microbes contributed to the aggressive behavior observed in introduced relative to native populations. Higher resource availability appeared to be the primary driver of regional growth differences. However, decreased interactions with both antagonistic and beneficial soil biota in the introduced populations was also detected. Together, these data support the increased resource availability and enemy release hypotheses as explanations for the invasion success of *B. rubens*.

Introduction

Exotic plant species are experiencing increased translocation to novel environments with the continued globalization of the past few centuries (Vitousek et al. 1997, Mack et al. 2000). Investigations into the mechanisms that determine successful invasion historically involved emphasis on aboveground phenotypic traits (Pyšek et al. 1995, Williamson and Fitter 1996, Bever et al. 2010). Plant-soil interactions recently emerged as a likely explanatory factor facilitating invasion success (Reinhart and Callaway 2006). However, understanding species-specific interactions and functional response of individual populations to these interactions remains lacking, thus limiting the formation of general principles that underlie observed patterns. The historical lack of consensus on causal mechanisms in plant invasion can partially be attributed to two commonly neglected factors: the interaction between plants and soil microbial communities and the importance of identifying genetic source or closely related regional populations.

Technical issues with the study of soil microbes historically posed difficulties in examining plant-soil microbial interactions, resulting in bias in theory toward the importance of aboveground, antagonistic (e.g. competition, predation, etc.) interactions (Bever et al. 2010). However, plant-soil interactions play a significant role in structuring plant communities (Allen and Allen 1980, Grime et al. 1987, Allen and Allen 1990, Mills and Bever 1998, van der Heijden et al. 1998, Packer and Clay 2000). These interactions are important at both regional and local scales (Allen and Boosalis 1983, Klironomos 2002, Callaway et al. 2004), influencing plant-plant competitive interactions and subsequent

community composition (Allen and Allen 1984, Allen and Allen 1990, Clay 1990, Allen et al. 1995).

Plant-soil feedbacks (PSFs) describe net differential plant growth responses due to interactions with antagonistic (negative) and mutualistic (positive) soil organisms (Bever et al. 1997). Negative feedbacks dominate in most ecosystems where coevolved microbial antagonists accumulate and constrain growth (Mills and Bever 1998, Kulmatiski et al. 2008). These feedbacks promote diversity by increasing niche stabilizing differences (Chesson 2000) via density-dependent antagonism (Janzen 1970, Connell 1971), thus limiting population sizes of otherwise competitively dominant species (Bever 2003, HillesRisLambers et al. 2012). Positive PSFs result from decreased antagonism, beneficial partnerships with mycorrhizae or N-fixing bacteria, increased access to resources, or indirect interactions that alter nutrient cycling (Reinhart and Callaway 2004, Reinhart and Callaway 2006, van der Putten et al. 2007). These processes increase host competitive ability and increases relative fitness differences among co-occurring species (Chesson 2000), which ultimately result in decreased diversity and dominance of one to a few plant species (Bever et al. 2012, HillesRisLambers et al. 2012). Such feedbacks are commonly observed among dominant native and invasive species (Klironomos 2002).

The vast majority of PSF studies in the field of invasion ecology treated the soil component as a 'black box', focusing instead on plant performance. While this approach revealed the general pattern of invasive species interacting differently with invaded relative to native range soils, it provides little to no information on *why* they differ. To elucidate these mechanisms light must be shed on this 'black box' in the form of species level

identification. In addition, many investigations emphasized the role of pathogenic/parasitic microbes in limiting host fitness within the native range (Klironomos 2002, Beckstead and Parker 2003, Reinhart et al. 2003, Callaway et al. 2004, Knevel et al. 2004, Agrawal et al. 2005). Consequently, authors commonly invoke the enemy release hypothesis – an increase in invader fitness due to 'release' from coevolved predators and pathogens (Elton 1958, Keane and Crawley 2002) – to simultaneously explain negative interactions in the native range and positive interactions in the invaded range. While this is a parsimonious explanation, it neglects the potentially important contribution of soil mutualists (i.e. arbuscular mycorrhizal fungi, AMF) to the promotion of aggressive growth observed in invasive populations. Rather than plants being released from 'enemies' of their native range, divergent growth habits and population dynamics may be the product of interactions with different soil mutualist communities (Richardson et al. 2000, Reinhart and Callaway 2004, Reinhart and Callaway 2006, Pringle et al. 2009, Sun and He 2010). In either case, species of soil microbes must be different and/or interact differently with hosts among respective regions to conclude that they promote invasion.

Here I used four previously analyzed populations (Chapter 2) of the locally important invasive grass *Bromus rubens* L. (Pavlick and Anderson 2007, = *B. madritensis ssp. rubens*, Fortune et al. 2008) to investigate divergence in plant-soil interactions as an explanation for invasion success. A description of the natural history of *B. rubens* can be found in chapter 1. In chapter 1, I demonstrated that introduced populations displayed evidence for post-introductory evolutionary divergence in important quantitative traits (e.g. biomass, height and relative growth rate), which translated into the increased competitive

abilities of introduced populations observed in chapter 2. In addition to these findings, correlation and selection analyses identified selective agents driving the observed divergence in these traits. My results suggested that abiotic conditions experienced in the introduced range selected for increased expression of traits correlated with seed production (Chapter1). Yet, it remains unknown how different plant-soil interactions among regionally isolated, but closely related, native and introduced populations may alter this behavior.

One crucial, yet often overlooked, aspect in the identification of causal mechanisms of invasion success is ensuring the lack of said mechanisms in the introduced range, or vice versa (Hierro et al. 2005). The best way to achieve this is to first identify 'source' and/or regional populations of close relation. This allows the investigator to accurately locate areas within the native region where mechanisms of interest are in action. I was able to accomplish this goal through information provided by two separate studies. In chapter 1, analyses of molecular associations among regional populations of *B. rubens* revealed two distinct groups. One group primarily contained individuals belonging to native populations (referred to as GC2, the 'native' group), although a few individuals of this group were also found within one introduced population (Riverside, referred to as GC_{ADMIX}). The other group contained the vast majority of individuals belonging to introduced populations and those belonging to one native population (together referred to as GC1, the 'invasive' group). Given the preponderance of GC1 individuals in the introduced range and only one population within the native range, it was concluded that this native population (El Pardo, referred to as $GC1_{NAT}$) was the source of introduced populations sampled in chapter 1. In addition to these data, previous work by Elizabeth Holmes, Michael F. Allen and Edith B.

Allen (*unpublished data*) provided data on soil microbial populations. This study used field-collected fine root samples of *B. rubens* from $GC1_{NAT}$ and $GC1_{INT}$ (Perris population, representative of most common introduced genotype) populations to identify *in situ* host-fungal interactions via high throughput sequencing of the internal transcribed spacer (ITS) region. Together this information allowed for explicit comparison of potential soil borne ecological mechanisms underlying invasion success between $GC1_{NAT}$ and $GC1_{INT}$, while also providing a survey on the responses of a common native and uncommon introduced population to soil components. By comparing the growth responses to soil components of each respective host genotype my objective was to determine whether host genetic identity, soil source and/or host-soil source interactions explain divergence in phenotypic expression among these regionally isolated populations.

I hypothesized that differences in native and introduced population responses to soils of each respective region contributed to the aggressive growth of *B. rubens* within the introduced range. Using this invasive plant host-soil system I tested two dominant hypotheses of invasive plant biology, the increased resource availability hypothesis (IRA, Davis et al. 2000) and the enemy release hypothesis (ERH, Darwin 1859, Elton 1958, Keane and Crawley 2002). I also hypothesized there might be a change in mutualism that could alter invasiveness (i.e. the enhanced mutualism hypothesis [EMH, Reinhart and Callaway 2004]). To do this I utilized a full factorial experimental design in which plants were grown in the presence or absence of soil microbial communities. If any of these hypotheses explained differential growth in sterilized and inoculated soils of regional origin, then it was expected that all populations would perform best in introduced soils

regardless of soil treatment. Evidence for the IRA would be found if all populations performed best in sterilized, introduced soils. Evidence for the ERH would be found if individuals were exposed to lower pathogen diversity (i.e. decreased diversity in potential pathogen/parasite communities) and actual infection, resulting in less inhibition in introduced soils. Finally, evidence for the EMH would be found if both root colonization and host performance were enhanced when in the presence of introduced soil communities.

Materials and Methods

Seed and soil collections

Seeds of the subset of populations chosen for this experiment were those previously analyzed (Chapter 2). Soils were collected from each respective population, bulked, thoroughly mixed and stored under ambient laboratory conditions. Prior to experimentation each respective soil was halved, with one half sterilized via autoclave (two cycles of steam sterilization at 121˚C for 60 minutes) and the other half left unsterilized for inoculation of soil communities.

Soils and microbial characteristics

Nutrient analyses were performed at the US Agricultural Research Service Station Reno, Nevada. The soils from the different source populations were different. In general, the sites from which the introduced populations were collected had higher available nutrient levels (Table 3.1).

Data on microbial community composition within the roots of field collected samples of the two most closely related genotypes ($GC1_{NAT}$ [El Pardo] and $GC1_{INT}$ [Perris]) was taken from Holmes et al. (*unpublished data*). It was unfortunate that information for all populations included in this experiment were not available. However, in chapter 1 $GC1_{NAT}$ was identified as the most likely source population for all but one population (GC_{ADMIX} [Riverside], which contained individuals from both genetic groups identified) sampled in the introduced range. The preponderance of GC1 individuals in introduced populations indicated that some degree of environmental filtering had occurred leading to the dominance of this genotype within the introduced range, followed by selection driven phenotypic trait change in these populations. Given these data, $GC1_{NAT}$ represents the ancestral population and $GC1_{INT}$ represents the dominant introduced (descendent) genotype of *B. rubens* included in this experiment. Therefore, any factors leading to invasion success must be shown to be different between these populations more than either of the remaining two test populations (Hierro et al. 2005).

The microbial community structure was different between native ($GC1_{NAT}$) and introduced ($GC1_{INT}$) rhizospheres. Total operational taxonomic units (OTU's) were higher in native relative to introduced soils (Fig 3.1). Importantly, there were far more Ascomycotina OTUs, many of which are facultative parasites/saprotrophs, in the native site. In addition, AMF OTUs found in fine roots were far more diverse in the native site than introduced collections (Table 3.2).

To determine potential and actual host-AMF interactions among the two most closely related host genotypes ($GC1_{NAT}$ [El Pardo] and $GC1_{INT}$ [Perris]) I quantified spore

abundance and intraradicle infection. AMF spores were isolated and counted per g^{-1} of soil. Spores were isolated via sucrose centrifugation and counts were made via visualization at 40x using a dissecting scope (Allen et al. 1979, Ianson and Allen 1986). These data demonstrate that introduced soils contained more AMF spores with the potential to infect hosts (Figure 3.2).

Live root samples were also collected from these two populations to assess *in situ* host-fungal interactions. Root infection from individuals involved in the experiment described below were also included to determine actual host-fungal interaction during said experiment. Intraradicle infection was assessed via staining with trypan blue and visualization at 400x magnification (Koske and Gemma 1989). The proportion of root length colonized by 'fine endophyte' hyphae, coarse AMF hyphae and/or pathogenic fungi was quantified by grid-intersect method and compared between populations (McGonigle et al. 1990). Field collected roots experienced higher infection rates than roots collected from the experiment, but the relative infection rates between populations only differed for 'other' (parasites/saprotrophs) fungi between field and experimental roots (Figure 3.3). Comparisons between the two populations show that native individuals experience higher rates of infection by coarse AMF (in both the field and the experiment) and 'other' hyphae (in the field but not the experiment), while introduced individuals experience higher rates of infection by 'fine' hyphae.

Experimental design

I used a full factorial design containing 4 seed populations, 4 soil sources and 2 microbial treatments with 10 replicates per treatment (N = 320 total plants). All pots were prepared in the following manner; 70g sterilized sand placed in bottom of pot, followed with 20g either sterile or 'raw' microbial treatments and topped with an additional 30g of sterilized sand. Seeds of each respective population were then planted 5 pot^{-1}, gently watered, then thinned to one individual pot^{-1} following a 10-day establishment period. Pots were randomly arranged within racks and transferred to a growth chamber (Percival growth chamber [Iowa, USA]) for the remainder of the experiment. Conditions in the chamber mimicked an average winter's day in a Mediterranean climate; 10:14 hour day: night cycle at temperatures of 24˚C:18˚C day:night, and light levels at 400μmol m^{-2} s^{-1}.

Growth duration was limited to 50 days to avoid individuals from getting 'pot-bound'. Plants were destructively harvested after day 50. Roots were cleaned of soil debris and total plant biomass was quantified after a drying period of three days at 70˚C.

Statistical analyses

Analyses conducted herein were generally the same as those conducted in chapter 2. Three sets of analyses were used to evaluate genetic host-soil interactions. First, log response ratios were derived for each trait of interest in order to compare the magnitude of microbial treatment effects across populations. This was done by taking the natural log of individual performance in the presence of microbial inoculate over the population mean response to growth in sterilized soils; relative response to microbial treatment *i* $\text{Ln}(\text{Trait}_i)$

= Ln($Trait_{microbe}$/$Trait_{sterile}$). Chapter 2 of this dissertation describes the rationale for using response ratios.

Fixed-factor analysis of variance (ANOVA) models with type-III sums of squares were run to determine the role soil nutrient availability, microbial communities and their origins had on plant performance. Regional soil models sought to determine the influence of region of seed origin (SDREG), region of soil origin (SLREG), soil microbial treatment (MIC), seed population | SDREG (SDPOP), soil population | SLREG (SLPOP) and their interactions on biomass production. Two additional fixed-factor ANOVAs were run on biomass response ratios. The first was the same as above with MIC factor omitted, response ratios inherently contain these effects. The second was a reduced model to explicitly determine the effect of seed population by soil population interactions. When significant differences were detected Tukey's HSD tests were conducted to determine differences among treatments. All models were performed in JMP® Pro 12.0.1 (SAS Institute Inc., Cary, NC, USA, 2015).

Results

Comparison of regional seed performance and regional soil origin effects

Region of seed origin explained much of the variation contained in the regional soil ANOVA model (Table 3.3). Introduced populations produced 25% more biomass than native populations across all treatments ($F = 21.26$, $p < 0.0001$; Figure 3.4 A). Performance was also 18% greater in introduced soils across all treatments ($F = 10.63$, $p = 0.001$; Figure 3.4 B). These patterns were driven by seed and soil populations within region. GC_{ADMIX}

(biomass $\bar{x}$ = 0.049 g [± 0.002]) individuals displayed the greatest performance, followed by $GC1_{INT}$ (biomass $\bar{x}$ = 0.041 g [± 0.002]), $GC1_{NAT}$ (biomass $\bar{x}$ = 0.039 g [± 0.002]) and GC2 (biomass $\bar{x}$ = 0.029 g [± 0.002]) (seed population within region F = 9.25, p = 0.0001), while growth was greatest in GC_{ADMIX} soils (soil population within region F = 7.59, p = 0.0006).

Biomass accumulation was decreased by 36% when individuals were grown in the presence of microbial inoculate across populations and soils (F = 42.58, p < 0.0001) (Figure 3.4 C). This factor explained the majority of variation captured in the regional soil model ANOVA (Table 3.3). Introduced populations were more inhibited by inoculation treatments (a decrease in biomass of 42%) than native populations (a decrease in biomass of 27%) (seed region x inoculation F = 4.73, p = 0.03; Figure 3.5 A). All populations performed best when grown in sterilized soil of introduced origin and worst when grown in the presence of microbial inoculate of native origin (seed origin x soil population x microbial treatment F = 3.45, p = 0.03). However, introduced populations tended to outperform native populations in each treatment.

Responses of regional seed populations to inoculation

Similar to the results from the untransformed data set, region of seed and soil origin, soil population and the interaction between region of seed origin and soil population were all significant factors (Table 3.4). Again, introduced populations were more inhibited by inoculation than native populations (introduced response $\bar{x}$ = -0.51 [± 0.04] vs. native $\bar{x}$ = -0.39 [± 0.04], F = 4.41, p = 0.04). While all populations were inhibited by microbial

inoculate to some degree, the effect was most severe when individuals were grown in the presence of native microbial communities across all populations (native inoculate effect $\bar{x}$ = -0.54 [± 0.04] vs. introduced $\bar{x}$ = -0.36 [± 0.04], F = 9.19, p = 0.003; Figure 3.6). These patterns were driven by the severity of inhibition at the soil population level, and the interaction between region of seed origin and soil population. Individuals grown with either $GC1_{NAT}$ ($\bar{x}$ = -1.25 [± 0.06]) or GC_{ADMIX} ($\bar{x}$ = -0.72 [± 0.06]) displayed negative responses to inoculation, while responses of individuals grown with $GC1_{INT}$ ($\bar{x}$ = 0.002 [± 0.06]) were neutral, and those grown with GC2 ($\bar{x}$ = 0.17 [± 0.06]) were positive (soil population F = 183.32, p < 0.0001; region of seed origin x soil population F = 4.52, p = 0.01) (Figure 3.7).

Seed population no longer explained a significant amount of variation observed in the reduced model. The effect of inoculation with respective soil populations explained the vast majority of variation (F = 130.04, p < 0.0001; Table 3.4). As above, strong negative responses to inoculation with $GC1_{NAT}$ ($\bar{x}$ = -1.25 [± 0.06]) and GC_{ADMIX} ($\bar{x}$ = -0.72 [± 0.06]), neutral responses to inoculation with $GC1_{INT}$ ($\bar{x}$ = 0.002 [± 0.06]) and positive responses to GC2 ($\bar{x}$ = 0.17 [± 0.06]) microbial communities were experienced by most individuals (Figure 3.6). However, the magnitude of these responses differed by seed population (seed population x soil population F = 2.78, p = 0.005; Figure 3.7).

Discussion

I hypothesized that phenotypic differences in native and introduced populations could be attributed to genotypic responses of populations to soils of each respective region. Through the use of this invasive plant host-soil system I controlled for post-introductory

evolution of invasive populations while simultaneously testing three hypotheses within invasive plant biology. By controlling for relatedness among native and introduced populations of *B. rubens*, I found the primary reason for differences in phenotypic expression were due to post-introductory evolution (Chapter 1). Results from the full factorial plant-soil growth assay indicated that nutrient availability, followed by decreased associations with both antagonistic and beneficial soil fungi contributed to differences in growth patterns between native and introduced populations. Overall this investigation provides evidence in support of the evolution invasiveness, increased resource availability and enemy release hypotheses.

Biomass production and post-introductory evolution

The observation of greater biomass production in introduced populations, regardless of soil treatment, indicated that phenotypic differences among regional populations were the result of factors other than plant-soil interactions. In chapter 1 I showed that selection pressure from abiotic resources likely promoted phenotypic trade-offs resulting in increased expression of traits correlated with seed production. Native populations of *B. rubens* experienced greater selection pressure relative to introduced populations. Together these observations suggest that colonizing populations experienced selection pressure directly upon arrival. The phenotypic differences among native and introduced populations included in this experiment may therefore be the product of ~130 years of selection on traits associated with more aggressive growth. While post-introductory evolution was clearly important, the observation of increased performance of

most populations in introduced soils suggests that both evolutionary and ecological factors promoted invasiveness in *B. rubens*.

The influence of soil resources

The increased resource availability (IRA) hypothesis posits that greater soil nutrients in introduced range soils are the primary cause of invasiveness (Davis et al. 2000). In a similar study investigating the contribution of soil components to the promotion of invasiveness in *Elymus caput-medusae,* increased soil nutrients were found to be the primary driver in that system (Morgan et al. 2017). Through the use of sterilized and unsterilized soil treatments, I found that both native and introduced populations of *B. rubens* produced the greatest biomass in sterilized soils of introduced origin. These results were similar to Morgan et al. (2017), and indicate that increased resource availability within introduced soils was the primary soil component contributing to the greater biomass production of *B. rubens*.

There are several background factors that could affect plant growth response to soil resources among regional soils. In the native range (Spain), the sites studied are slightly basic and high in $CaCO_3$, which tends to immobilize P ($CaPO_4$) (Allen and Allen *personal observation*). Introduced (California) soils with *B. rubens*, are younger, granitic-based soils and tend to be slightly acidic and higher in P. The site where GC2 (Los Cuadros) population seed was collected is also higher in organic matter, resulting in greater immobilization of what nutrients are present. In addition, investigations have found that N deposition in introduced range soils greatly increases fertility and exacerbates invasive behavior of *B.*

rubens and other invasive grasses (Fenn et al. 2003). Therefore, both geological and anthropogenic factors explain differences in nutrient availability between regional soils.

The influence of antagonistic microbial interactions

The enemy release hypothesis (ERH) predicts that increased growth and reproduction observed in invasive populations is due to the absence of coevolved antagonists from the native range (Darwin 1859, Elton 1958, Keane and Crawley 2002). The parsimonious nature of this hypothesis has attracted the attention of many investigators within the field of invasive plant biology, and remains one of the most commonly cited mechanisms of invasion success (Colautti et al. 2004).

In the invasive plant-soil interaction literature the ERH is particularly attractive. The commonality of more negative responses of plant hosts to native relative to introduced soil communities prompted many investigators to cite the ERH as the causal mechanism for these observations (Callaway et al. 2004, Colautti et al. 2004, Kulmatiski et al. 2008). Yet without information on the soil microbial communities found in each respective range, accurate conclusions could not be made.

The initial background microbial populations were vastly different between $GC1_{NAT}$ (El Pardo) and $GC1_{INT}$ (Perris). The diversity of fungi in the native population rhizosphere ($GC1_{NAT}$) was higher than the introduced population ($GC1_{INT}$) rhizosphere. The primary differences were the large diversity of Ascomycetes and Basidiomycetes, many of which are parasites, found in association with the native host population ($GC1_{NAT}$). In addition to these data, native host populations experienced greater levels of

infection by fungi other than AMF. Together, this information and the negative growth responses to native soil microbial populations supports the enemy release hypothesis.

The influence of host-AMF interactions

The enhanced mutualisms hypothesis (EMH) postulates that differences in host mutualist interactions among regional populations of invasive species promotes greater growth and reproduction in introduced ranges (Reinhart and Callaway 2004). Such increases in plant vigor may be due to modification of interactions with the same mutualistic species and/or interactions with differing mutualistic species which provide increased benefit. This is a fairly new and understudied hypothesis within invasive plant biology, but may explain some patterns observed in introduced ranges (Pringle et al. 2009).

AMF community structure differed between the two populations . Six AMF species (*Glomus hoi, Gl. irregular, Gl. etunicatum, Gl. claroideum* [=*Claroideoglomus* sp], *Gl. itraradices* and *Entrophospora infrequens*) were identified in native (Spanish) soils while only one taxon of *Gl. claroideum* was found in introduced (Californian) soils (Holmes et al. *unpublished data*). In this experiment, native individuals ($GC1_{NAT}$) experienced greater colonization by coarse AMF hyphae, while introduced individuals were more readily colonized by fine AMF hyphae. Given that both populations ($GC1_{NAT}$ and $GC1_{INT}$) displayed negative responses to native soils, it appears that the benefit derived from a diverse mutualistic community did not overcome the negative impacts of the diverse parasitic community. Finally, neutral responses to inoculation with introduced microbes suggest that mutualist loss was not as important as enemy release in this experiment.

Overall patterns and conclusions

Both positive and negative responses to soil microbial populations can be found in the literature, with little mechanistic understanding of why one or the other predominates in any system (Kulmatiski et al., 2008). In this experiment, both native and introduced seed performed best in sterilized soil of introduced origin and were less inhibited by interaction with introduced microbial inoculate. The causal mechanisms primarily driving these patterns were the negative responses all populations had in the presence of the diverse microbial communities associated with $GC1_{NAT}$ soils, the neutral to positive response of all populations to the species poor microbial communities associated with $GC1_{INT}$ soils, and the overall increase in nutrient availability within introduced soils.

GC2 (Los Cuadros) was the only population to display positive responses to intra-population soil microbial communities. Such responses may be due to species-specific host-microbial interactions reflecting adaptation to this specific soil. GC2 soils were low in available P, but likely had high bound inorganic P and high organic P. AMF produce both acid and alkaline phosphatases that increase P uptake of organic P (Allen et al. 1981) and also increase respiration and organic acid production that weather bound P, such as $CaPO_4$ (Jurinak et al. 1986, Knight et al. 1989). In addition, it is possible that the high diversity of other fungi included mutualistic endophytes that also promoted, rather than depressed growth. It is interesting to note that this population represents 'native' genotypes identified in chapter 1. In that chapter the majority of populations belonging to GC2 displayed intermediate to high levels of growth performance. Given those observations, and the positive soil interactions found in this investigation, it is interesting that very few

individuals of the GC2 genotype have not successfully established in the introduced range. It may be the case that GC2 individuals remain in the 'lag' phase of invasion, and may become invasive sometime in the future.

GC_{ADMIX} (Riverside) individuals displayed moderate inhibition in their own soils. The response of these individuals to soil microbial communities of other populations were generally similar to individuals belonging to both native and introduced GC1 populations. Further, this was the only population found in the introduced range that contained individuals belonging to each genetic cluster identified in chapter 1. As with GC2, it may be that individuals within the area sampled have not yet begun to express invasive characters and may be maintained by moderate to high levels of soil nutrients.

Populations within the GC1 genetic cluster showed very different responses to inoculation with intra-population soil microbial communities and provide explanatory evidence for the aggressive growth performance observed in this system. The two populations belonging to this group are closely related, yet regionally isolated, and may be expected to interact with soil communities in similar ways. That was indeed the case, as both $GC1_{NAT}$ (El Pardo) and $GC1_{INT}$ (Perris) both displayed large negative responses to inoculation with soil communities belonging to the native population and neutral to positive responses to soil communities belonging to the introduced population. Through the use of background information on available soil resources and microbial community composition these data support the increased resource availability and enemy release hypotheses. The role of mutualism requires further testing.

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Table 3.1. Soil characteristics from sites tested in this study.

Region	Population	Total N (%)	Organic C (%)	KCL NH_4 (mg/kg)	KCL NO_3 (mg/kg)	Bicarb-extract P (mg/kg)
Native						
	GC2	0.18	8.34	1.05	0.18	0.49
	$GC1_{NAT}$	0.06	0.68	0.82	0.01	0.20
Introduced						
	$GC1_{INT}$	0.13	1.47	2.58	3.98	0.62
	GC_{ADMIX}	0.11	1.26	1.23	4.64	0.54

Table 3.2. List of arbuscular mycorrhizal fungi identified from ITS sequences from field-collected fine roots of *B. rubens* (Holmes et al. *unpublished data*).

Population	AMF species identified
$GC1_{NAT}$ (El Pardo)	
	Glomus hoi
	Glomus irregulare
	Glomus etunicatum
	Glomus claroideum
	Glomus intraradices
	Entrophospora infrequens
$GC1_{INT}$ (Perris)	
	Glomus claroideum

Table 3.3. Results of regional soil ANOVA models on biomass production and relative response ratios of individuals grown in the presence of respective soil components. Factors were region of seed origin (SDREG), region of soil origin (SLREG), soil microbial treatment (MIC), seed population | SDREG (SDPOP), soil population | SLREG (SLPOP) and their interactions on biomass production and response to respective soil components. *F*-ratios and *p*-values are provided. Dashes (--) indicate no data available.

	Untransformed data		Relative response data	
Regional soil models	Biomass		Ln(Biomass)	
	F-ratio	*p*-value	*F*-ratio	*p*-value
Seed region (SDREG)	21.26	<0.0001	4.41	0.04
Soil Region (SLREG)	10.63	0.001	9.19	0.003
Microbial treatment (MIC)	42.59	<0.0001	--	--
Seed population | SDREG (SDPOP)	9.25	0.0001	0.56	0.57
Soil population | SLREG (SLPOP)	7.59	0.0006	183.33	<0.0001
SDREG x SLREG	0.0003	0.99	1.51	0.22
SDREG x MIC	4.73	0.03	--	--
SLREG x MIC	0.08	0.78	--	--
SLREG x SDPOP	0.12	0.89	2.12	0.12
SDREG x SLPOP	1.47	0.23	4.52	0.01
SDREG x SLREG x MIC	0.51	0.47	--	--
SLREG x SDPOP x MIC	0.52	0.59	--	--
SDREG x SLPOP x MIC	3.45	0.03	--	--

Table 3.4. Results of ANOVA model on relative response of seed populations (SDPOP), soil populations (SLPOP) and their interaction to respective soil microbial treatments. *F*-ratios, *p*-values and least square means (± SE) are provided. Letters above mean values indicate differences based upon Tukey's HSD tests.

		Ln(Biomass)	
Population soil models		*F*-ratio	*p*-value
	Seed population (SDPOP)	1.89	0.13
	Soil population (SLPOP)	130.04	<0.0001
	SDPOP x SLPOP	2.78	0.005
Least sq means (± SE)			
	Seed population		
	GC2	-0.34±0.06	
	$GC1_{NAT}$	-0.43±0.06	
	$GC1_{INT}$	-0.51±0.06	
	GC_{ADMIX}	-0.51±0.06	
	Soil population		
	GC2	0.17^{A}±0.06	
	$GC1_{NAT}$	-1.25^{C}±0.06	
	$GC1_{INT}$	0.001^{A}±0.06	
	GC_{ADMIX}	-0.72^{B}±0.06	
	SDPOP x SLPOP		
	GC2 x GC2	0.50^{A}±0.12	
	GC2 x $GC1_{NAT}$	-1.17^{EF}±0.12	
	GC2 x $GC1_{INT}$	-0.14^{BC}±0.12	
	GC2 x GC_{ADMIX}	-0.56^{CD}±0.12	
	$GC1_{NAT}$ x GC2	-0.05^{ABC}±0.12	
	$GC1_{NAT}$ x $GC1_{NAT}$	-1.04^{DEF}±0.12	
	$GC1_{NAT}$ x $GC1_{INT}$	-0.05^{ABC}±0.12	
	$GC1_{NAT}$ x GC_{ADMIX}	-0.59^{CD}±0.12	
	$GC1_{INT}$ x GC2	0.17^{AB}±0.12	
	$GC1_{INT}$ x $GC1_{NAT}$	-1.33^{F}±0.12	
	$GC1_{INT}$ x GC_{1INT}	0.08^{AB}±0.12	
	$GC1_{INT}$ x GC_{ADMIX}	-0.97^{DEF}±0.12	
	GC_{ADMIX} x GC2	0.07^{AB}±0.12	
	GC_{ADMIX} x $GC1_{NAT}$	-1.46^{F}±0.12	
	GC_{ADMIX} x $GC1_{INT}$	0.11^{AB}±0.12	
	GC_{ADMIX} x GC_{ADMIX}	-0.76^{DE}±0.12	

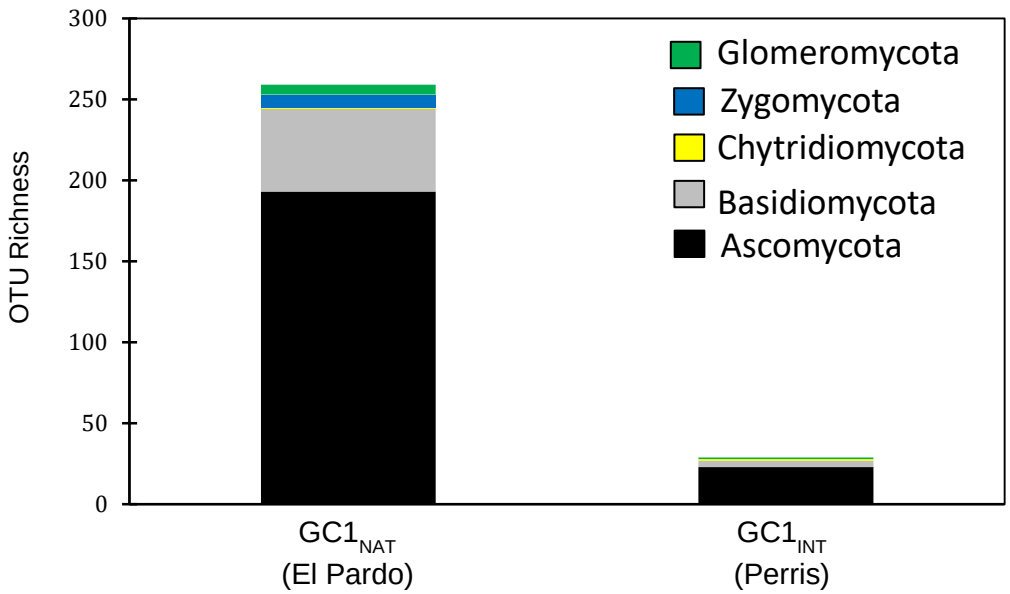

Figure 3.1. Total OTU richness based on ITS sequences for the two most closely related, yet regionally isolated, genotypes included in this study (Holmes et al. *unpublished data*).

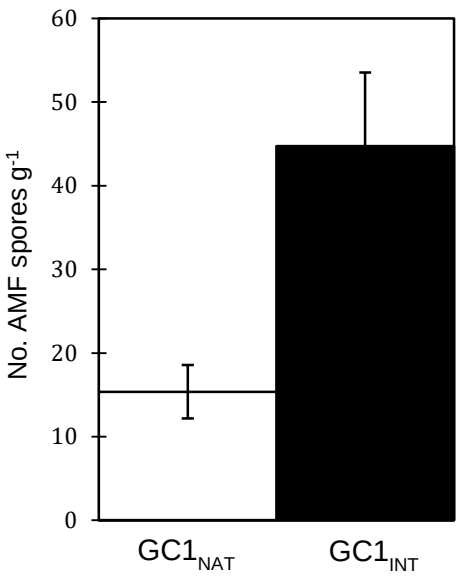

Figure 3.2. Mean spore count g^{-1} quantified from field collected soils of the two most closely related, yet regionally isolated, genotypes ($GC1_{NAT}$ = El Pardo, $GC1_{INT}$ = Perris).

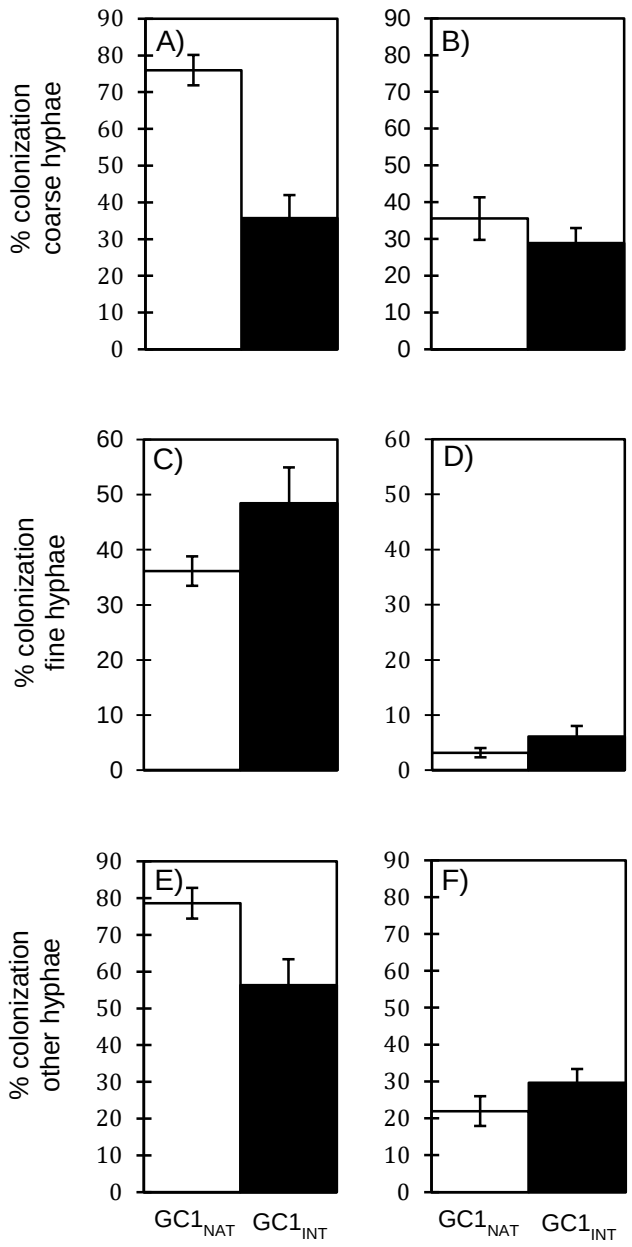

Figure 3.3. Mean fungal colonization within the roots of the two most closely related, yet regionally isolated, genotypes ($GC1_{NAT}$ = El Pardo, $GC1_{INT}$ = Perris). Graphs A, C and E display intraradicle colonization within individuals collected in the field. Graphs B, D and F display intraradicle colonization within individuals post-experiment.

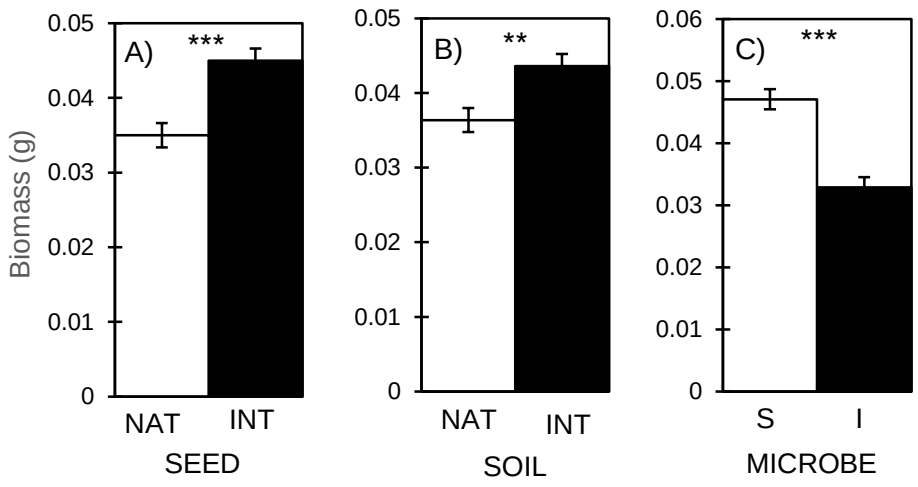

Figure 3.4. Comparative mean (±SE) growth performance (biomass) of A) regional seed populations across soil treatments (NAT = native seed populations, INT = introduced seed), B) performance in regional soils across populations (NAT = native soils, INT = introduced soils) and C) performance in microbial treatments across populations and soils (S = sterile soils, I = inoculated soils). Asterisks indicate significant differences based upon fixed-factor ANOVAs. Levels of significance were the following: ** $p < 0.01$, *** $p < 0.001$.

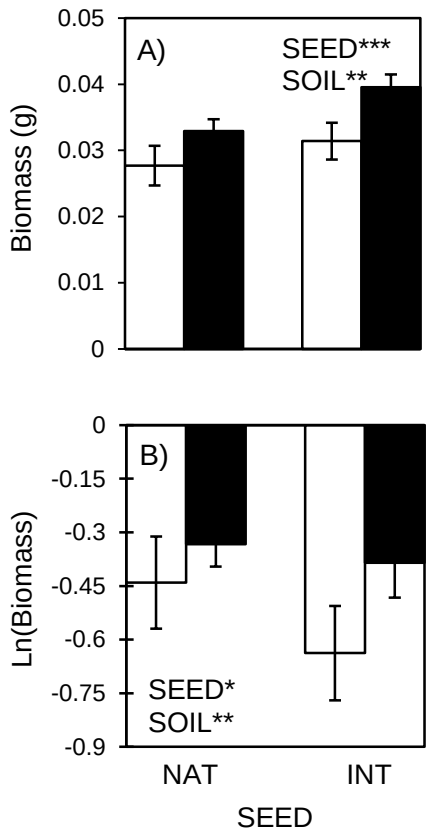

Figure 3.5. Comparative mean (± SE) growth performance (biomass) of A) regional seed populations in each regional soil (NAT = native seed populations, INT = introduced seed; white bars indicate native soils, black bars indicate introduced soils) and B) response ratios for regional population growth in the presence of regional inoculate (white bars indicate native inoculate, black bars indicate introduced inoculate). Asterisks beside 'SEED' and 'SOIL' indicate significance of these terms from fixed-factor ANOVAs. Levels of significance were the following: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

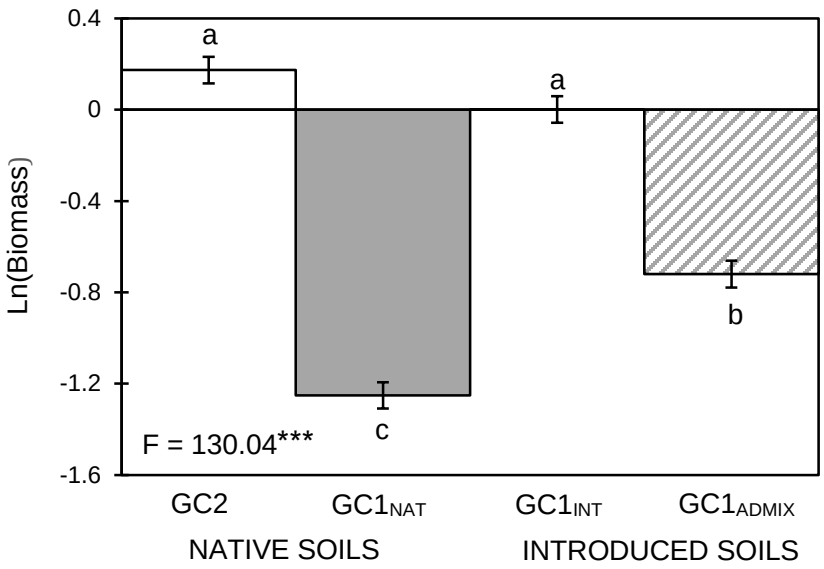

Figure 3.6. Comparative mean (± SE) growth performance (biomass) response ratios across all populations in each respective soil (GC2 = Los Cuadros [white], $GC1_{NAT}$ = El Pardo [gray], $GC1_{INT}$ = Perris [black] and GC_{ADMIX} = Riverside [hashed]). F-ratio from fixed-factor ANOVA provided. Lowercase letters indicate significant differences based upon Tukey's HSD test. Levels of significance were the following: *** $p < 0.001$.

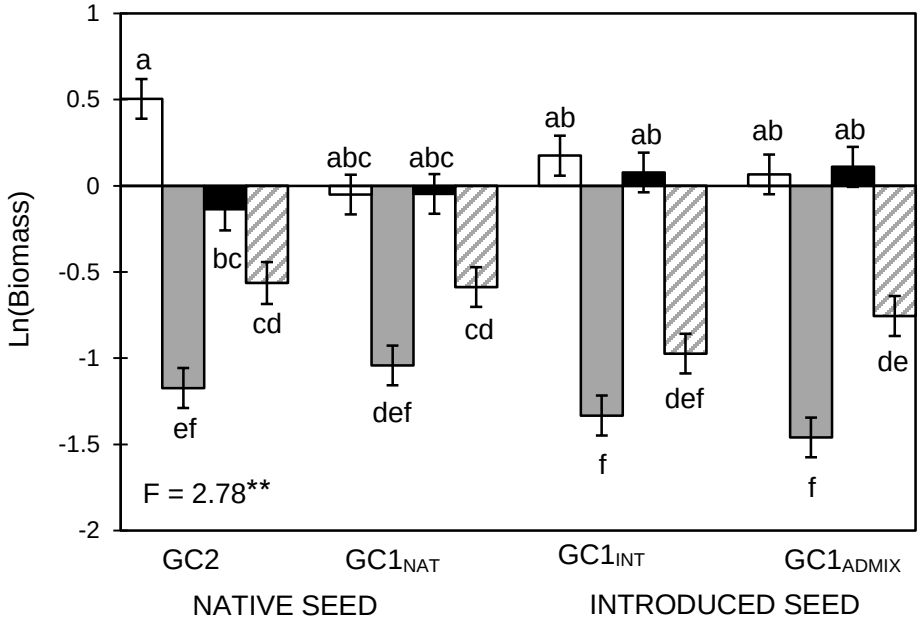

Figure 3.7. Comparative mean (± SE) growth (biomass) response ratios for all populations in each respective soil (GC2 = Los Cuadros [white], $GC1_{NAT}$ = El Pardo [gray], $GC1_{INT}$ = Perris [black] and GC_{ADMIX} = Riverside [hashed]). F-ratio from fixed-factor ANOVA provided. Seed population indicated below graph. Lowercase letters indicate significant differences based upon Tukey's HSD test. Levels of significance were the following; ** $p < 0.01$.

Conclusions

Biologists have long recognized that invasive plant species provide a 'natural' example of dramatic ecological and evolutionary change (Darwin 1859). Early investigations into the mechanisms that determine successful invasion focused on identification of ecological predictors and subsequent control strategies (Pyšek et al. 1995, Williamson and Fitter 1996). While advances by these and other studies were certainly significant, an overwhelming emphasis on ecological explanations for invasion success may have restricted the formulation of general principles within invasion biology. Recent integration of evolutionary concepts into a field previously dominated by ecological theory is now providing a more holistic view of factors contributing to invasion success (Ellstrand and Schierenbeck. 2000, Sakai et al. 2001, Lee 2002). An increasing number of investigations have demonstrated that post-introductory evolutionary dynamics are critical to understanding invasion in many systems (reviewed in Felker-Quinn et al. 2013). In addition to these promising patterns, a recent shift in ecological investigations of invasion plants away from above- to belowground interactions revealed a consistent pattern in which invasive populations are facilitated in introduced range soils, while inhibited in native range soils (Reinhart and Callaway 2006). Given the consistency of these two patterns it is likely that post-introductory trait changes may impact ecological patterns of host-soil interactions, and vice versa, in important ways. The degree to which these forces interact in invasive plant systems has not been thoroughly investigated (terHorst and Zee 2016). I explored the evolutionary dynamics of post-introductory trait change, implications of

observed trait changes and the contribution of regional plant-soil interactions in the locally important invasive grass *Bromus rubens* L. (Pavlick and Anderson 2007, = *B. madritensis ssp. rubens*, Fortune et al. 2008) to gain a more comprehensive understanding of factors promoting invasion in this species.

Post-introductory divergence among native and introduced populations of *B. rubens* was found to be the primary promoter of aggressive, invasive behavior in this system. Similar to other investigations on the evolution of invasive characters, large losses of molecular diversity occurred following introduction (Novak and Mack 2005, Dlugosch and Parker 2008). In my investigation this was represented by the prevalence of one successful genotype found within the introduced range and only one population in the native range. Given the success of introduced populations belonging to this group, it was inferred that some degree of environmental filtering had occurred. But this was not the case of an 'ideal weed' entering a new, suitable habitat (Baker 1965). The patterns displayed in my common garden experiment revealed that considerable differences between ancestral and descendent populations were the result of a novel set of selection pressures.

A trade-off between phenotypic traits provided evidence for selection driven change. Native populations employed a leafy-high photosynthetically active growth strategy, while introduced populations displayed a larger-more rapid growth strategy. These observations were not in agreement with large-scale investigations of phenotypic trait correlations (Wright et al. 2004), but overall provided support that native and introduced species occupy different regions of the leaf economics spectrum (Leishman et al. 2007). Correlational and selection analyses further revealed that the evolution of

invasiveness demonstrated in this system did not comply with the biotic assumptions of the evolution of increased competitive ability (EICA) hypothesis. I found that traits correlated with increased fitness were consistently selected upon in both native and introduced populations, but that differences in irradiance between regions was the primary promoter of a shift in growth strategies observed between regional populations.

Phenotypic selection analyses revealed two interesting patterns. One was the greater magnitude of selection pressure on native populations to increase the expression of traits commonly associated with aggressive growth. The second was the lack of significant selection pressure to alter trait expression in introduced populations. These patterns suggested that post-introductory selection acts early in the invasion process, and that adaptation to novel abiotic environments contributed to the invasiveness in *B. rubens*.

Scaling up from population level observations of differentiation, I next explored the implications of post-introductory change in *B. rubens* with an intraspecific competition experiment. The evolution of increased competitive ability (EICA) hypothesis postulates that, in the absence of coevolved antagonists, post-introductory selection on reallocation of resources away from once useful, defensive traits toward increased growth and reproduction explains invasiveness (Blossey and Nötzold 1995). I tested the competitive aspect of the EICA with populations of known backgrounds to determine if increased competitive ability was the product of translocation of an 'ideal weed' or whether divergence in competitive behavior occurred post-introduction. Introduced populations continued to display greater overall performance relative to native populations. Importantly, the greater performance of introduced individuals also translated into

increased competitive effects on neighbors. The competitive dominance displayed by both introduced populations indicated that divergence in competitive abilities did occur between closely related ancestral (native) and descendent (introduced) populations, but that it was likely the product regional differences in selection pressure, not genotypic similarity to the native, 'source' population.

Patterns from the previous two experiments suggest that post-introductory evolution of increased growth performance and competitive ability were important to the success of *B. rubens* as an invader. These data provide support for consistent observations of evolutionary change as an explanation for invasion success (Felker-Quinn et al. 2013). Building on this data, I next explored how another consistent pattern within invasion ecology contributed to the invasiveness of *B. rubens*. This experiment sought to determine how differences in regional soil components further influenced the growth of introduced populations in this system. A full factorial growth experiment, with each population grown in the presence and absence of respective soil microbial communities, was employed to tease apart the relative importance of soil resource availability, antagonistic and beneficial microbial populations on host performance. This allowed me to test two dominant hypotheses in invasive plant biology, the increased resource availability hypothesis (IRA, Davis et al. 2000) and the enemy release hypothesis (ERH, Darwin 1859, Elton 1958, Keane and Crawley 2002), as well as the enhanced mutualism hypothesis (EMH, Reinhart and Callaway 2006), as possible mechanisms of successful invasion.

I hypothesized that differences in phenotypic expression in native and introduced populations could be explained by host responses to soils of each respective region. The

primary difference in growth performance remained post-introductory change in introduced populations. However, each soil component did contribute to the patterns observed in this experiment. All populations performed best in sterilized soil of introduced origin. This was explained by greater overall resource availability in introduced relative to native soils. In contrast, all populations performed the worst when grown in the presence soil microbial populations originating from the native, 'source' population. Data on microbial species composition and root colonization by beneficial and potentially antagonistic fungi explained these patterns. Soil microbial populations from native soils were 9 times as diverse as introduced soils. The primary differences were the high diversity of Ascomycotina and Basidiomycotina (many of which are parasites), and Glomeromycotina (mutualist fungi) in native relative introduced range soils. Colonization data further demonstrated that native individuals were more readily infected by potentially parasitic and beneficial fungi in the native range. Taken together these data provide support for the IRA (Davis et al. 2000) and the ERH (Elton 1958, Keane and Crawley 2002) hypotheses, while data for the EMH (Reinhart and Callaway 2006) was inconclusive.

Efforts to identify general principles in invasive plant biology were repeatedly frustrated by context dependencies in the past (Catford et al. 2009). Two consistent mechanisms have now been identified which explain divergent growth behavior between native and introduced populations of invasive species. The first is the prevalent observation of evolutionary differentiation among native and introduced populations of many exotic species (Felker-Quinn et al. 2013). The second is the ubiquity of positive plant-soil interactions in introduced ranges and negative plant-soil interactions in native ranges.

Through integration of these two promising directions in invasion biology my work sought to gain a more comprehensive view of the mechanisms leading to successful invasion in the exotic grass *B. rubens*. Evidence for the role of post-introductory evolution towards more aggressive growth was found in all experiments. As predicted, both native and introduced populations also performed the best in introduced and worst in native range soils. This work contributes to the growing body of evidence in support of evolutionary change as the primary driver of invasion. It also provides additional support for the role of plant-soil interactions in the invasion process. Through detailed inspection of regional soil components this work was also able to provide mechanistic explanations for observed host responses. Together these results suggest that a more holistic view of mechanisms underlying invasion success may rely on continued integration of evolutionary and ecological theory. The unfortunate implication of this work is that traits commonly found in invasive species may not be reliable predictors of successful invasion, suggesting that seemingly benign, exotic species may hold the potential to become noxious invaders at some unknown time in the future.

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