Title
Factors shaping the evolution of emergence timing and its community-level impacts in seasonal environments

Author
Waterton, Joseph Sebastian

Publication Date
2019

UNIVERSITY OF CALIFORNIA SAN DIEGO

Factors shaping the evolution of emergence timing and its community-level impacts in seasonal environments

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Biology

by

Joseph Sebastian Waterton

Committee in charge:

Professor Elsa Cleland, Chair
Professor Joshua Kohn
Professor Susan Mazer
Professor Justin Meyer
Professor Jonathan Shurin
Professor Jennifer Smith

2019

The Dissertation of Joseph Sebastian Waterton is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

____

____

____

____

____

____

Chair

University of California San Diego

2019

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

Thank you to the many students who assisted with this research, and without whom this entire body of work would not have been possible: Martin Arceo, Justin Bain, Gary Bair, Danelle Baronia, Nia Bock, Jon Bricke, Laurel Brigham, Edward Chen, Alexis Cugini, Julia Gaudio, Sandra Hamilton, Carline Hua, Kirk Hutchison, Kristy Lanz, Vanessa Lundsten, and Jennifer Oberhoffer at UCSD, Sean Jarrell-Hurtado at UC Davis. I would also like to thank technicians in the Cleland Lab, Andrew Heath and Elizabeth Premo, for their hard work in the lab, field, and greenhouse.

Thank you to the staff at the UC Natural Reserve System and Hopland Research and Extension Center who in 2015 helped me collect seeds of *Stipa pulchra* and *Bromus diandrus* which were used in many of the experiments in this dissertation: Tim Brown, Virginia Boucher, Don Canestro, Peter Connors, Gage Dayton, Isabelle Kay, Robert Keiffer, Kate McCurdy, Wendy Morris, Mark Readdie, Cris Sandoval, Jackie Sones, Vincent Voegeli, Shane Waddell.

Thank you to Joanna Dunn and Alyona Bobkova for your administrative support over the years, and for helping to organize the EBE breakfasts which were something I looked forward to each week.

Thank you to my committee, Joshua Kohn, Justin Meyer, Susan Mazer, Jon Shurin, and Jennifer Smith, for your valuable feedback and advice. My research and writing have benefitted hugely from your experience.

Thank you to my advisor, Elsa Cleland, for accepting me into your lab and making me feel so welcome in a new country and environment. Thank you for all the helpful advice, not just on scientific matters, but also professional skills and personal development, careers, concerts and much more. You are a fantastic mentor and teacher, and I have been very fortunate to have your support over the past few years.

Thank you to past and present graduate students in the Cleland lab, Ellen Esch, Chandler Puritty, and Liz Ryan, for helping me in so many ways, whether it was giving feedback on a talk, helping in the field, or simply being a friendly face around the office. It has been awesome being part of such a great group of scientists. Thank you also to EBE students in other labs, past and present, for your support, particularly Marika Schulhof, who I was lucky to have as a peer mentor, as well as my cohort, Bahram Kheradmand, Ida Naughton, Audrey Proenca, Jess Gambel, and Hamanda Cavalheri.

Thank you to my friends from the UK, particularly those who made the long journey to visit me in San Diego, including Graham Kimber, Will Lloyd, Pat Traynor and JL Loewenthal. Thank you also to the great friends I made in San Diego, particularly Rob Pulido, Jon Bricke, Brian Lewis, Gabby Hands, Anna Guzikowski, Helen Pu, Matt Douglas. Thank you also Nikki Calisa for putting up with me this past year or so.

Thank you to my brother and sister-in-law, Pedro and Léanne, for being such great travel companions and friends. Your support and advice were so reassuring when navigating the uncharted territory that was graduate school. Thank you most of all to my parents, Kevin and Mercedes, for your love and support while I was on the other side of world. None of this would have been possible without you.

Funding was provided by University of California, San Diego Division of Biological Sciences; Jean Marie Messier Memorial Fund Fellowship; Institute for the Study of Ecological Effects of Climate Impacts Graduate Student Researcher Fellowship; Frontiers of Innovation Scholars Program; David V. Goeddel Endowed Graduate Fellowship.

Chapter 1, in full, has been submitted for publication of the material. Waterton, J., Mazer, S. J., Meyer, J.R., and E.E. Cleland. The dissertation author is the primary investigator and author of this paper.

Chapter 2, in full, is currently being prepared for submission for publication of the material. Waterton, J., Mazer S. J. and E.E. Cleland. The dissertation author is the primary investigator and author of this paper.

Chapter 3, in full, has been submitted for publication of the material. Waterton, J., and E.E. Cleland. The dissertation author is the primary investigator and author of this paper.

Chapter 4, in full, has been published in the journal *Ecology & Evolution*. Waterton, J., and E.E. Cleland. The dissertation author is the primary investigator and author of this paper.

VITA

2013	Bachelor of Arts in Biological Sciences, St Hugh’s College, Oxford
2019	Doctor of Philosophy in Biology, University of California San Diego, La Jolla, CA

PUBLICATIONS

Waterton, J., and E. E. Cleland. 2016. Trade-off between early emergence and herbivore susceptibility mediates exotic success in an experimental California plant community. Ecology and Evolution 6(24): 8942-8953.

ABSTRACT OF THE DISSERTATION

Factors shaping the evolution of emergence timing and its community-level impacts in seasonal environments

by

Joseph Sebastian Waterton
Doctor of Philosophy in Biology
University of California San Diego, 2019
Professor Elsa Cleland, Chair

Phenology, the timing of life-history transitions, is a crucial component of plant adaptation in seasonal environments because it determines the environmental conditions that each developmental stage interacts with. Adaptive evolution in phenological traits will contribute to the long-term persistence of plant species in the face of climate change and plant species invasions. The timing of emergence from seed is a highly adaptive phenological trait and has sizeable impacts on plant community structure, yet the potential for this trait to evolve and the subsequent effects on plant communities is unclear. The goal of my dissertation research is therefore to improve our understanding of how various factors shape the evolution of emergence timing and its impacts on plant communities. Using two California grasses, the native perennial

Stipa pulchra (Hitchc.) Barkworth and the exotic annual *Bromus diandrus* (Roth), and a suite of other native and exotic herbaceous species as a study system, I carried out a series of field and greenhouse experiments that form the basis of each dissertation chapter. In Chapter 1, I demonstrate that a genetic trade-off between within- and among-year emergence timing can constrain evolutionary responses to joint selection on both traits, resulting in Pareto optimal trait combinations in more arid environments. In Chapter 2, I demonstrate that variation in the surrounding plant community influences the strength of selection for earlier emergence, although the selective effect of communities can vary between species. I also show that the strength of selection for earlier emergence can be influenced by the origin of neighbors, suggesting that changes in community composition are likely to alter patterns of selection on this trait. In Chapter 3, I demonstrate that mammalian herbivory weakens directional selection for earlier emergence in competition independently of reductions in mean fitness. In Chapter 4, I demonstrate that species that emerge earlier in the growing season are more susceptible to mammalian herbivory, particularly when herbivory occurs early in the growing season, and that early-season herbivory increases the relative performance of later-emerging native species when growing with earlier-emerging exotics. Together, these results demonstrate that the evolution of emergence timing is constrained by a genetic trade-off between its within-year and among-year components. Furthermore, these results demonstrate that biotic interactions, such as competition and herbivory, play an important role in determining selection on emergence timing within species and the community-level impacts of variation in emergence timing among species.

INTRODUCTION

The importance of plant phenology in a changing world

Phenology, the seasonal timing of life-history transitions such as growth and reproduction, is an integral component of plant adaptation to seasonal environments (Forrest and Miller-Rushing 2010). Phenology determines the potential for plants to carry out essential functions, such as acquiring resources and reproducing; thus, plants experience strong selection to match the timing of life-history transitions to favorable conditions in the biotic and abiotic environment. This has resulted in the evolution of physiological mechanisms that integrate information on the surrounding environment, such as temperature, precipitation, and photoperiod, to regulate phenological traits (Forrest and Miller-Rushing 2010, Wadgymar et al. 2018). Individual-level phenological responses to the environment in turn influence a wide range of biological phenomena at different scales, including interspecific interactions (Kudo and Ida 2013) and the amount of carbon sequestered by entire ecosystems (Hu et al. 2010).

The study of plant phenology has become of particular importance in the context of anthropogenic climate change. Shifts in seasonal phenology are among the most conspicuous impacts of climate change on living organisms. Climate change is proceeding at an unprecedented rate, with globally averaged land and ocean temperatures increasing by an estimated 0.85 °C between 1880 -2012 as well as changing patterns of precipitation (IPCC 2013). Advances in leaf-out and flowering times resulting from warmer spring temperatures been observed across plant taxa in regions with seasonal climates (Parmesan and Yohe 2003, Anderson et al. 2012, CaraDonna et al. 2014). Greater phenological tracking with climate change is associated with increased plant performance (Cleland 2012), highlighting the importance of shifts in plant phenology for species persistence.

Invasive plants also pose a severe threat to native plant species in the ecosystems that they invade (Sala et al. 2000). Differences in seasonal phenology are a key mechanism that that promote successful invasion by exotic plant species (Wolkovich and Cleland 2011, Fridley 2012). Notably, earlier seasonal phenology can allow species to pre-empt resources and thus competitively suppress later-active species, a form of seasonal priority effect (Wainwright et al. 2012, Thomson et al. 2017). This advantage of earlier seasonal phenology has been implicated as a key factor contributing to the success of many exotic species (Pérez-Fernández et al. 2000, Reynolds et al. 2001, Deering and Young 2006, Abraham et al. 2009, Wainwright et al. 2012, Wainwright and Cleland 2013, Wolkovich et al. 2013, Thomson et al. 2017). Furthermore, such advantages that exotic species gain from earlier seasonal phenologies may be exacerbated by climate change, with several studies documenting greater advancement of seasonal phenology in exotic species relative to co-occurring natives (Wolkovich et al. 2013, Willis et al. 2015, Zettlemoyer et al. 2019). Therefore, the long-term persistence of native species will likely depend not only on their own capacity to shift seasonal phenologies, but also the relative capacity of exotics to shift seasonal phenologies.

Phenological traits are often highly plastic, in that their expression is dependent on environmental conditions, but the mechanisms that regulate them have a strong genetic basis (Mazer and LeBuhn 1999). Consequently, both phenotypic plasticity as well as genetic changes have contributed to observed phenological shifts in response to climate change (Franks et al. 2007, Anderson et al. 2012). However, with projected increases in average temperatures of 2° C (or greater) by 2100 accompanied by further alterations of precipitation regimes (IPCC 2013), it is widely expected that adaptive evolution will be necessary for longer-term species persistence (Jump and Peñuelas 2005). This importance of *in situ* adaptation is heightened because migration of plants to higher latitudes and elevations to track climate is hampered by habitat fragmentation (Jump and Peñuelas 2005).

The potential for the adaptive evolution of phenological traits to keep pace with climate change will depend on multiple factors. A lack of genetic variation in phenological traits under selection due to climate change will prevent adaptive evolution (Etterson 2004). Habitat loss and fragmentation can reduce genetic variation in traits and thus may limit the adaptive potential of plant populations (Aguilar et al. 2019). Even if substantial genetic variation exists within populations, phenological traits have been shown to be phylogenetically conserved (Norden et al. 2009, Davies et al. 2013, Xu et al. 2014) which may limit the range of the potential evolutionary changes that can occur. Furthermore, genetic correlations between phenological traits and others can impede adaptation to climate change if the direction of combined selection on both traits is not concordant with the direction of the correlation (Etterson and Shaw 2001). Plant phenology is also under strong selection from interactions with pollinators, herbivores, and competitors (Brody 1997, Pilson 2000, Weinig 2000, Sandring and Agren 2009, Sletvold et al. 2010), which may therefore shift traits away from the trait values favored by climate change alone.

Emergence timing as a key component of plant phenology

The transition from seed to seedling is the first major life history transition in plant development. As the first phenological trait expressed during development, emergence timing therefore determines the environmental conditions with which plants interact, a form of niche construction (Donohue et al. 2005). As a result, emergence timing strongly influences plant fitness as well as patterns of selection on traits expressed later in development (reviewed in Donohue et al. 2010).

In seasonal environments, the timing of emergence is highly adaptive when evaluated both within and among growing seasons (Donohue et al. 2010). Within a growing season, fitness may be maximized by emerging at a specific time dependent on various aspects of the biotic and abiotic environment. The general environment exerts “hard” selection that is independent of the phenotypes of competitors (Weis et al. 2015). For example, emerging early within a growing season can increase the risk of growing before the onset of reliably tolerable abiotic conditions, such as exposure to a prolonged dry period (Wainwright et al. 2012) or a late frost (Skálová et al. 2011). In contrast, emerging late may leave insufficient time for growth and reproduction before favorable conditions deteriorate (Dickman et al. 2019). Emergence timing within a growing season is also under “soft selection” that is exerted by the social environment and thus depends on the phenotypes of competitors (Weis et al. 2015). Emerging earlier than competitors provides longer windows for growth and reproduction before favorable conditions deteriorate, as well the potential to pre-empt resources and suppress the growth of individuals that emerge later (i.e. priority effects) (Vannette and Fukami 2014). Accordingly, meta-analysis shows that earlier emergence consistently enhances fitness, largely driven by greater fecundity (Verdú and Traveset 2005).

The spread of offspring emergence across growing seasons can also strongly influences parental fitness (Clauss and Venable 2000). In the case that the environmental conditions within a growing season become unfavorable to growing plants, preventing germination in a fraction of offspring seeds can allow them the opportunity to potentially emerge in more favorable conditions. This strategy, known as bet-hedging, is highly adaptive in unpredictably variable environments where the risk of reproductive failure is high for actively growing plants (Tielbörger et al. 2012, Gremer and Venable 2014, Gremer et al. 2016). Bet-hedging allows plants to maximize long-term fitness (the geometric mean of fitness contributions over time) by reducing temporal variance in reproductive success. Such interannual fluctuations in reproductive success can result from both density-independent and density-dependent effects (Gremer et al. 2016).

The selective forces acting on emergence timing cause plants to germinate in response to specific environmental cues that indicate the arrival of favorable conditions (Bewley et al. 2013). Germination is the process by which dry seeds take up water and part of the embryo (typically the radicle) penetrates surrounding structures (Bewley et al. 2013). Besides water, seeds require sufficient temperature and oxygen (Bewley et al. 2013, Baskin and Baskin 2014). The seeds of many species also require certain quantities or qualities of light to initiate germination (Bewley et al. 2013, Baskin and Baskin 2014). The importance of each cue for triggering germination often depends on factors that are most limiting to growth (Baskin and Baskin 2014). For example, in Mediterranean-type climates where moisture is the major limiting factor on growth, germination occurs mostly in the autumn and winter when the majority of annual precipitation occurs (Bartolome 1979). Seeds can also accelerate germination in the presence of other seeds or seedlings, which is likely an adaptive plastic response to competitive environments (Dyer et al. 2000, Tielbörger and Prasse 2009).

Seed dormancy prevents viable seeds from germinating despite the presence of favorable environmental conditions (Bewley et al. 2013). This can prevent seeds from germinating if favorable conditions are unlikely to persist (Koornneef et al. 2002). For example, in Mediterranean-type climates, germination in response to a summer rainstorm can be maladaptive, as there is a low probability that seedlings will survive until the arrival of the winter growing season where precipitation is sufficient for growth and reproduction (Wainwright et al.

2012); therefore, dormancy mechanisms that block germination until the winter can allow individuals to germinate at favorable more favorable times. Dormancy can also allow the maintenance of a persistent seed-bank as part of bet-hedging strategies that insure against reproductive failure (Clauss and Venable 2000). Finally, dormancy allows more time for seeds to be dispersed, allowing germination events of offspring seeds to have greater spatial and temporal distributions (Bewley et al. 2013).

Emergence timing within and among growing seasons controls the climatic conditions that plants experience following dispersal; therefore, this trait is expected to play a key role in phenological climate tracking (Walck et al. 2011). While plasticity in emergence timing may promote short-term persistence, adaptive evolution is predicted to be necessary for persistence over longer timescales (Walck et al. 2011). However, in comparison to other phenological traits such as flowering time, which are the focus of many studies assessing evolutionary responses to climate change (e.g. Franks et al. 2007, Anderson et al. 2012), relatively few studies have tested for evolutionary change in emergence timing response to recent climate change. Dickman et al. (2019) observed the evolution of earlier emergence in response to drought in the annual *Mimulus laciniatus*, a shift consistent with adaptation to shortened windows of favorable conditions. Further support for the need to adaptively evolve emergence timing in response to climate change is provided by numerous studies showing clinal patterns of emergence timing across climatic gradients (reviewed in Cochrane et al. 2015).

Earlier emergence relative to co-occurring natives has been implicated as a key factor contributing to the dominance of herbaceous exotic invaders (Pérez-Fernández et al. 2000, Reynolds et al. 2001, Deering and Young 2006, Abraham et al. 2009, Wainwright et al. 2012, Wainwright and Cleland 2013, Gioria and Pyšek 2016, Thomson et al. 2017). This strategy of earlier emergence allows exotic species to pre-empt resources and suppress later-emerging natives through priority effects (Wainwright et al. 2012, Thomson et al. 2017). More flexible emergence cues may also allow exotics to shift emergence earlier than natives in response to climate change, and thus potentially increase their competitive advantage over natives (Pérez-Fernández et al. 2000, Wainwright and Cleland 2013). This highlights the need to develop our understanding of factors that shape the community-level impacts of variation in emergence timing.

Dissertation overview

A major goal of my dissertation is to further our understanding of factors shaping the evolution of emergence timing in seasonal systems, and thus improve our understanding of the potential for adaptive evolution in this trait to keep pace with climate change. An additional goal is to further our understanding of factors that shape the community-level impacts of variation in emergence time among species. Chapters 1, 2 and 3 are focused on understanding factors that shape the evolution of emergence timing in plant populations. In Chapter 1, I investigate how a genetic trade-off between within- and among-year emergence timing can constrain adaptative evolution in response to joint selection on both traits. In Chapter 2, I investigate how the composition of the surrounding community influences selection on emergence time and the underlying mechanisms. In Chapter 3, I investigate whether interactions with mammalian herbivores weaken directional selection for earlier emergence. In Chapter 4, I investigate whether earlier-emerging species are more susceptible to mammalian herbivory early in the growing season, and whether this increased susceptibility can increase the relative performance of later-emerging native species.

Study system

I used as a study system two widespread California grasses, the native perennial *Stipa pulchra* (Hitchc.) Barkworth and the exotic *Bromus diandrus* (Roth), as well as a suite of other California native and exotic herbaceous species.

The California Floristic Province is a global plant biodiversity hotspot, with exceptionally high species diversity and endemism (Burge et al. 2016). Since European settlement in the 18th century, California has experienced widespread invasion by exotic plant species, particularly exotic annual grasses from Eurasia (Heady 1977). The successful establishment and spread of exotic annual grasses was in large part a result of chronic cattle grazing (HilleRisLambers et al. 2010). Exotic species tend to emerge earlier than native species in response to seasonal cues (Reynolds et al. 2001, Deering and Young 2006, Abraham et al. 2009, Wainwright et al. 2012, Wainwright and Cleland 2013), and differ in a suite of other functional traits (Funk et al. 2016)

Much of California has a Mediterranean-type climate, with mild, wet winters and warm dry summers, and is also characterized by high levels of geographic variation in climate. Southern regions tend to be warmer and drier, but with greater interannual variability in precipitation, than northern regions (Pratt and Mooney 2013). Anthropogenic climate change is altering climate patterns across the state. Predictions for climatic shifts in California by the end of the century differ between emission scenarios and climate models. Temperatures are expected to increase, with estimates of changes in mean annual temperatures ranging from 1.5C to 5.3C (Hayhoe et al. 2004, Cayan et al. 2008). These will be accompanied by more frequent and severe heatwaves (Hayhoe et al. 2004). Predicted changes in precipitation regimes have been less consistent, with various models predicting small changes (~10%) in mean precipitation occurring in different directions (Cayan et al. 2008, Berg and Hall 2015); however, interannual variability in precipitation is expected to increase with extremely wet and dry winters becoming more frequent (Berg and Hall 2015), and large storm events may also become more frequent (Dettinger 2011). Increases in interannual variability in precipitation in coastal California have been observed in recent decades, with northern regions experiencing the greatest increases (Pratt and Mooney 2013).

Stipa pulchra (purple needlegrass) is a native perennial bunchgrass found in woodland, chaparral, and grassland from Baja California to northern California (Baldwin et al. 2012). Analyses of neutral genetic markers shows that *S. pulchra* harbors relatively low genetic variation within populations but high genetic differentiation among populations, likely due to high rates of self-fertilization (reported selfing rates ≈ 1) (Larson et al. 2001). Consistent with this, quantitative traits in *S. pulchra* show evidence of ecotypic differentiation among populations (Knapp and Rice 1998). *Bromus diandrus* (great brome or ripgut brome) is an exotic annual grass species native to Eurasia. This species is found in many habitats across California, and is particularly dominant in disturbed areas, such as abandoned agricultural fields (Stromberg and Griffin 1996). *B. diandrus* is also largely self-fertilizing (reported selfing rates > 0.99) (Kon and Blacklow 1990).

Chapter summaries

A major goal of my dissertation research, addressed in Chapters 1, 2 and 3, is to further our understanding of factors that shape the evolution of emergence timing in seasonal systems. An additional goal, addressed in Chapter 4, is to further our understanding of factors shaping the community-level impacts of variation in emergence time among species.

In Chapter 1, I investigate the potential for a genetic trade-off between earlier emergence within years and greater spread of emergence among years to constrain adaptive evolution in response to joint selection for both. I collected seeds of *S. pulchra* and *B. diandrus* from multiple maternal lines in source populations across an aridity gradient in the Mediterranean climate region of California. I used a common garden approach, whereby I subjected seeds of each maternal line to approximately 5 months of high or low watering in the greenhouse. I monitored emergence and assayed nongerminated seeds for viability to determine the fraction of seed persistence, a metric of potential among-year emergence. In both focal species, maternal lines with higher fractions of persistent seeds had later mean emergence, consistent with a genetic trade-off between the traits that can constrain adaptive evolution in each. Source population aridity was not a strong predictor of emergence traits when each was considered individually. However, when considered jointly, in both species the source populations that were Pareto optimal for early emergence and high seed persistence fractions occupied significantly more arid sites than non-Pareto optimal populations. This is consistent with constrained evolution in response to joint selection for earlier emergence and higher seed persistence fractions in more arid sites. In *S. pulchra*, seed persistence increased in response to low watering in the greenhouse, suggesting that plasticity could mitigate the costs of the genetic trade-off in more arid environments. In contrast, seed persistence of *B. diandrus* decreased in response to low watering in the greenhouse, which may be a detrimental plastic response in arid environments.

In Chapter 2, I investigate how variation in the surrounding plant community influences phenotypic selection on emergence time. I carried out a field experiment in which I evaluated phenotypic selection on emergence time in *S. pulchra* and *B. diandrus* when growing in competition with 6 other native and exotic grasses in individual species and mixed species treatments. I found that community composition significantly altered the strength but not the direction of selection on emergence time in both focal species, and that the strength of selection exerted by each competitive neighborhood was not concordant in both focal species. Stronger selection for earlier emergence in *S. pulchra* was weakly associated with later-emergence of neighbors and greater light interception in the surrounding community, but variation in the strength of selection in *B. diandrus* was not explained by any measures of community context. Selection for earlier emergence was stronger in the exotic *B. diandrus* when growing with exotic neighbors, but neighbor origin did not significantly influence selection for earlier emergence in the native *S. pulchra*. In both focal species, there was no significant effect of neighbor diversity on the strength of selection for earlier emergence. These results suggest that community composition is an important force shaping selection on emergence timing, and thus changes in community composition resulting from species invasions and climate change are likely to alter patterns of selection on this trait.

In Chapter 3, I investigate whether mammalian herbivores weaken directional selection for earlier emergence. I carried out a field experiment in which I evaluated phenotypic selection in *S. pulchra* and *B. diandrus* growing in close competition with and without mammalian herbivore exclusion. I found that mammalian herbivory weakened the strength of selection for earlier emergence in both focal species independently of reductions in mean fitness. Thus, herbivores can contribute to the maintenance of variation in emergence timing despite the consistent competitive advantage of earlier emergence. However, mammalian herbivory could slow adaptive evolutionary responses to drivers of global change that favor earlier seasonal phenologies.

In Chapter 4, I investigate whether species that emerge earlier in the growing season, and thus benefit from seasonal priority effects, are more susceptible to early-season mammalian herbivory. Furthermore, I investigate whether early-season mammalian herbivory can increase the relative performance of later-emerging natives when growing in competition with earlier-emerging exotic species. I carried out a field experiment in which I monitored the emergence and growth of 12 focal herbaceous species (six each native and exotic) in monoculture and polyculture, whilst experimentally excluding mammalian herbivores both early and later in the growing season. I found that earlier emerging species achieved greater biomass by the end of the experiment, but were more negatively impacted by herbivory, particularly in the early part of the growing season. This greater impact of early-season herbivory on early-active species lead to a reduction in the competitive advantage of exotic species growing in polyculture and improved the performance of later-emerging natives. These results show that early-season mammalian herbivory is likely to play an important role in maintaining the performance of late-emerging species in the face of consistent competitive advantages gained by species that emerge earlier.

References

Abraham, J. K., J. D. Corbin, and C. M. D'Antonio. 2009. California native and exotic perennial grasses differ in their response to soil nitrogen, exotic annual grass density, and order of emergence. Plant Ecology **201**:445-456.

Aguilar, R., E. J. Cristobal-Perez, F. J. Balvino-Olvera, M. de Jesus Aguilar-Aguilar, N. Aguirre-Acosta, L. Ashworth, J. A. Lobo, S. Marten-Rodriguez, E. J. Fuchs, G. Sanchez-Montoya, G. Bernardello, and M. Quesada. 2019. Habitat fragmentation reduces plant progeny quality: a global synthesis. Ecol Lett **22**:1163-1173.

Anderson, J. T., D. W. Inouye, A. M. McKinney, R. I. Colautti, and T. Mitchell-Olds. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proc Biol Sci **279**:3843-3852.

Baldwin, B. G., D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken, editors. 2012. The Jepson manual: vascular plants of California. 2nd edition. University of California Press, Berkeley.

Bartolome, J. W. 1979. Germination and seedling establishment in California annual grassland. Journal of Ecology **67**:273-281.

Baskin, C. C., and J. M. Baskin. 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego.

Berg, N., and A. Hall. 2015. Increased interannual precipitation extremes over California under climate change. Journal of Climate **28**:6324–6334.

Bewley, J. D., K. J. Bradford, H. H. W. M., and H. Nonogaki. 2013. Seeds: Physiology of Development, Germination and Dormancy. 3rd edition. Springer.

Brody, A. K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. Ecology **78**:1624-1631.

Burge, D. O., J. H. Thorne, S. P. Harrison, B. C. O'Brien, J. P. Rebman, J. R. Shevock, E. R. Alverson, L. K. Hardison, J. D. RodrÍguez, S. A. Junak, T. A. Oberbauer, H. Riemann, S. E. Vanderplank, and T. Barry. 2016. Plant Diversity and Endemism in the California Floristic Province. Madroño **63**:3-206.

CaraDonna, P. J., A. M. Iler, and D. W. Inouye. 2014. Shifts in flowering phenology reshape a subalpine plant community. Proceedings of the National Academy of Sciences **111**:4916–4921.

Cayan, D. R., E. P. Maurer, M. D. Dettinger, M. Tyree, and K. Hayhoe. 2008. Climate change scenarios for the California region. Climatic Change **87**:21-42.

Clauss, M. J., and D. L. Venable. 2000. Seed germination in desert annuals: an empirical test of adaptive bet hedging.

Cleland, E. E. 2012. Phenological tracking enables positive species responses to climate change.

Cochrane, A., C. J. Yates, G. L. Hoyle, and A. B. Nicotra. 2015. Will among-population variation in seed traits improve the chance of species persistence under climate change? Global Ecology and Biogeography **24**:12-24.

Davies, T. J., E. M. Wolkovich, N. J. Kraft, N. Salamin, J. M. Allen, T. R. Ault, J. L. Betancourt, K. Bolmgren, E. E. Cleland, B. I. Cook, T. M. Crimmins, S. J. Mazer, G. J. McCabe, S. Pau, J. Regetz, M. D. Schwartz, and S. E. Travers. 2013. Phylogenetic conservatism in plant phenology. Journal of Ecology **101**:1520-1530.

Deering, R. H., and T. P. Young. 2006. Germination speeds of exotic annual and native perennial grasses in California and the potential benefits of seed priming for grassland restoration. Grasslands **16**:14-16.

Dettinger, M. 2011. Climate change, atmospheric rivers, and floods in California - a multimodel analysis of storm frequency and magnitude changes. Journal of the American Water Resources Association **47**:514-523.

Dickman, E. E., L. K. Pennington, S. J. Franks, and J. P. Sexton. 2019. Evidence for adaptive responses to historic drought across a native plant species range. Evolutionary Applications.

Donohue, K., L. Dorn, C. Griffith, E. Kim, A. Aguilera, C. R. Polisetty, and J. Schmitt. 2005. Niche construction through germination cueing: life-history responses to timing of germination in Arabidopsis thaliana. Evolution **59**:771-785.

Donohue, K., R. Rubio de Casas, L. Burghardt, K. Kovach, and C. G. Willis. 2010. Germination, postgermination adaptation, and species ecological ranges. Annual Review of Ecology, Evolution, and Systematics **41**:293–319.

Dyer, A. R., A. Fenech, and K. J. Rice. 2000. Accelerated emergence in interspecific competitive neighbourhoods. Ecology Letters **3**:523-529.

Etterson, J. R. 2004. Evolutionary potential of Chamaecrista fasciculata in relation to climate change. II. Genetic architecture of three populations reciprocally planted along an environmental gradient in the Great Plains. Evolution **58**:1459-1471.

Etterson, J. R., and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. Science **294**:151-154.

Forrest, J., and A. J. Miller-Rushing. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. Philos Trans R Soc Lond B Biol Sci **365**:3101-3112.

Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences USA **104**:1278-1282.

Fridley, J. D. 2012. Extended leaf phenology and the autumn niche in deciduous forest invasions. Nature **485**:359-362.

Funk, J. L., R. J. Standish, W. D. Stock, and F. Valladares. 2016. Plant functional traits of dominant native and invasive species in mediterranean- climate ecosystems. Ecology **97**:75-83.

Gioria, M., and P. Pyšek. 2016. Early bird catches the worm: germination as a critical step in plant invasion. Biological Invasions **19**:1055-1080.

Gremer, J. R., S. Kimball, and D. L. Venable. 2016. Within-and among-year germination in Sonoran Desert winter annuals: bet hedging and predictive germination in a variable environment. Ecol Lett **19**:1209-1218.

Gremer, J. R., and D. L. Venable. 2014. Bet hedging in desert winter annual plants: optimal germination strategies in a variable environment. Ecology Letters **17**:380-387.

Hayhoe, K., D. Cayan, C. B. Field, P. C. Frumhoff, E. P. Maurer, N. L. Miller, S. C. Moser, S. H. Schneider, K. N. Cahill, E. E. Cleland, L. Dale, R. Drapek, R. M. Hanemann, L. S. Kalkstein, J. Lenihan, C. K. Lunch, R. P. Neilson, S. C. Sheridan, and J. H. Verville. 2004. Emissions pathways, climate change, and impacts on California. Proceedings of the National Academy of Sciences USA **101**:12422-12427.

Heady, H. F. 1977. Valley grassland. Pages 491–514 *in* M. G. Barbour and J. Major, editors. Terrestrial vegetation in California. Wiley, New York.

HilleRisLambers, J., S. G. Yelenik, B. P. Colman, and J. M. Levine. 2010. California annual grass invaders: the drivers or passengers of change? Journal of Ecology **98**:1147-1156.

Hu, J. I. A., D. J. P. Moore, S. P. Burns, and R. K. Monson. 2010. Longer growing seasons lead to less carbon sequestration by a subalpine forest. Global Change Biology **16**:771-783.

IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA.

Jump, A. S., and J. Peñuelas. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters **8**:1010-1020.

Knapp, E. E., and K. J. Rice. 1998. Comparison of isozymes and quantitative traits for evaluating patterns of genetic variation in purple needlegrass (Nassella pulchra). Conservation Biology **12**:1031–1041.

Kon, K. F., and W. M. Blacklow. 1990. Polymorphism, outcrossing and polyploidy in *Bromus diandrus* and *B. rigidus* Australian Journal of Botany **38**:609–618.

Koornneef, M., L. Bentsink, and H. Hilhorst. 2002. Seed dormancy and germination. Current Opinion in Plant Biology **5**:33-36.

Kudo, G., and T. Y. Ida. 2013. Early onset of spring increases the phenological mismatch between plants and pollinators. Ecology **94**:2311-2320.

Larson, S. R., E. Cartier, C. L. McCracken, and D. Dyer. 2001. Mode of reproduction and amplified fragment length polymorphism variation in purple needlegrass (Nassella pulchra): utilization of natural germplasm sources. Molecular Ecology **10**:1165-1177.

Mazer, S. J., and G. LeBuhn. 1999. Genetic Variation in Life-History Traits: Heritability Estimates within and Genetic Differentiation among Populations. Pages 85-171 *in* T. O. Vuorisalo and P. K. Mutikainen, editors. Life History Evolution in Plants. Kluwer

Academic, Dordrecht, The Netherlands.

Norden, N., M. I. Daws, C. Antoine, M. A. Gonzalez, N. C. Garwood, and J. Chave. 2009. The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. Functional Ecology **23**:203-210.

Parmesan, C., and G. Yohe. 2003. Aglobally coherent fingerprint of climate change impacts across natural systems. Nature **421**:37-42.

Pérez-Fernández, M. A., B. B. Lamont, A. L. Marwick, and W. G. Lamont. 2000. Germination of seven exotic weeds and seven native species in south-western Australia under steady and fluctuating water supply. Acta Oecologica **21**:323-336.

Pilson, D. 2000. Herbivory and natural selection on flowering phenology in wild sunflower, Helianthus annuus. Oecologia **122**:72-82.

Pratt, J. D., and K. A. Mooney. 2013. Clinal adaptation and adaptive plasticity in Artemisia californica: implications for the response of a foundation species to predicted climate change. Global Change Biology **19**:2454–2466.

Reynolds, S. A., J. D. Corbin, and C. M. D’Antonio. 2001. The effects of litter and temperature on the germination of native and exotic grasses in a coastal California grassland. Madroño **48**:230-235.

Sala, O. E., F. S. Chapin III, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Sandring, S., and J. Agren. 2009. Pollinator-mediated selection on floral display and flowering time in the perennial herb Arabidopsis lyrata. Evolution **63**:1292-1300.

Skálová, H., L. Moravcová, and P. Pyšek. 2011. Germination dynamics and seedling frost resistance of invasive and native Impatiens species reflect local climatic conditions. Perspectives in Plant Ecology, Evolution and Systematics **13**:173–180.

Sletvold, N., J. M. Grindeland, and J. Ågren. 2010. Pollinator-mediated selection on floral display, spur length and flowering phenology in the deceptive orchid Dactylorhiza lapponica. New Phytol **188**:385-392.

Stromberg, M. R., and J. R. Griffin. 1996. Long-term patterns in coastal California grasslands in relation to cultivation, gophers, and grazing. Ecological Applications **6**:1189–1211.

Thomson, D. M., R. A. King, and E. L. Schultz. 2017. Between invaders and a risky place: Exotic grasses alter demographic tradeoffs of native forb germination timing. Ecosphere **8**:e01987.

Tielbörger, K., M. Petrů, and C. Lampei. 2012. Bet-hedging germination in annual plants: a sound empirical test of the theoretical foundations. Oikos.

Tielbörger, K., and R. Prasse. 2009. Do seeds sense each other? Testing for density-dependent germination in desert perennial plants. Oikos **118**:792-800.

Vannette, R. L., and T. Fukami. 2014. Historical contingency in species interactions: towards niche-based predictions. Ecology Letters **17**:115-124.

Verdú, M., and A. Traveset. 2005. Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. Ecology **86**:1385–1394.

Wadgymar, S. M., J. E. Ogilvie, D. W. Inouye, A. E. Weis, and J. T. Anderson. 2018. Phenological responses to multiple environmental drivers under climate change: insights from a long-term observational study and a manipulative field experiment. New Phytol **218**:517-529.

Wainwright, C. E., and E. E. Cleland. 2013. Exotic species display greater germination plasticity and higher germination rates than native species across multiple cues. Biological Invasions **15**:2253-2264.

Wainwright, C. E., E. M. Wolkovich, and E. E. Cleland. 2012. Seasonal priority effects: implications for invasion and restoration in a semi-arid system. Journal of Applied Ecology **49**:234-241.

Walck, J. L., S. N. Hidayati, K. W. Dixon, K. Thompson, and P. Poschlod. 2011. Climate change and plant regeneration from seed. Global Change Biology **17**:2145–2161.

Weinig, C. 2000. Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. Evolution **54**:124-136.

Weis, A. E., K. M. Turner, B. Petro, E. J. Austen, and S. M. Wadgymar. 2015. Hard and soft selection on phenology through seasonal shifts in the general and social environments: A study on plant emergence time. Evolution **69**:1361-1374.

Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, J. B. Losos, and C. C. Davis. 2015. Favorable climate change response explains non-native species' success in Thoreau's Woods. PLoS One **5**:e8878.

Wolkovich, E. M., and E. E. Cleland. 2011. The phenology of plant invasions: a community ecology perspective. Frontiers in Ecology and the Environment **9**:287-294.

Wolkovich, E. M., T. J. Davies, H. Schaefer, E. E. Cleland, B. I. Cook, S. E. Travers, C. G. Willis, and C. C. Davis. 2013. Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. American Journal of Botany **100**:1407-1421.

Xu, J., W. Li, C. Zhang, W. Liu, and G. Du. 2014. Variation in seed germination of 134 common species on the Eastern Tibetan Plateau: phylogenetic, life history and environmental correlates. PLoS One **9**:e98601.

Zettlemoyer, M. A., E. H. Schultheis, and J. A. Lau. 2019. Phenology in a warming world: differences between native and non-native plant species. Ecol Lett **22**:1253-1263.

CHAPTER 1

Genetic trade-off drives Pareto optimality of within- and among-year emergence timing in response to increasing aridity

Abstract

Adaptation to current and future climates can be constrained by genetic trade-offs between traits that limit the combinations of trait values that can evolve. The timing of seedling emergence strongly influences plant fitness in seasonal environments, but adaptation may be constrained by a trade-off between within- and among-year emergence timing. Consequently, selection for earlier within-year emergence and greater among-year emergence spread — as is expected in arid environments — may result in combinations of trait values for which gains in either function can only be achieved with a loss in the other (i.e. Pareto optimal). We sourced seeds of two grasses, *Stipa pulchra* and *Bromus diandrus*, from multiple maternal lines within populations across an aridity gradient in California and subjected them to 5 months of watering in the greenhouse. We monitored emergence and assayed nongerminated seeds for viability to determine seed persistence, a metric of potential among-year emergence. In both species, maternal lines with higher fractions of persistent seeds had later mean emergence, indicating a genetic trade-off between the traits. Source population aridity was not a strong predictor of emergence traits when each was considered individually. However, when considered jointly, in both species the source populations that were Pareto optimal for early emergence and high seed persistence fractions occupied significantly more arid sites than non-Pareto optimal populations. Our results demonstrate how Pareto optimality can be applied to the joint responses of associated traits when evaluating adaptation to climate.

Introduction

Plant populations are often adapted to their local climate (e.g. Franks et al. 2007, Fournier-Level et al. 2011, Colautti and Barrett 2013, Wadgymar et al. 2017), and anthropogenic climate change is expected to further require species to adaptively evolve to persist in novel conditions (Jump and Peñuelas 2005, Hoffmann and Sgro 2011). However, adaptive evolution can be constrained by many factors, including genetic trade-offs between fitness-related traits that limit the combinations of trait values that can evolve (Etterson and Shaw 2001, Caruso et al. 2005). To further our understanding of how plant populations adapt to current and future climate, it is therefore important to characterize trade-offs between traits that can constrain adaptive responses.

The timing of seedling emergence determines the environmental conditions experienced by plants following dispersal, strongly influencing fitness as well as patterns of selection on traits expressed later in development (Donohue et al. 2010). As a result, emergence timing is a key trait influencing adaptation to local conditions as well as potential adaptation in response to anthropogenic climate change (Donohue et al. 2010, Walck et al. 2011, Cochrane et al. 2015). In seasonal ecosystems, the timing of emergence is a complex trait composed of two potentially independent traits affecting fitness: (1) within-year emergence timing; (2) among-year emergence spread (defined here as the fraction of viable seeds persisting among years). Earlier within-year emergence is associated with increased fitness, resulting from longer windows for growth and reproduction as well the potential to pre-empt resources and suppress the growth of late-arrivers (Verdú and Traveset 2005). However, a number of factors could favor later emergence in response to the onset of seasonal germination cues. For example, earlier within-year emergence may increase the risk of growing before the onset of reliably tolerable conditions, for example by exposing individuals to a prolonged dry period (Wainwright et al. 2012), or a late frost (Skálová et al. 2011). Additionally, earlier within-year emergence can increase susceptibility to mammalian herbivores, potentially through increased apparency (Waterton and Cleland 2016). Spreading emergence among years requires that: (1) not all seeds germinate in a given year, and (2) that some ungerminated seeds survive in the soil. In general, greater among-year emergence is favored in variable environments that result in higher variance in fitness among years (Gremer et al. 2016).

A genetic trade-off between earlier within-year emergence and greater among-year emergence may constrain the range of possible trait combinations that can evolve in plant populations. Dormancy prevents seeds from germinating in conditions that would otherwise be sufficient, thereby delaying emergence and increasing the number of ungerminated seeds (Baskin and Baskin 2014, Long et al. 2015). Additionally, dormancy may further promote among-year emergence by increasing the resistance of seeds to ageing in soils (reviewed in Long et al. 2015). Quantitative trait loci (QTLs) that influence primary dormancy have been shown to colocate with QTLs affecting both germination fraction and within-year emergence time under field conditions, with increased dormancy associated with lower germination fractions and later emergence (Huang et al. 2010). Nondormant seeds can also persist across years in the soil if germination cues (e.g. water, light, temperature) are not met (Long et al. 2015), and more stringent cue requirements generally result in lower overall germination fractions and later germination among seeds that do germinate (Bewley et al. 2013). Thus, we expect among-year emergence spread to be positively associated with mean within-year emergence time. While trade-offs are typically characterized as negative relationships between traits or functions, this expected positive relationship between emergence timing traits is interpretable as a trade-off because larger values of within-year emergence time reflect a decrease in potential growing time and competitive ability (i.e. a loss in function). Consistent with this potential constraint on adaptive evolution within species, it has been shown that, across different species occupying similar habitats, earlier emergence is associated with lower soil seed persistence (Saatkamp et al. 2011).

In scenarios where selection favors both earlier within-year emergence and greater among-year emergence, a trade-off between them will prevent plant populations from fully optimizing both. Adaptive responses will instead be bounded by a set of trait combinations for which a gain in one function (i.e. earlier within-year emergence or greater among-year emergence) can only be achieved with a loss in the other (Fig. 1.1). All such trait combinations can be described as “Pareto optimal”, a concept originating from the fields of economics and engineering that has only recently been applied to biological systems (Shoval et al. 2012). Thus, the hypothesized trade-off would result in the evolution of emergence timing trait values that appear maladaptive when considered separately but are in fact Pareto optimal when considered together (Fig. 1.1). However, plasticity in emergence timing traits in response to environmental conditions that select for earlier within-year emergence and greater among-year emergence could mitigate the costs of the constraint if plastic responses are consistent with the direction of selection (i.e. adaptive plasticity) (Simons 2014).

Aridity gradients in Mediterranean climate regions are ideal for investigating a potential trade-off between within- and among-year emergence. In such regions, water availability is the major control over seasonal plant growth and is a key factor shaping the evolution of emergence timing within and among years (e.g. Arroyo et al. 2006, Petrů and Tielbörger 2008, Torres-Martínez et al. 2017). Plant populations towards the drier ends of aridity gradients are expected to experience shorter windows of favorable conditions as well as greater interannual variability in conditions than populations occupying more mesic sites. As a result, more arid sites might select for earlier emergence within years (Sexton et al. 2011, Dickman et al. 2019), greater spread of emergence among years (Venable and Brown 1988, Arroyo et al. 2006, Petrů and Tielbörger 2008), or both simultaneously. Additionally, adaptive plasticity in emergence timing in response to soil moisture could promote population persistence. For example, lower soil moisture conditions may decrease the proportion of seeds that emerge (Bewley et al. 2013) and increase the survival of non-emerging seeds (Mordecai 2012, Long et al. 2015), contributing to greater among-year emergence in drier, potentially less favorable conditions. However, lower soil moisture might also cause later within-year emergence (Bewley et al. 2013) which may be maladaptive in arid environments. Studying how traits vary along aridity gradients is particularly important because it provides insights into adaptive responses to climatic conditions which are consistent with the direction of climate change (Pratt and Mooney 2013). Mediterranean ecosystems around the world are projected to become increasingly arid, with many areas expected to experience warmer and drier average conditions as well as increased interannual variability in precipitation (Seager et al. 2007, Alpert et al. 2008, IPCC 2013, Berg and Hall 2015, Yoon et al. 2015).

We investigated the potential for a genetic trade-off between within- and among-year emergence to constrain adaptive responses to aridity in two widespread California grasses, the native perennial *Stipa pulchra* (Hitchc.) Barkworth and the exotic annual *Bromus diandrus* (Roth). We also investigated whether adaptive plasticity in response to drier conditions could mitigate the costs of such a constraint. We tested the following hypotheses: (1) across maternal lines, both within and among populations, greater potential for among-year emergence is associated with later emergence; (2) increasing aridity of source populations is associated with either earlier emergence or greater potential among-year emergence (but not both) and/or Pareto optimality for combinations of earlier emergence or greater potential among-year emergence; (3) low watering results in later emergence and greater potential for among-year emergence.

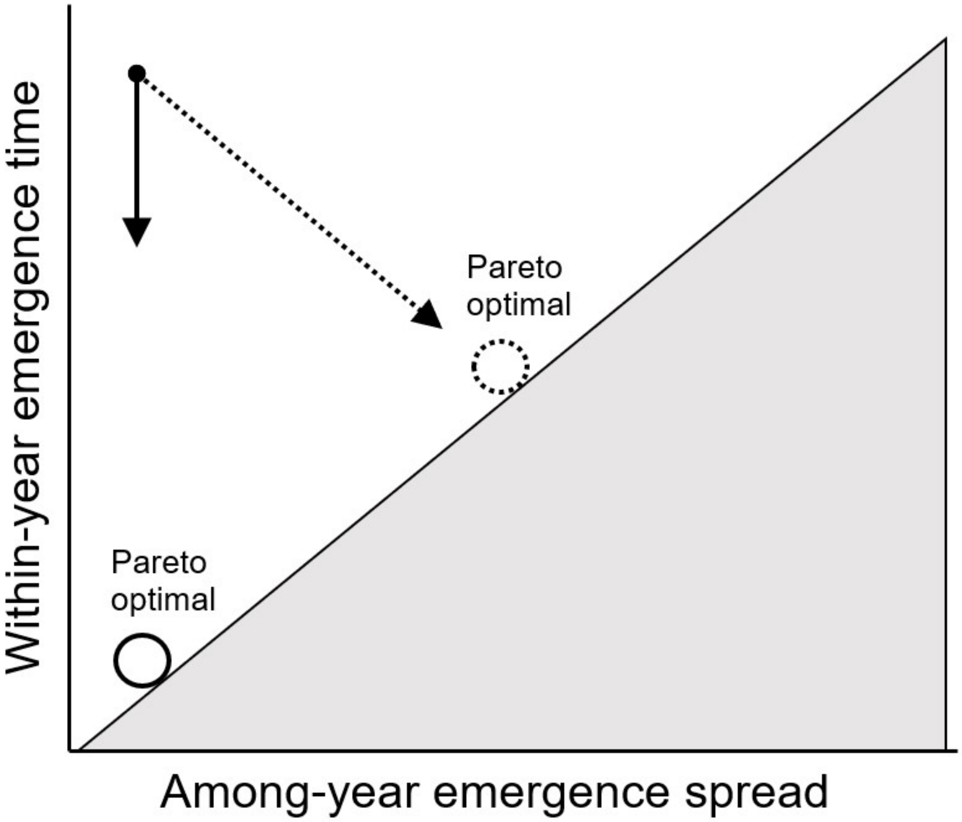

Figure 1.1: Hypothesized constraint to the evolution of both earlier within-year emergence and greater among-year emergence spread resulting from a trade-off between the two traits. Solid and dashed arrows are vectors representing the relative strength of selection for earlier within-year emergence and greater among-year emergence in two different environments, and circles represent the corresponding trait values that evolve. Non-feasible trait combinations resulting from the trade-off between the two traits are represented by the gray shaded area. Adaptive responses are constrained to trait combinations at which neither function can be improved simultaneously (i.e. Pareto optimal). In this example, both environments result in the evolution of Pareto optimal combinations, but the environment with stronger selection for earlier within-year emergence (dashed arrow) results in the evolution of later emergence.

Materials and methods

Study system

Coastal California is characterized by a steep gradient in aridity that is consistent with projections of future climate change in the region, with southern regions tending to be warmer and drier, but with greater interannual variability in precipitation, than northern regions (Pratt and Mooney 2013). Since European settlement in the 18th century, exotic annual grasses have become particularly dominant in California, displacing much of the native flora (Heady 1977). The two widespread grasses used in this study, the native perennial *S. pulchra* and the exotic annual *B. diandrus*, are therefore representative of two key functional groups in California grasslands that differ with respect to origin and life history strategy.

Stipa pulchra (purple needlegrass) is a native perennial bunchgrass found in woodland, chaparral, and grassland from Baja California to northern California (Baldwin et al. 2012). A study of neutral genetic markers shows that *S. pulchra* harbors relatively low genetic variation within populations but high genetic differentiation among populations, likely due to high rates of self-fertilization (reported selfing rates ≈ 1) (Larson et al. 2001). Consistent with this, quantitative traits in *S. pulchra* show evidence of ecotypic differentiation among populations (Knapp and Rice 1998), although no studies have assessed both within-year emergence time and among-year emergence.

Bromus diandrus (great brome or ripgut brome) is an exotic annual grass species native to Eurasia. This species is found in many habitats across California, and is particularly dominant in disturbed areas, such as abandoned agricultural fields (Stromberg and Griffin 1996). *B. diandrus* is also largely self-fertilizing (reported selfing rates > 0.99) (Kon and Blacklow 1990).

Source populations and field sampling

In April 2015, we collected seeds of *S. pulchra* from 13 populations and *B. diandrus* from 8 populations (Fig. 1.2). At each site, we collected seeds from 20 plants (hereafter referred to as maternal lines) situated in open flat areas and spaced at least 5m apart. We stored seeds at 4 °C until planting.

For each source population, we calculated a historical aridity index (historical AI) as the ratio of mean annual precipitation to mean annual potential evapotranspiration (P/PET) for the years 1985 – 2014 (values closer to zero indicate greater aridity). We then calculated, for each population, the deviation in aridity between the year of seed collection and the long-term mean (referred to here as the deviation AI), which we estimated as the difference between P/PET for May 2014 – April 2015 and the historical AI. Negative values of the deviation AI indicate that the year of collection was drier than the historical average while positive values indicate a wetter than average collection year. We retrieved temperature and precipitation data for calculating AIs from the PRISM Climate Group database (prism.oregonstate.edu/). We estimated potential evapotranspiration using temperature and latitude data with the Thornthwaite equation (Thornthwaite 1948), in the R package *SPEI* (Beguería and Vicente-Serrano 2017). Climate data for each source population are provided in Appendix Table 1A.1.

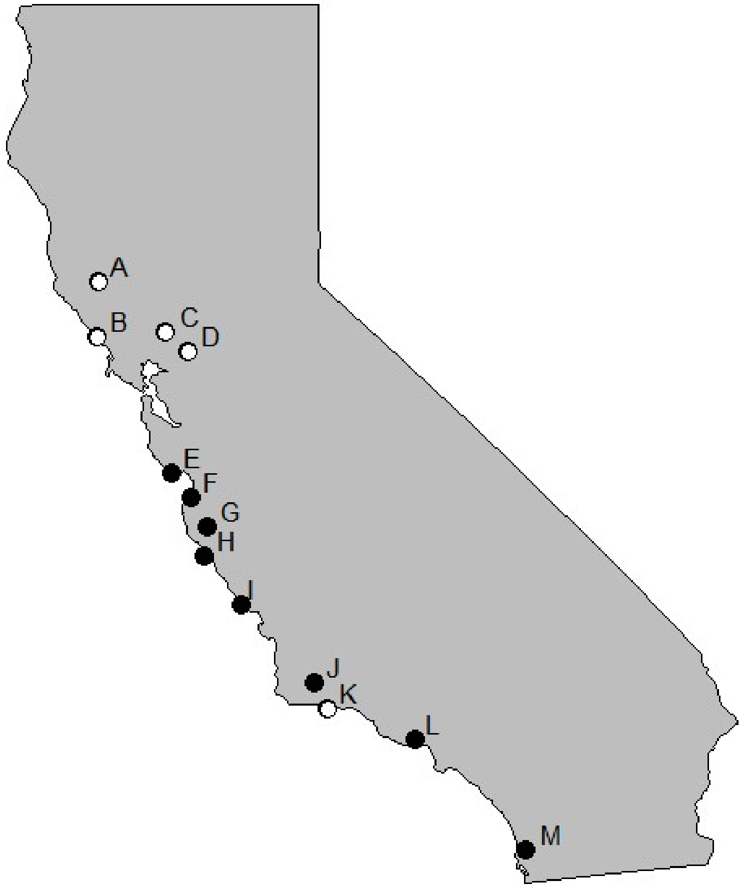

Figure 1.2: *S. pulchra* and *B. diandrus* source populations. *S. pulchra* was collected from all thirteen sites. Filled circles represent sites where *S. pulchra* and *B. diandrus* were collected. A) Hopland Research and Extension Center; B) Bodega Marine Reserve; C) Quail Ridge Reserve; D) Jepson Prairie Reserve; E) Younger Lagoon Reserve; F) Fort Ord Natural Reserve; G) Hastings Natural History Reservation; H) Landels-Hill Big Creek Reserve; I) Kenneth S. Norris Rancho Marino Reserve; J) Sedgwick Ranch Reserve; K) Coal Oil Point Natural Reserve; L) Stunt Ranch Santa Monica Mountains Reserve; M) Elliott Chaparral Reserve.

Greenhouse experiment

The experiment was conducted at the University of California, San Diego Biology Field Station greenhouses (32.885° N, 117.230° W). For every maternal line in each source population, we randomly chose six *S. pulchra* seeds and five *B. diandrus* seeds to plant in two watering treatments, "high" and "low", for a total of 1600 *B. diandrus* seeds (8 populations × 20 maternal lines × 2 watering treatments × 5 seeds per treatment) and 3120 *S. pulchra* seeds (13 populations × 20 maternal lines × 2 watering treatments × 6 seeds per treatment). We weighed seeds with awns attached, avoiding any that appeared empty or nonviable.

We planted individual seeds to a depth of 1cm into RLC4 "cone-tainers" (Stuewe & Sons, Inc., Tangent OR) filled with dry 70/30 Topsoil (Agriservice, Inc., Oceanside, CA), a mix of 70% sandy loam soil with 30% humic compost (pH ≈ 7.5). For each species, we arranged cone-tainers so that each rack contained one seed from every maternal line, with 6 racks per watering treatment for *S. pulchra* and 5 racks per watering treatment for *B. diandrus*. All water was delivered by overhead irrigation. We planted seeds into dry soil to allow all seeds the opportunity to initiate germination simultaneously when water was eventually applied. We first planted *S. pulchra* seeds over several days until 1 March when watering began (Day 0 for *S. pulchra*). We later planted *B. diandrus* seeds over several days until 17 March when watering began (Day 0 for *B. diandrus*). We watered seeds of both species until soil saturation on their respective Days 0 and 2 to simulate large early season rain events, and subsequently imposed the separate watering treatments on Day 4. The high watering treatment received 3 times as much water as the low treatment, which approximately represents the difference in mean annual precipitation between the wettest and driest source populations (Appendix Table 1A.1). Plants in the low watering treatment initially received 10mm of water every four days. Plants in the high watering treatment received the same 10mm pulse every four days plus an additional 20mm delivered two days after each 10mm pulse. For both species, we doubled the amount of water supplied in each pulse for both treatments beginning 22 April to compensate for warm greenhouse conditions. We rotated cone-tainer racks every 4 days to account for potential spatial variation in greenhouse conditions. All plants received ambient light throughout the experiment. Temperature data inside the greenhouse were unavailable during the experiment; however, mean temperature for this period at the experiment site extracted from the PRISM database was 17.7 °C. Subsequent measurements for a comparable period in 2019 showed that temperatures inside the greenhouse are on average 1°C warmer than outside (personal observation), and thus plants experienced temperatures closer to the warmest source populations (Appendix Table 1A.1).

We monitored cone-tainers daily and recorded the date of emergence for each individual. Total emergence of *B. diandrus* was low until Day 10 (< 4% of seeds planted), likely due to drying soils. Therefore, beginning on Day 10, we watered *B. diandrus* cone-tainers until soil saturation daily for 4 days before restarting the separate watering treatments. We retrieved non-emerged seeds from the soil over several days beginning Days 141 and 139 for *B. diandrus* and *S. pulchra*, respectively. To facilitate the retrieval of seeds, we watered daily during this collection period to soften soils; therefore, seedlings emerging during this period were considered as persistent in analyses. We rinsed intact seeds with ethanol to surface sterilize them, allowed them to air dry and stored them in coin envelopes at 4 °C until they were scored for viability in July 2017 using a tetrazolium assay (AOSA/SCST 2010).

Statistical analyses

We conducted all statistical analyses using R version 3.5.2 (R Core Team 2018). Due to the low total emergence of *B. diandrus* until Day 10 (< 4%), we calculated days to emergence in this species from the start of the consecutive four-day watering pulse that began on Day 10. In the initial cohort with low emergence, we observed the earliest emerging individuals after four days; therefore, we assigned individuals emerging before Day 14 this same earliest emergence time of four days. In both species, emergence time was right-skewed and therefore square-root transformed to improve normality of residuals (Simons and Johnston 2006).

To minimize potential bias introduced by maternal lines and source populations with low levels of seed viability, we excluded maternal lines in which, across both watering treatments, fewer than 50% of planted seeds either emerged or remained persistent, and we excluded source populations with fewer than 10 maternal lines meeting this "viability" threshold. No populations of *B. diandrus* were excluded, but 10 maternal lines were excluded in total, leaving 150 maternal lines in the analyses reported here. For *S. pulchra*, the following 5 source populations were excluded entirely: Fort Ord (8/20 "viable" maternal lines), Hastings (5/20 "viable" maternal lines), Hopland (6/20 "viable" maternal lines), Jepson (3/20 "viable" maternal lines), and Younger Lagoon (4/20 "viable" maternal lines). A total of 17 maternal lines were excluded from across the remaining 8 populations of *S. pulchra*, leaving 143 in the analyses reported here. We suspect the low viability from the excluded populations was due to collection earlier than optimal for seed ripening in this species.

We first fit generalized linear mixed models (GLMMs) and linear mixed models (LMMs) to test for the overall effects of watering treatment, population, and seed mass on the fates of individual seeds, with maternal line included as a random effect in all models. We fit LMMs to test for the effects of each factor on emergence time (“emergence time”). We fit GLMMs, using binomial error distributions and logit link functions to test for the effects of factors on the probability of seed persistence (“persistence”). Because persistence is dependent on both germination and mortality in non-emerging seeds, we also fit separate GLMMs to test the effect of each factor on the probability of emerging (“emergence probability”) and the probability of mortality in non-emerging seeds (“non-emerging mortality”). Significance of watering effects indicates plasticity in emergence timing traits. We included seed mass as a covariate to account for potential effects of maternal provisioning. We tested for differences in plastic responses among populations by evaluating the significance of watering treatment × population interactions. However, GLMMs containing watering treatment × population interactions failed to converge; therefore, we tested these interactions in separate GLMs that excluded maternal line as a random effect. All population × watering treatment interactions in LMMs and GLMs were non-significant (Appendix Table 1A.2), thus we evaluated the significance of main effects in GLMMs and LMMs without interaction terms.

To test for a trade-off between emergence time and seed persistence fractions in each species, we fit LMMs in which, across both watering treatments, mean emergence time in maternal lines was predicted by the fraction of viable persistent seeds, with source population treated as a random effect. We calculated mean emergence time and seed persistence fraction across watering treatments to maximize sample sizes within maternal lines. Where the fraction of persistent seeds in maternal lines was a significant predictor of mean emergence time, we tested whether this relationship occurred within populations or whether it was driven by covariation in population means. For this, we calculated for each maternal line the deviations in mean emergence time and seed persistence fraction from their respective source population means by subtracting mean source population values from maternal line values. We then fit the same LMM as before, replacing the raw maternal line values with the deviations from their source population means. A significant positive relationship between deviation values indicates that greater fractions of persistent seeds predict longer mean time to emergence independently of differences in mean values among source populations. Additionally, where the fraction of persistent seeds in maternal lines was a significant predictor of mean emergence time, we tested whether seed mass was mediating the relationship by replacing raw emergence time values in the original LMM with the residuals from a regression of mean emergence time against mean seed mass in maternal lines. One limitation of these analyses is that maternal lines in which 100% of living seeds remained persistent in soil cannot be included as they have no associated emergence time; however, this occurred in only two maternal lines of *B. diandrus*. We specified LMMs using the R package *nlme* (Pinheiro et al. 2018) and GLMMs using the R package *glmmADMB* (Fournier et al. 2012). We evaluated the significance of fixed effects with Type II tests using the *car* package (Fox and Weisberg 2011).

Where population was a significant source of variation in emergence time and the probability of seed persistence, we tested for associations between emergence timing traits and each AI (historical and deviation) for each source population. Significant effects of historical AI are consistent with adaptive evolution to aridity, while significant effects of deviation AI indicate that the aridity experienced by maternal plants in the year of collection (relative to long-term norms) influences seed behavior. We used linear regressions to test the relationships between AIs and the population means for each trait individually, averaged across both watering treatments. We determined the Pareto optimal populations for combinations of early emergence and high seed persistence fractions (i.e. the set of populations for which no other single population possesses both earlier emergence and greater seed persistence fraction) and tested whether aridity was significantly different in Pareto optimal vs non-Pareto optimal populations. We emphasize that populations are described as Pareto optimal in the context of our sampled source populations and may not represent the globally Pareto optimal populations. We determined the Pareto optimality of combinations of mean population trait values using the *psel* function in the R package *rPref* (Roocks 2016), with the preference object set to simultaneously optimize for low emergence time and high seed persistence fractions with both traits weighted equally. We tested whether Pareto optimal populations were more historically arid than non-Pareto optimal populations using one-tailed permutation tests with 10,000 repeats. We had no *a priori* hypothesis of how deviation AI would influence emergence traits so tested whether this was significantly different in Pareto optimal populations vs. non-Pareto optimal populations using two-tailed permutation tests with 10,000 repeats.

Results

Sources of variation in emergence time and potential among-year emergence

In both species, time to emergence differed significantly between populations (*S. pulchra*: $\chi^2(7) = 17.0$, $P = 0.02$; *B. diandrus*: $\chi^2(7) = 33.5$, $P < 0.001$; Table 1.1) and tended to decrease with seed mass (*S. pulchra*: $\chi^2(1) = 37.7$, $P < 0.001$; *B. diandrus*: $\chi^2(1) = 14.5$, $P < 0.001$; Table 1.1). Watering treatment did not have a significant effect on emergence time in either species (*S. pulchra*: $\chi^2(1) = 1.44$, $P = 0.23$; *B. diandrus*: $\chi^2(1) = 0.40$, $P = 0.53$; Table 1.1). Similarly, in both species, population was a significant source of variation for the probability of remaining persistent (*S. pulchra*: $\chi^2(7) = 14.9$, $P = 0.04$; *B. diandrus*: $\chi^2(7) = 52.7$, $P < 0.001$; Table 1.1). Larger seeds were overall less likely to remain persistent (*S. pulchra*: $\chi^2(1) = 6.41$, $P = 0.01$; *B. diandrus*: $\chi^2(1) = 4.37$, $P = 0.04$; Table 1.1), resulting from an increased probability of emergence (*S. pulchra*: $\chi^2(1) = 96.9$, $P < 0.001$; *B. diandrus*: $\chi^2(1) = 39.4$, $P < 0.001$; Table 1.1). For *S. pulchra*, seeds were more likely to persist in the low watering treatment ($\chi^2(1) = 3.70$, $P = 0.05$; Table 1.1), driven by a lower probability of emergence ($\chi^2(1) = 3.85$, $P = 0.05$; Table 1.1; Fig. 1.3) and lower mortality among non-emerging seeds ($\chi^2(1) = 3.92$, $P = 0.05$; Table 1.1; Fig. 1.3). In contrast, *B. diandrus* seeds were more likely to persist in the high watering treatment ($\chi^2(1) = 5.68$, $P = 0.02$; Table 1.1; Fig. 1.3), driven by greater mortality among non-emerging seeds in the low watering treatment ($\chi^2(1) = 9.97$, $P = 0.002$; Table 1.1; Fig. 1.3).

Table 1.1: Results of LMMs (emergence time) and GLMMs (persistence, emergence probability, non-emerging mortality) evaluating the effects of watering treatment, population and seed mass on the outcomes of individual seeds in *S. pulchra* and *B. diandrus*. The watering treatment "effect" column indicates the overall effect of the low vs. high treatment on each seed outcome where $P < 0.05$. The seed mass "effect" column indicates the overall effect of increasing seed mass on each seed outcome where $P < 0.05$.

S. pulchra	Population			Watering treatment				Seed mass			
	df	χ^2	*P*	*df*	χ^2	*p*	*Effect (low)*	*df*	χ^2	*P*	*Effect (larger)*
Emergence time	7	17.0	**0.02**	1	1.44	0.23	--	1	37.7	**<0.001**	**Decrease**
Persistence	7	14.9	**0.04**	1	3.70	**0.05**	**Increase**	1	6.41	**0.01**	**Decrease**
Emergence probability	7	83.1	**<0.001**	1	3.85	**0.05**	**Decrease**	1	96.9	**<0.001**	**Increase**
Non-emerging mortality	7	11.8	0.11	1	3.92	**0.05**	**Decrease**	1	0.01	0.92	--
B. diandrus	Population			Watering treatment				Seed mass			
	df	χ^2	*P*	*df*	χ^2	*P*	*Effect (low)*	*df*	χ^2	*P*	*Effect (larger)*
Emergence time	7	33.5	**<0.001**	1	0.40	0.53	--	1	14.5	**<0.001**	**Decrease**
Persistence	7	52.7	**<0.001**	1	5.68	**0.02**	**Decrease**	1	4.37	**0.04**	**Decrease**
Emergence probability	7	86.3	**<0.001**	1	0.03	0.85	--	1	39.4	**<0.001**	**Increase**
Non-emerging mortality	7	4.09	0.77	1	9.97	**0.002**	**Increase**	1	11.5	**<0.001**	**Decrease**

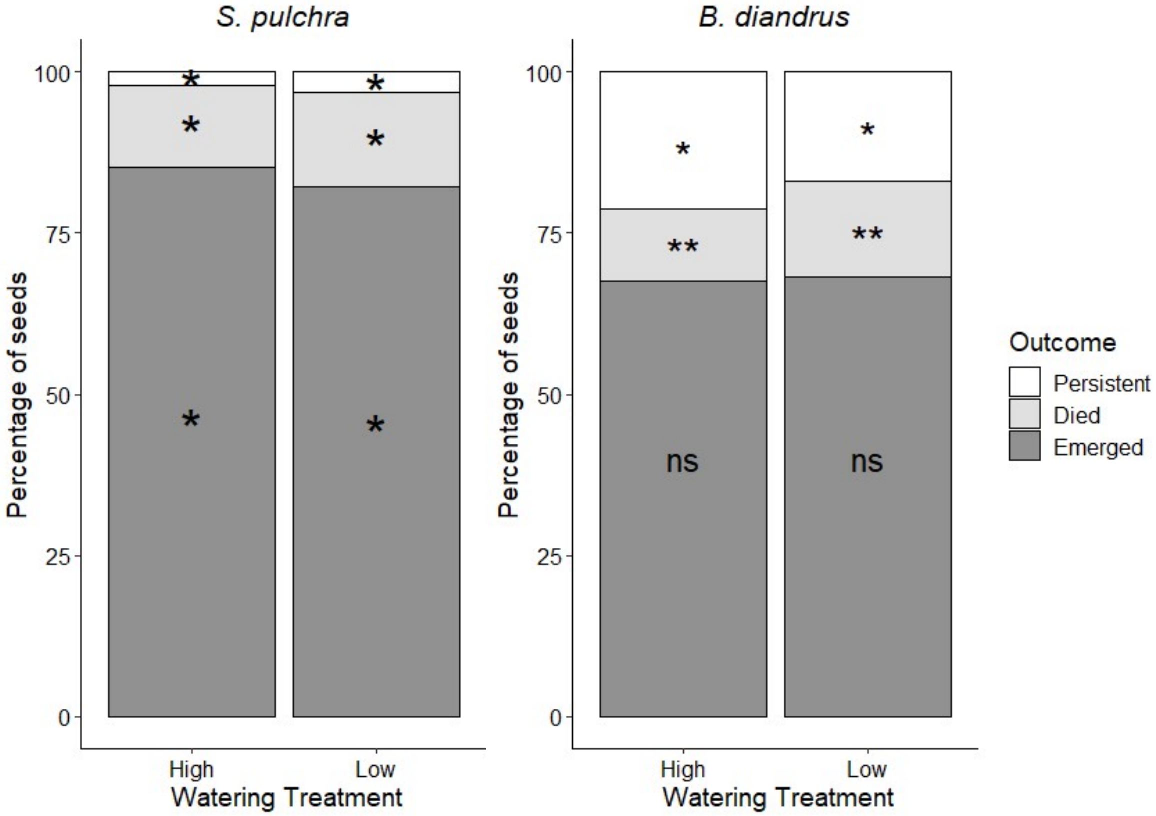

Figure 1.3: Influence of watering treatment on the outcomes of individual seeds in *S. pulchra* and *B. diandrus*. Symbols in the centre of each stacked bar indicate significance of watering treatment from GLMMs (ns > 0.05; * < 0.05; ** < 0.01).

Trade-off between within- and among-year emergence

Across all maternal lines, higher fractions of persistent seeds were associated with later mean emergence in both species (*S. pulchra*: $\chi^2(1) = 34.1$, $P < 0.001$; *B. diandrus*: $\chi^2(1) = 11.4$, $P < 0.001$; Figs. 1.4A, C). Higher seed persistence fractions resulted in larger increases in mean emergence time in *S. pulchra* (coefficient = 2.92) compared to *B. diandrus* (coefficient = 1.54). For both species, deviations of seed persistence fractions in maternal lines from their respective population means showed a significant positive relationship with deviations of mean emergence time (*S. pulchra*: $\chi^2(1) = 31.9$, $P < 0.001$; *B. diandrus*: $\chi^2(1) = 10.5$, $P = 0.001$; Figs. 1.4B, D). Thus, the positive relationship between seed persistence fraction and mean emergence time occurred within populations and independently of covariation between population means. Across, all maternal lines results were qualitatively the same after removing variation in emergence timing associated with seed mass (*S. pulchra*: $\chi^2(1) = 33.4$, $P < 0.001$; *B. diandrus*: $\chi^2(1) = 10.4$, $P = 0.001$), indicating that seed mass does not mediate the observed relationship between seed persistence fraction and emergence time.

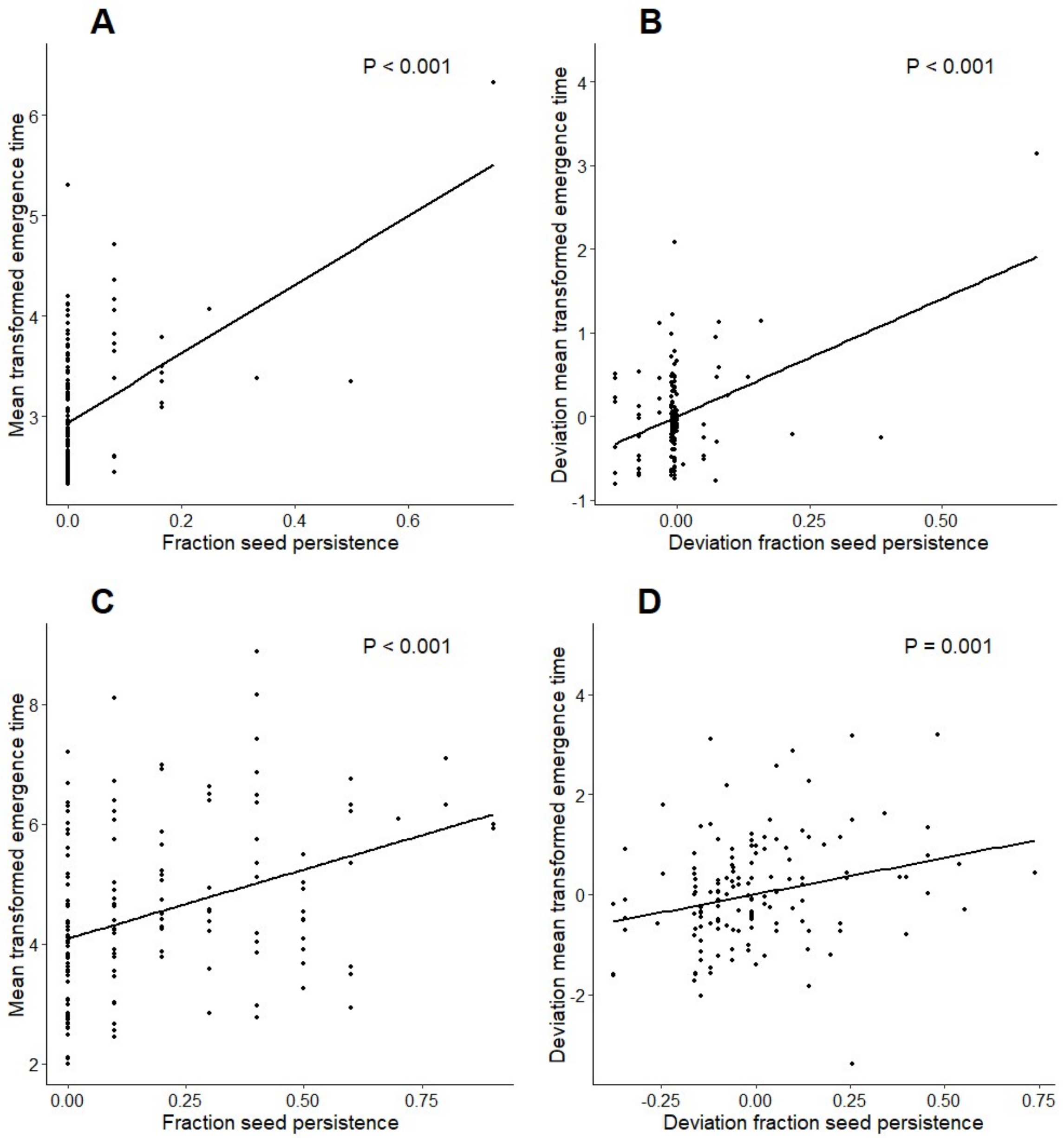

Figure 1.4: Scatter plots of seed persistence fraction in relation to emergence time for *S. pulchra* (A, B) and *B. diandrus* (C, D). Points in (A) and (C) represent values of mean transformed emergence time and seed persistence fraction of maternal lines. Points in (B) and (D) represent the deviation of mean values in maternal lines from their respective source populations.

Associations between emergence timing traits and site-level aridity

For the native *S. pulchra*, historical AI of source populations did not significantly predict emergence time ($F_{(1)} = 0.004$, $P = 0.95$; Fig. 1.5A) or seed persistence fraction ($F_{(1)} = 2.76$, $P = 0.15$; Fig. 1.5B). However, seeds from the two driest sites exhibited the highest persistence fractions, consistent with this species experiencing greater probabilities of "bad" growing seasons in these most arid sites (Fig. 1.5B), and stronger chronic selection favoring higher inter-annual dormancy. When considering both seed traits together, the four populations with Pareto optimal trait combinations had the most historically arid climates, and this difference was statistically significant ($P = 0.01$; Fig. 1.5C). Deviation AI did not significantly predict time to emergence ($F_{(1)} = 0.35$, $P = 0.58$) or seed persistence fraction ($F_{(1)} = 3.42$, $P = 0.11$), and did not differ significantly between Pareto optimal combinations and non-Pareto optimal source populations ($P = 0.20$).

For the exotic *B. diandrus*, historical AI of source populations was a significant predictor of emergence time ($F_{(1)} = 9.19$, $P = 0.02$; Fig. 1.5D), with earlier emergence in drier sites consistent with the interpretation that, where the duration of favorable conditions for plant growth is relatively short, natural selection favors earlier emergence. However, seed persistence fraction in *B. diandrus* was not associated with historical site-level aridity ($F_{(1)} = 1.09$, $P = 0.34$; Fig. 1.5E). Considering both seed traits together, the populations with Pareto optimal trait combinations were the first, second and fourth most arid, and these were significantly more arid than the remaining populations ($P = 0.02$; Fig. 1.5F). Deviation AI did not significantly predict time to emergence ($F_{(1)} = 1.31$, $P = 0.30$) or seed persistence fraction ($F_{(1)} = 0.42$, $P = 0.54$), and did not differ significantly between Pareto optimal combinations and non-Pareto optimal source populations ($P = 0.22$).

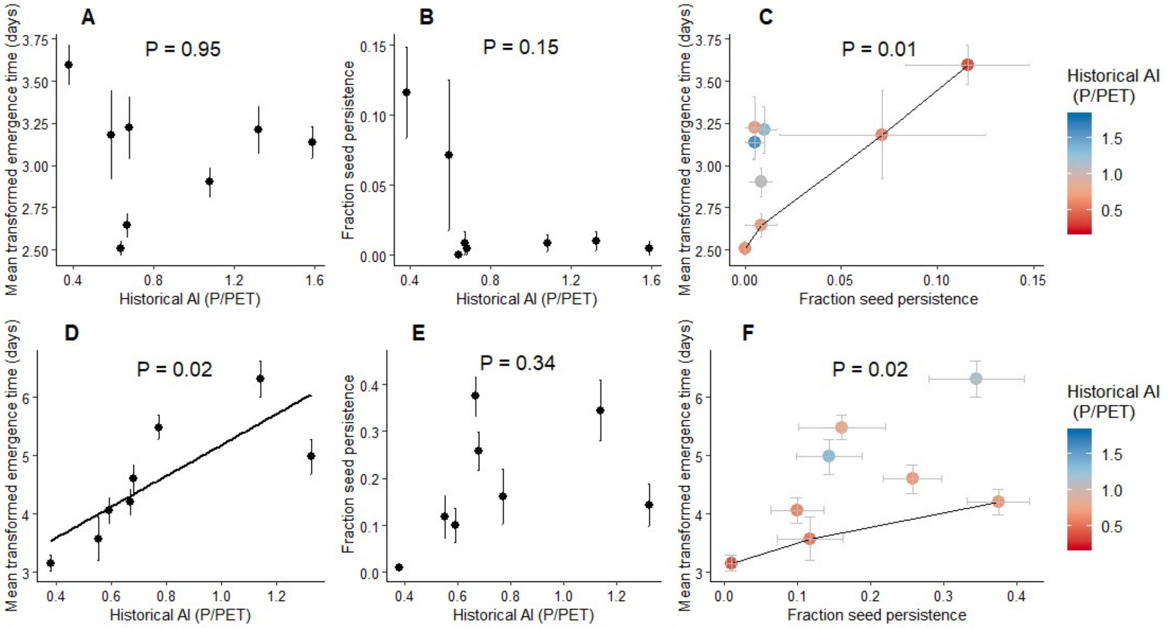

Figure 1.5: Associations between historical aridity of source populations and emergence timing traits in *S. pulchra* (*A*, *B*, *C*) and *B. diandrus* (*D*, *E*, *F*), for individual traits and trait combinations. Points represent values of mean transformed emergence time and seed persistence fraction for populations. Horizontal and vertical bars denote one standard error of the mean, where *n* is the number of maternal lines within each population. Black lines in (*C*) and (*F*) connect populations that are Pareto optimal for earlier mean emergence and greater seed persistence fractions, and *P* values represent the results of permutation tests testing for differences in aridity between Pareto optimal and non-Pareto optimal source populations.

Discussion

The timing of emergence within years and the spread of emergence among years are key traits influencing fitness in seasonal environments (Donohue et al. 2010). Our results support the existence of a genetic trade-off between within- and among-year emergence timing that can constrain adaptive evolution in each trait. We demonstrate that this trade-off can result in emergence timing trait values along an environmental gradient that appear maladaptive when considered separately but are in fact Pareto optimal when considered in combination. Furthermore, plastic responses in emergence timing traits to environmental conditions in a given year may mitigate the costs associated with this constraint. Our findings highlight the importance of considering emergence timing both within and among years when evaluating their adaptive significance in natural populations.

Trade-off between within- and among-year emergence

Maternal lines of *S. pulchra* and *B. diandrus* with higher fractions of persistent seeds (greater potential among-year emergence) emerged later, supporting a genetic trade-off between the timing of emergence within and among years that is likely to constrain adaptive evolution in each trait. Within- and among-year emergence were positively associated independently of differences in mean trait values among source populations. This relationship could result from variation among maternal lines in the conditions that enforce dormancy and those that cause emergence in nondormant seeds (or both together); however, determining the underlying mechanisms was beyond the scope of this study.

Dormancy and germination requirements often vary within species (e.g. Smith et al. 2000, Huang et al. 2010). However, both are strongly influenced by environmental factors such as seed maturation environment and maternal provisioning (Roach and Wulff 1987, Gómez

2004, Halpern 2005, Bewley et al. 2013, Fernández-Pascual et al. 2013, Baskin and Baskin 2014). Our experiment used field collected seeds, and therefore environmental variation within and among source population sites may have contributed to variation in emergence traits among maternal lines. However, we found that the observed relationship occurred independently of the mean seed mass of maternal lines, suggesting that it was not mediated by variation in maternal provisioning. Furthermore, as shown by the significant, positive relationship between the deviations of mean emergence time and seed persistence fractions in maternal lines from source population means (Figs. 1.4B, D), this relationship occurred within populations that likely experienced relatively similar seed maturation conditions. We also found that the deviation from long-term average aridity in the year of collection, a measure of the seed maturation conditions, did not predict emergence timing traits. Fernández-Pascual et al. (2013), compared dormancy in seeds of *Centaurium somedanum* collected from separate wild populations to seeds collected from a second generation grown in the greenhouse and found that differences in seed maturation environment in source populations did not mask genetically based differences in dormancy. Thus, despite the potential for variation in seed maturation environment and maternal provisioning, within and among source populations, to influence values of emergence traits in our experiment, our results are consistent with a genetic basis for the trade-off between within- and among-year emergence among maternal lines.

A limitation of our experimental approach is that seeds that remained persistent could not be assessed for either emergence time or for continued soil persistence as the tetrazolium assay is lethal to seeds. Further work is needed to characterize the within-year emergence time of seeds that persist among years, as this will have important implications for a trade-off between the two traits. For example, relatively early emergence of persistent seeds in subsequent years would result in a weaker trade-off when assessed across years compared to within a single year. However, few studies investigate emergence traits of seeds persisting over multiple years (but see Philippi 1993), and we are aware of no studies assessing emergence time in persistent seeds.

Associations between emergence timing traits and site-level aridity

Increasing aridity might select for earlier emergence (Sexton et al. 2011, Dickman et al. 2019), greater spread of emergence among years (Venable and Brown 1988, Petrů and Tielbörger 2008), or both simultaneously. In the native perennial *S. pulchra*, neither emergence trait was significantly associated with historical aridity of source populations when considered individually. However, of the populations sampled, those that were Pareto optimal for early emergence and high among-year emergence experienced more historically arid climates. This pattern is consistent with arid climates selecting for both earlier within-year emergence and greater among-year emergence, with variation among source populations in the relative strength of selection for each. In *B. diandrus*, increasing aridity of source populations was associated with earlier emergence under greenhouse conditions and, of the populations sampled, those with Pareto optimal trait combinations were also more arid. This result is consistent with increasing aridity selecting for earlier emergence and greater among-year emergence, with stronger selection for earlier emergence in the most arid environments.

Results from studies of emergence timing traits across putatively similar environmental gradients are notably inconsistent (reviewed in Cochrane et al. 2015). However, such studies typically test for associations between environmental variables and individual emergence timing traits and are therefore unlikely to characterize scenarios in which selection is acting on both traits together. Our results for *S. pulchra* in particular illustrate how Pareto optimality within the context of associations between emergence timing traits can explain the presence varied, seemingly maladaptive individual trait values across sites experiencing putatively more similar climate. To our knowledge, this is the first study in which Pareto optimality has been applied to correlated traits across an environmental gradient to detect signatures of joint selection. We suggest this is a promising approach for untangling how genetic trade-offs among traits can constrain patterns of adaptive evolution.

The combinations of emergence timing trait values that evolve in response to increasing aridity may depend on multiple factors, including selective constraints imposed by life history strategy (Rees et al. 2006) and the steepness of the trade-off between within- and among-year emergence. As an annual, *B. diandrus* might experience relatively strong selection to emerge early, facilitating the completion of its life cycle before favorable conditions deteriorate. Although Pareto optimal populations of *B. diandrus* occupied significantly more arid sites than non-Pareto optimal populations, the trend of earlier emergence with increasing aridity of the source population site suggests that increased variance in fitness among years is not the major selective effect of increasing aridity within the range of populations sampled for this experiment. We expect that this pattern would not persist beyond some threshold of aridity; annual plant communities in desert ecosystems characterized by exceptionally high interannual variability in precipitation, and thus variance in fitness among years, typically have both high among-year emergence and diversified emergence within years (e.g. Gremer et al. 2016). For *S. pulchra*, a perennial species that does not typically flower in its first year, increasing aridity might not always result in selection for earlier emergence and thus the relative strength of selection for earlier emergence vs. greater among-year emergence may differ among populations to a greater extent than in annual counterparts. Furthermore, the steeper relationship between within- and among-year emergence in *S. pulchra* may mean that evolving greater among-year emergence carries a greater cost, which might contribute to the lower among-year emergence compared to *B. diandrus*. However, with only two species, we are unable to determine the factors influencing the combinations of within- and among-year emergence that evolve.

Plastic responses of emergence timing traits

We expected that drier conditions would cause later emergence and increased probability of seed persistence. Watering treatment had no effect on emergence time in either species; this is unsurprising as a considerable proportion of emergence occurred in response to initial pulses that were the same across treatments. However, seed persistence fractions in each focal species responded plastically to watering treatment, albeit in opposite directions. *S. pulchra* had greater potential for among-year emergence in the low watering treatment, while potential among-year emergence in *B. diandrus* was greater in the high watering treatment (Fig. 1.3). The greater potential for among-year emergence in the low watering treatment for the native perennial *S. pulchra* was partly driven by lower emergence probability, suggesting that higher moisture is required to initiate germination in this species (Bewley et al. 2013). The lower mortality of non-emerging seeds in the low watering treatment may result from reduced seed ageing or reduced microbial activity in drier soils (Long et al. 2015). In contrast, seed persistence in *B. diandrus* was lower in the low watering treatment, resulting from greater mortality among non-emerging seeds in the low watering treatment (Fig. 1.3). While drier soils may directly increase mortality of non-emerging *B. diandrus* due to the desiccation of vulnerable seedlings in the soil, the probability of successfully emerging did not differ significantly between treatments. Therefore, we suspect that higher mortality in the low watering treatment was driven instead by increased rates of seed ageing in warmer soils (due to lower latent heat loss) (Long et al. 2015). This also suggests that the ageing of *S. pulchra* seeds, which were more likely to persist in the low watering treatment, is less sensitive to high soil temperatures.

Emergence traits that plastically respond to cues that reflect the favorability of growing season conditions can be adaptive in variable environments (Simons 2014). For *S. pulchra*, greater potential for among-year emergence in drier conditions (Fig. 1.3) is consistent with the effect of increasing source population aridity, and thus likely represents adaptive plasticity that could mitigate the costs of the constraint on emergence timing traits. However, the adaptive nature of this plastic response to wetter conditions has likely been altered by exotic plant species invasions. In California, herbaceous exotic species are relatively more abundant in wetter years (e.g. Copeland et al. 2016, Puritty et al. 2019), and emergence in such conditions can reduce the establishment of perennial species due to more intense competition (Esch et al. 2018). In *B. diandrus*, potential among-year emergence was lower under drier conditions (Fig. 1.3), which might be maladaptive in more arid climates (Venable and Brown 1988, Petrů and Tielbörger 2008). However, our results suggest that increasing aridity most strongly selects for early within-year emergence (cf. Sexton et al. 2011, Dickman et al. 2019), and therefore this plastic response to dry conditions might impact fitness to a lesser extent than if greater aridity of source populations were instead associated with greater among-year emergence.

Conclusions

The timing of emergence within and among years are associated traits that must be considered simultaneously when investigating adaptation to current and future environmental conditions. Failure to do so may lead researchers to incorrectly characterize patterns of selection acting on each emergence timing trait, resulting in less accurate predictions of adaptive responses to environmental change. The concept of Pareto optimality has only recently been applied to biological systems (Shoval et al. 2012) but is useful in the context of trade-offs that can generate seemingly maladaptive individual trait values. We suggest that a similar approach applied to other associated fitness-related traits could be fruitful for understanding patterns of trait variation along environmental gradients and thus predicting adaptation to future environmental change.

Acknowledgements

We thank Kristy Lanz for carrying out tetrazolium assays. We also thank Chandler Puritty and Elizabeth Premo for assistance in the greenhouse. This work was supported by an Institute for the Study of Ecological Effects of Climate Impacts (ISEECI) Graduate Student Researcher Fellowship and Jeanne M. Messier Memorial Fellowship to J.W.

Chapter 1, in full, has been submitted for publication of the material. Waterton, J., Mazer, S.J., Meyer, J.R., and E.E. Cleland. The dissertation author is the primary investigator and author of this paper.

Appendix 1A

Table 1A.1: Climate data for each source population. Historical climate variables were calculated for the years 1985 – 2014 and collection year variables were calculated for May 2014 – April 2015.

Site	Latitude	Historical mean annual precipitation (mm)	Historical mean annual temperature (°C)	Historical annual PET (mm)	Historical aridity index	Collection year precipitation (mm)	Collection year mean temperature (°C)	Collection year PET (mm)	Collection year aridity index	Collection year deviation aridity index
Hopland	39.02	997	14.79	758	1.315	835	16.58	873	0.957	-0.357
Bodega	38.42	1087	13.35	683	1.590	797	14.64	759	1.050	-0.540
Quail Ridge	38.48	806	14.40	746	1.081	578	15.85	834	0.694	-0.387
Jepson Prairie	38.27	520	15.91	800	0.650	342	17.33	892	0.384	-0.267
Younger Lagoon	36.95	764	13.08	669	1.141	657	15.21	808	0.812	-0.329
Fort Ord	36.69	379	13.76	684	0.554	316	15.23	777	0.406	-0.147
Hastings	36.38	559	14.43	725	0.771	395	16.43	853	0.464	-0.307
Landels-Hill	36.07	944	14.53	713	1.324	573	16.33	829	0.691	-0.633
Kenneth S. Norris	35.53	459	13.67	675	0.680	318	15.77	813	0.391	-0.290
Sedgwick	34.69	541	16.62	807	0.670	245	17.93	900	0.272	-0.398
Coal Oil Point	34.42	469	15.25	733	0.640	220	16.74	834	0.263	-0.377
Stunt Ranch	34.09	497	17.68	839	0.593	221	19.57	979	0.225	-0.367
Elliott Chaparral	32.89	316	17.63	832	0.380	200	19.48	979	0.205	-0.176

Table 1A.2: Results of LMMs (emergence time) and GLMs (persistence, emergence probability, non-emerging mortality) evaluating the effects of watering treatment, population, watering treatment × population interaction, and seed mass on the outcomes of individual seeds in *S. pulchra* and *B. diandrus*. The watering treatment "effect" column indicates the overall effect of the low vs. high treatment on each seed outcome where $P < 0.05$. The seed mass "effect" column indicates the overall effect of increasing seed mass on each seed outcome where $P < 0.05$.

S. pulchra	Population			Watering treatment				Population × watering treatment			Seed mass			
	df	χ^2	*P*	*df*	χ^2	*P*	*Effect (low)*	*df*	χ^2	*P*	*df*	χ^2	*P*	*Effect (larger)*
Emergence time	7	17.0	**0.02**	1	1.44	0.23	--	7	7.30	0.40	1	36.9	**<0.001**	**Decrease**
Persistence	7	72.3	**<0.001**	1	3.34	0.07	--	7	3.73	0.81	1	7.47	**0.006**	**Decrease**
Emergence probability	7	99.5	**<0.001**	1	3.65	0.06	--	7	7.64	0.37	1	123.1	**<0.001**	**Increase**
Non-emerging mortality	7	60.4	**<0.001**	1	3.56	0.06	--	7	3.39	0.85	1	0.03	0.86	--
B. diandrus	Population			Watering treatment				Population × watering treatment			Seed mass			
	df	χ^2	*P*	*df*	χ^2	*P*	*Effect (low)*	*df*	χ^2	*P*	*df*	χ^2	*P*	*Effect (larger)*
Emergence time	7	33.4	**<0.001**	1	0.40	0.53	--	7	6.87	0.44	1	13.2	**<0.001**	**Decrease**
Persistence	7	162.2	**<0.001**	1	4.72	**0.03**	**Decrease**	7	5.06	0.65	1	4.73	**0.03**	**Decrease**
Emergence probability	7	219.7	**<0.001**	1	0.02	0.90	--	7	5.59	0.59	1	54.25	**<0.001**	**Increase**
Non-emerging mortality	7	20.5	**0.005**	1	10.6	**0.001**	**Increase**	7	2.49	0.87	1	15.6	**<0.001**	**Decrease**

References

Alpert, P., S. O. Krichak, H. Shafir, D. Haim, and I. Osetinsky. 2008. Climatic trends to extremes employing regional modeling and statistical interpretation over the E. Mediterranean. Global and Planetary Change **63**:163–170.

AOSA/SCST. 2010. Tetrazolium Testing Handbook. 2010 edition. Association of Official Seed Analysts and the Society of Commercial Seed Technologists.

Arroyo, M. T., P. Chacon, and L. A. Cavieres. 2006. Relationship between seed bank expression, adult longevity and aridity in species of Chaetanthera (Asteraceae) in central Chile. Annals of Botany **98**:591-600.

Baldwin, B. G., D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken, editors. 2012. The Jepson manual: vascular plants of California. 2nd edition. University of California Press, Berkeley.

Baskin, C. C., and J. M. Baskin. 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego.

Beguería, S., and S. M. Vicente-Serrano. 2017. SPEI: Calculation of the standardised precipitation-evapotranspiration index [R package version 1.7].

Berg, N., and A. Hall. 2015. Increased interannual precipitation extremes over California under climate change. Journal of Climate **28**:6324–6334.

Bewley, J. D., K. J. Bradford, H. H. W. M., and H. Nonogaki. 2013. Seeds: Physiology of Development, Germination and Dormancy. 3rd edition. Springer.

Caruso, C. M., H. Maherali, A. Mikulyuk, K. Carlson, and R. B. Jackson. 2005. Genetic variance and covariance for physiological traits in Lobelia: are there constraints on adaptive evolution? Evolution **59**:826-837.

Cochrane, A., C. J. Yates, G. L. Hoyle, and A. B. Nicotra. 2015. Will among-population variation in seed traits improve the chance of species persistence under climate change? Global Ecology and Biogeography **24**:12-24.

Colautti, R. I., and S. C. H. Barrett. 2013. Rapid adaptation to climate facilitates range expansion of an invasive plant. Science **342**:364-366.

Copeland, S. M., S. P. Harrison, A. M. Latimar, E. I. Damschen, A. Eskelinen, B. Fernandez-Going, M. J. Spasojevic, B. L. Anacker, and J. H. Thorne. 2016. Ecological effects of extreme drought on Californian herbaceous plant communities. Ecological Monographs **86**:295-311.

Dickman, E. E., L. K. Pennington, S. J. Franks, and J. P. Sexton. 2019. Evidence for adaptive responses to historic drought across a native plant species range. Evolutionary Applications.

Donohue, K., R. Rubio de Casas, L. Burghardt, K. Kovach, and C. G. Willis. 2010. Germination, postgermination adaptation, and species ecological ranges. Annual Review of Ecology, Evolution, and Systematics **41**:293–319.

Esch, E. H., A. C. Ashbacher, C. W. Kopp, E. E. Cleland, and S. Schwinning. 2018. Competition reverses the response of shrub seedling mortality and growth along a soil moisture gradient. Journal of Ecology **106**:2096-2108.

Etterson, J. R., and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. Science **294**:151-154.

Fernández-Pascual, E., B. Jiménez-Alfaro, J. Caujapé-Castells, R. Jaén-Molina, and T. E. Díaz. 2013. A local dormancy cline is related to the seed maturation environment, population genetic composition and climate. Annals of Botany **112**:937-945.

Fournier-Level, A., A. Korte, M. D. Cooper, M. Nordborg, J. Schmitt, and A. M. Wilczek. 2011. A map of local adaptation in Arabidopsis thaliana. Science **334**:86-89.

Fournier, D. A., S. H. J., J. Ancheta, J. Ianelli, A. Magnusson, M. Maunder, A. Nielsen, and J. Sibert. 2012. Generalized linear mixed models using 'AD Model Builder' [R package version 0.8.3.3].

Fox, J., and S. Weisberg. 2011. An {R} Companion to Applied Regression. 2nd edition. Sage, Thousand Oaks, CA.

Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences USA **104**:1278-1282.

Gómez, J. 2004. Bigger is not always better: Conflicting selective pressures on seed size in Quercus ilex. Evolution **58**:71-80.

Gremer, J. R., S. Kimball, and D. L. Venable. 2016. Within-and among-year germination in Sonoran Desert winter annuals: bet hedging and predictive germination in a variable environment. Ecol Lett **19**:1209-1218.

Halpern, S. L. 2005. Sources and consequences of seed size variation in Lupinus perennis (Fabaceae): Adaptive and non-adaptive hypotheses. American Journal of Botany **92**:205-213.

Heady, H. F. 1977. Valley grassland. Pages 491–514 *in* M. G. Barbour and J. Major, editors. Terrestrial vegetation in California. Wiley, New York.

Hoffmann, A. A., and C. M. Sgro. 2011. Climate change and evolutionary adaptation. Nature **470**:479-485.

Huang, X., J. Schmitt, L. Dorn, C. Griffith, S. Effgen, S. Takao, M. Koornneef, and K. Donohue. 2010. The earliest stages of adaptation in an experimental plant population: Strong selection on QTLS for seed dormancy. Molecular Ecology **19**:1335-1351.

IPCC. 2013. Annex I: Atlas of Global and Regional Climate Projections.*in* G. J. van Oldenborgh, M. Collins, J. Arblaster, J. H. Christensen, J. Marotzke, S. B. Power, M. Rummukainen, and T. Zhou, editors. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jump, A. S., and J. Peñuelas. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters **8**:1010-1020.

Knapp, E. E., and K. J. Rice. 1998. Comparison of isozymes and quantitative traits for evaluating patterns of genetic variation in purple needlegrass (Nassella pulchra). Conservation Biology **12**:1031–1041.

Kon, K. F., and W. M. Blacklow. 1990. Polymorphism, outcrossing and polyploidy in *Bromus diandrus* and *B. rigidus* Australian Journal of Botany **38**:609–618.

Larson, S. R., E. Cartier, C. L. McCracken, and D. Dyer. 2001. Mode of reproduction and amplified fragment length polymorphism variation in purple needlegrass (Nassella pulchra): utilization of natural germplasm sources. Molecular Ecology **10**:1165-1177.

Long, R. L., M. J. Gorecki, M. Renton, J. K. Scott, L. Colville, D. E. Goggin, L. E. Commander, D. A. Westcott, H. Cherry, and W. E. Finch-Savage. 2015. The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. Biological Reviews **90**:31-59.

Mordecai, E. A. 2012. Soil moisture and fungi affect seed survival in California grassland annual plants. PLoS One **7**:e39083.

Petrů, M., and K. Tielbörger. 2008. Germination behaviour of annual plants under changing climatic conditions: Separating local and regional environmental effects. Oecologia **155**:717–728.

Philippi, T. 1993. Bet-hedging germination of desert annuals: Beyond the first year. The American Naturalist **142**:474-487.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2018. nlme: Linear and Nonlinear Mixed Effects Models [R package version 3.1-137].

Pratt, J. D., and K. A. Mooney. 2013. Clinal adaptation and adaptive plasticity in Artemisia californica: implications for the response of a foundation species to predicted climate change. Global Change Biology **19**:2454–2466.

Puritty, C. E., E. H. Esch, S. P. Castro, E. M. Ryan, D. A. Lipson, and E. E. Cleland. 2019. Drought in Southern California coastal sage scrub reduces herbaceous biomass of exotic species more than native species, but exotic growth recovers quickly when drought ends. Plant Ecology **220**:151-169.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rees, M., D. Z. Childs, C. J. Metcalf, K. E. Rose, A. W. Sheppard, and P. J. Grubb. 2006. Seed dormancy and delayed flowering in monocarpic plants: selective interactions in a stochastic environment. The American Naturalist **168**:E53-E71.

Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. Annual Review of Ecology, Evolution, and Systematics **18**:209-235.

Roocks, P. 2016. Computing Pareto frontiers and database preferences with the rPref package. The R Journal **8**:393-404.

Saatkamp, A., L. Affre, T. Dutoit, and P. Poschlod. 2011. Germination traits explain soil seed persistence across species: the case of Mediterranean annual plants in cereal fields. Annals of Botany **107**:415-426.

Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H. Huang, N. Harnik, A. Leetmaa, N. Lau, C. Li, J. Velez, and N. Naik. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science **316**:1181–1184.

Sexton, J. P., S. Y. Strauss, and K. J. Rice. 2011. Gene flow increases fitness at the warm edge of a species' range. Proceedings of the National Academy of Sciences USA **108**:11704-11709.

Shoval, O., H. Sheftel, G. Shinar, Y. Hart, O. Ramote, A. Mayo, E. Dekel, K. Kavanagh, and U. Alon. 2012. Evolutionary trade-offs, Pareto optimality, and the geometry of phenotype space. Science **336**:1157-1160.

Simons, A. M. 2014. Playing smart vs. playing safe: the joint expression of phenotypic plasticity and potential bet hedging across and within thermal environments. Journal of Evolutionary Biology **27**:1047-1056.

Simons, A. M., and M. O. Johnston. 2006. Environmental and genetic sources of diversification in the timing of seed germination: Implications for the evolution of bet hedging. Evolution **60**:2280-2292.

Skálová, H., L. Moravcová, and P. Pyšek. 2011. Germination dynamics and seedling frost resistance of invasive and native Impatiens species reflect local climatic conditions. Perspectives in Plant Ecology, Evolution and Systematics **13**:173–180.

Smith, S. E., E. Riley, J. L. Tiss, and D. M. Fendenheim. 2000. Geographical variation in predictive seedling emergence in a perennial desert grass. Journal of Ecology **88**:139-149.

Stromberg, M. R., and J. R. Griffin. 1996. Long-term patterns in coastal California grasslands in relation to cultivation, gophers, and grazing. Ecological Applications **6**:1189–1211.

Thornthwaite, C. W. 1948. An approach toward a rational classification of climate. Geographical Review **38**:55-94.

Torres-Martínez, L., P. Weldy, M. Levy, and N. C. Emery. 2017. Spatiotemporal heterogeneity in precipitation patterns explain population-level germination strategies in an edaphic specialist. Annals of Botany **119**:253-265.

Venable, D. L., and J. S. Brown. 1988. The selective interactions of dispersal, dormancy and seed size as adaptations for reducing risk in variable environments. The American Naturalist **131**:360–384.

Verdú, M., and A. Traveset. 2005. Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. Ecology **86**:1385–1394.

Wadgymar, S. M., S. C. Daws, and J. T. Anderson. 2017. Integrating viability and fecundity selection to illuminate the adaptive nature of genetic clines. Evolution Letters **1**:26-39.

Wainwright, C. E., E. M. Wolkovich, and E. E. Cleland. 2012. Seasonal priority effects: implications for invasion and restoration in a semi-arid system. Journal of Applied Ecology **49**:234-241.

Walck, J. L., S. N. Hidayati, K. W. Dixon, K. Thompson, and P. Poschlod. 2011. Climate change and plant regeneration from seed. Global Change Biology **17**:2145–2161.

Waterton, J., and E. E. Cleland. 2016. Trade-off between early emergence and herbivore susceptibility mediates exotic success in an experimental California plant community. Ecology and Evolution **6**:8942-8953.

Yoon, J. H., S. Y. Wang, R. R. Gillies, B. Kravitz, L. Hipps, and P. J. Rasch. 2015. Increasing water cycle extremes in California and in relation to ENSO cycle under global warming. Nature Communications **6**:8657.

CHAPTER 2

When the neighborhood matters: contextual selection on emergence time in native and exotic California grasses

Abstract

The timing of seedling emergence has large impacts on plant fitness because it determines the environment with which a plant interacts throughout development. A major component of this environment is the surrounding plant community, and earlier emergence provides a consistent competitive advantage that contributes to the dominance of many early-emerging exotic plant species worldwide. Despite this, little is known about how variation in community composition shapes selection on emergence time. To investigate this, we characterized phenotypic selection on emergence time in two California grasses, the native *Stipa pulchra* and exotic *Bromus diandrus*, in the field when growing in competition with 6 other native and exotic grasses in individual species and mixed species treatments. We found that community composition significantly altered the strength but not the direction of selection on emergence time in both focal species, and the strength of selection exerted by each competitive neighborhood was not concordant in both focal species. Stronger selection for earlier emergence in *S. pulchra* was weakly associated with later-emergence of neighbors and greater light interception in the surrounding community, but variation in the strength of selection in *B. diandrus* was not explained by any of our measures of community context. Selection for earlier emergence was stronger in the exotic *B. diandrus* when growing with exotic neighbors, but neighbor origin did not significantly influence selection for earlier emergence in the native *S. pulchra*. In both focal species, there was no significant effect of neighbor diversity on the strength of selection for earlier emergence. Our results demonstrate that community composition

is an important factor shaping selection on emergence time, although further work will be required to characterize the underlying mechanisms. Spatial variation in community composition could therefore contribute to the maintenance of variation in emergence time within and among plant populations. Furthermore, the potential for emergence time to adaptively evolve in response to drivers of global change is likely to be mediated by the effects of the surrounding community on the process of natural selection.

Introduction

The surrounding plant community is a major component of the biotic environment with which plant populations interact. While the ecological consequences of interactions between plant species within communities have long been a major focus of research, the evolutionary consequences of such interactions have historically received less attention (Thorpe et al. 2011). In recent years, numerous studies have shown that the surrounding plant community can influence the evolutionary trajectories of species embedded within them (e.g. Callaway et al. 2005, Lau 2008, Leger 2008, Lankau 2012, Beans and Roach 2015). Such studies are increasingly important because drivers of global change — in particular species introductions — are drastically changing the composition of plant communities worldwide (Kuebbing et al. 2013, van Kleunen et al. 2015). Yet despite the large potential for shifts in community composition to drive eco-evolutionary feedbacks that influence their structure and function (Post and Palkovacs 2009), we still have a limited understanding of how community composition affects the strength or direction of selection exerted on many key fitness-related traits.

The timing of seedling emergence is the earliest phenological trait expressed in development, strongly influencing fitness as well as selection acting on subsequently expressed traits (Donohue 2002). In seasonal environments, emergence time is under strong selection by the general environment (i.e., “hard selection”), whereby the fitness associated with emergence time is independent of the phenotypes of competitors (Weis et al. 2015). For example, emerging early within a growing season can increase the risk of herbivore impacts (Waterton and Cleland 2016), or growing before the onset of reliably tolerable abiotic conditions, such as exposure to a prolonged dry period (Wainwright et al. 2012) or a late frost (Skálová et al. 2011). Consequently, similar to other phenological traits — such as flowering phenology (Franks et al. 2007, Anderson et al. 2012) — emergence time is expected to evolve in concert with climate change (Walck et al. 2011, Cochrane et al. 2015) and evidence of such shifts in emergence time has already been observed (Dickman et al. 2019).

The fitness value associated with emergence time also depends strongly on the phenotypes of competitors (i.e. “soft selection”) (Weis et al. 2015). Emerging earlier than competitors provides longer windows for growth and reproduction before favorable conditions deteriorate, as well the potential to pre-empt resources and suppress the growth of individuals that emerge later (i.e., priority effects) (Vannette and Fukami 2014). Accordingly, meta-analysis shows that earlier emergence tends to enhance individual fitness (Verdú and Traveset 2005). This competitive advantage of early emergence is considered to be a key factor contributing to the dominance of many exotic species and their sizeable impacts on the communities they invade (Abraham et al. 2009, Gioria et al. 2018). However, few studies have characterized how plant community composition itself shapes selection on emergence time (but see Weinig 2000).

A key mechanism by which community composition might influence selection on emergence time is by determining the extent to which priority effects cause fitness differences among individuals emerging at different times. Priority effects occur when the outcome of an interaction between species depends on the order of arrival, typically with earlier arrivers competitively suppressing later ones (Vannette and Fukami 2014). Numerous studies have demonstrated that species identity influences the strength of priority effects when arrival timing is manipulated experimentally (Dickson et al. 2012, Cleland et al. 2015, Wilsey et al. 2015, Stuble et al. 2016, Goodale and Wilsey 2018). However, under natural conditions the direction of priority effects experienced by individuals emerging at different times will likely depend on the relative emergence time of the surrounding community.

Stronger priority effects resulting from differences in arrival order relative to surrounding community are likely to generate stronger selection on emergence time. Priority effects are generally strong when: (1) competitors have high niche overlap, (2) early arrivers have large impacts on the environment experienced by later-arriving competitors (i.e., large competitive effect; Lavorel and Garnier 2002), (3) the growth of late arrivers is sensitive to variation in the environment (i.e., large competitive response; Lavorel and Garnier 2002) (Vannette and Fukami 2014). The environmental impact exerted by the surrounding community might be mediated by the uptake of multiple different resources above- or belowground. However, greater uptake of resources for which competition is most size-asymmetric, such as light (Schwinning and Weiner 1998), may result in particularly strong priority effects for late-emerging plants. Neighbor origin may also shape selection on emergence time by influencing the strength of priority effects. For example, different studies have found that exotic species (Dickson et al. 2012, Wilsey et al. 2015, Stuble et al. 2016, Goodale and Wilsey 2018) or native species (Seabloom et al. 2003) exert stronger priority effects, which might lead to stronger selection for earlier emergence. An additional factor that may influence selection on emergence time is the diversity of neighbors in a community. The presence of multiple neighbor species might increase the strength of priority effects (and thus selection for earlier emergence) through complementarity in resource uptake or a greater probability of containing species that exert strong priority effects (i.e. a sampling effect) (Fargione and Tilman 2005).

We carried out a field experiment to investigate how community composition influences phenotypic selection on emergence time in two widespread California grasses, the native *Stipa pulchra* (Hitchc.) Barkworth and exotic *Bromus diandrus* (Roth). The California Floristic Province has experienced widespread invasion by exotic plant species, particularly annual grasses, which have displaced many native species since the arrival of European settlers in the 18th century (Heady 1977). California exotics differ from their native counterparts in multiple traits relating to resource acquisition and life-history strategy, including a tendency for exotics to exhibit annual life-histories (Raven and Axelrod 1978), earlier emergence and faster growth than co-occurring natives (Reynolds et al. 2001, Deering and Young 2006, Abraham et al. 2009, Wainwright et al. 2012, Wainwright and Cleland 2013, Vaughn and Young 2015, Waterton and Cleland 2016), a pattern observed in other Mediterranean-climate regions (Funk et al. 2016). While it is widely recognized that earlier emergence is a key factor contributing to the competitive dominance of exotic species in California and elsewhere, little is known about how exotic species themselves alter patterns of selection on emergence time. Furthermore, the functional differences between natives and exotics provide an opportunity to explore general mechanisms by which community composition determines selection on emergence time. We hypothesized that community composition would influence the strength of selection for earlier emergence, and that selection would be influenced by one or more of the following: (1) neighbor emergence time and light interception (2) the origin of neighbors in the surrounding community; (3) the presence of multiple vs. single neighbor species.

Methods

Study site

The field experiment was carried out on an experimental field at the University of California San Diego Biological Field Station. The site is flat, regularly tilled, and is classified as having sandy clay loam soil. The climate is Mediterranean, with most precipitation typically falling between November and May, during which the majority of seedling emergence occurs. The site is typically dominated by exotic annual species including *Hordeum murinum* (Poaceae), *Erodium cicutarium* (Geraniaceae), and *Malva parviflora* (Malvaceae).

Focal species

Stipa pulchra (purple needlegrass) is a perennial bunchgrass native to California that is found in woodland, chaparral, and grassland from Baja California to northern California (Baldwin et al. 2012). A study of neutral genetic markers shows that *S. pulchra* has high rates of self-fertilization (selfing rate ≈ 1) (Larson et al. 2001).

Bromus diandrus is an annual grass native to Eurasia that has become naturalized in California since European settlement (Jackson 1985). This species is found in many habitats across California, and is particularly dominant in disturbed areas, such as abandoned agricultural fields (Stromberg and Griffin 1996). *B. diandrus* is also largely self-fertilizing (reported selfing rates > 0.99) (Kon and Blacklow 1990).

Field experiment

We carried out the field experiment between February 2017 and May 2018, lasting one growing season for the annual *B. diandrus* and two growing seasons for the native perennial *S. pulchra*. We analysed phenotypic selection on emergence time in of nine competitor identity treatments (Table 2.1). These consisted of six single species treatments that were sown with seeds of three native and three exotic grass species (taxonomically balanced at the genus level), two mixed species treatments sown with seeds of each of all three native and all three exotic species, and a control treatment without additional seeds sown in.

Table 2.1: Neighbor identity treatments in the field experiment. No neighbors were planted in the control treatment. The native and exotic mix treatments contained all three native and exotic species planted together each at a third of the densities as in single species treatments.

Neighbor identity	Abbreviation	Origin	Life history strategy
Bromus carinatus	BRCA	Native	Perennial
Festuca microstachys	FEMI	Native	Annual
Hordeum brachyantherum	HOBR	Native	Perennial
Native mix	NAMIX	Native	--
Bromus hordeaceus	BRHO	Exotic	Annual
Festuca myuros	FEMY	Exotic	Annual
Hordeum murinum	HOMU	Exotic	Annual
Exotic mix	EXMIX	Exotic	--
Control	CON	--	--

We used field collected seeds for focal species, with *B. diandrus* seeds sourced from Elliott Chaparral Reserve (32.889° N, 117.091° W) and *S. pulchra* seeds sourced from Sedgwick Reserve (34.692° N 120.043° W). To capture variation in emergence time occurring within each population, we used seeds collected from 12 maternal plants (hereafter referred to as maternal lines) spaced at least 5m apart. Focal seeds were collected in April 2015. For each focal species, we used 84 seeds in each of the nine neighbor identity treatments, with seven seeds from each maternal line (1512 focal seeds across both species in total). For each maternal line, we selected seeds that outwardly appeared and randomly assigned them to neighbor identity treatments. We weighed focal seeds individually prior to the experiment to account for effects of maternal provisioning which can influence emergence and subsequent growth (Roach and Wulff 1987). We obtained neighbor seeds through a commercial supplier (S&S Seeds, Carpinteria, California), with the exception of *H. murinum* seeds which we collected in bulk from the study site in February 2015. We stored all seeds in a refrigerator at 4° C until used in this experiment.

We established seven replicate 0.9 m × 1.05 m plots per neighbor identity treatment, arranged in blocks of nine plots that contained one plot for each neighbor identity treatment. Adjacent blocks were spaced 1.2 m apart and adjacent plots within each block were spaced 0.6 m apart. We randomly assigned neighbor identity treatments to plots in each block to account for possible spatial effects in the field. Prior to starting the experiment, we watered and tilled all plots to deplete the standing seed bank. In each plot, we planted 12 seeds of each focal species, one seed per maternal line. We planted seeds of both focal species into each plot, but with each focal species planted into two separate 6 × 2 arrangements in each plot half. We spaced adjacent conspecific focal individuals 15 cm from each other and left a 15 cm margin to plot edges. We left a 30 cm wide strip in the middle of each plot separating the focal individuals of each species. Because it was not possible to monitor the emergence of neighbor seeds for entire plots, we established 20 × 20 cm squares in the center of each plot which we used to monitor neighbor emergence and to estimate the density of emerging neighbors per square meter. We sowed seeds of neighbors evenly across plots at a target density of 900 emerging neighbors per square meter, with total seed numbers adjusted for emergence percentages observed in prior greenhouse trials. The density of seeds for each of the three species that we planted in mixed species treatments was therefore one third of their density in single species treatments. Seeds of focal species were planted inside 1.9 cm diameter PVC rings (0.5 cm depth, pressed into the soil) and marked with a plastic stake. We sowed neighbor seeds on 14 February 2017 and planted focal individuals by block over 14 -15 February in anticipation of a rain event that was forecasted for 17 February. We covered PVC rings while sowing competitor seeds to ensure that only focal individuals germinated within the rings and hence focal emergence could be accurately monitored. We planted focal seeds to a depth of 1 cm in the center of each PVC ring. The soil was dry while we planted all seeds; therefore, we gently applied 3 l of water across each plot on 16 February prior to the forecasted rain event on 17 February to help settle seeds into the soil and initiate germination. We supplied 3 l of water evenly across each plot twice a week, two days apart, until 13 May in the 2017 growing season and until 20 April in the 2018 growing season, except for weeks during which there was significant forecasted rain event. We carefully removed any non-grass weeds from plots as they emerged throughout the experiment.

We monitored the emergence of focal individuals and neighbors until 18 March 2017, at which point we had observed no individuals emerging for three consecutive days. We were unable to monitor any focal or neighbor emergence on 27 February due to a severe rainstorm; therefore, some individuals recorded as having emerged on 28 February may have emerged a day earlier. In the two mixed species treatments, we recorded each day the number of emerging neighbors for the three constituent species combined. We calculated the number of days to emergence from 16 February when we first watered plots.

We characterized water uptake from the soil in each neighbor identity treatment by measuring volumetric water content (VWC) from 0-15 cm depth using a FieldScout 150 soil moisture probe (Spectrum Technologies Inc., Aurora, Illinois USA) at five points in each plot (each corner and the center) over four consecutive days following a rain event on 8 May. To calculate the proportion of photosynthetically active radiation (PAR) intercepted by neighbor treatments, we measured PAR at waist height and at ground level along both diagonals of plots using an AccuPAR LP-80 PAR Ceptometer (Decagon Devices Inc., Washington USA) on 18 May on a clear afternoon.

We harvested seeds of *S. pulchra* and *B. diandrus* throughout the spring of 2017 once seeds had reached maturity but were still firmly attached to culms. At the end of the 2017 growing season, we harvested all neighbor aboveground biomass in plots around surviving *S. pulchra* plants from 31 July - 2 August 2017. We dried competitor biomass for each plot at 40 °C for three days before weighing. Beginning 18 December 2017, we re-established plots around the halves of plots containing the perennial *S. pulchra* and weeded them carefully so as not to disturb remaining focal individuals. On 18 January 2018, we re-sowed neighbor seeds in each plot, adjusting seed densities from the first growing season with the aim of achieving the original target density of 900 emerging individuals per square meter. We collected *S. pulchra* seeds throughout spring 2018 and harvested the aboveground biomass of each individual on 17 May 2018. We did not repeat measurements of community context in remaining plot halves during the 2018 growing season.

Statistical analyses

We conducted all statistical analyses using R version 3.6.1 (R Core Team 2019). We estimated broad-sense heritabilities of seed mass (as weighed prior to the experiment) and emergence time across treatments as the variance associated with maternal line (V_G) divided by the total phenotypic variance (V_P). We extracted variance components using restricted maximum likelihood (REML) models fit using the package *lme4* (Bates et al. 2015). For seed mass, we included maternal line as a random effect. For emergence time, we included both block and maternal line as random effects.

For each plot, we calculated the number of days to reach 50% of total neighbor emergence, the percentage of PAR intercepted, mean soil VWC (across the four days it was measured), total neighbor aboveground biomass, and density of emerging neighbors per square meter. We tested whether each measure of environmental context differed significantly between neighbor identity treatments (excluding the control treatment) with one-way ANOVAs.

For the annual *B. diandrus*, we used the total weight of seeds produced as a measure of fecundity. For the perennial *S. pulchra*, we used aboveground biomass after two growing seasons as a measure of fecundity. We used biomass rather than seed production as a measure of fecundity in the perennial *S. pulchra* because zero-values for seed production could be driven by both mortality or a failure to reproduce, and thus could mask differences in potential future reproduction. In contrast, zero values for biomass are only driven by mortality (i.e. the plant did not survive to harvest). Aboveground biomass of *S. pulchra* was strongly positively correlated with the total weight of seeds produced across the two growing seasons ($r = 0.86$, $P < 0.001$; Appendix Fig. 2A.1), hence we regarded it to be an appropriate estimate of fecundity. We modeled fitness using generalized linear mixed effects models (GLMMs) fit using the *mixed_model* function in the package *GLMMadaptive* (Rizopoulos 2019). Total seed production in *B. diandrus* and aboveground biomass in *S. pulchra* were zero-inflated (due to mortality) and over-dispersed; therefore, we modeled fitness using hurdle negative binomial mixed effects models that integrate both survival and fecundity fitness components. These models proceed in two parts, specifying a logistic regression for generating zero values (whether or not seeds or biomass were harvested due to mortality) and a negative binomial regression for non-zero values (seed mass or biomass given that any were harvested). We used hurdle models to estimate unconditional expected fitness; this represents the expected fitness for each individual while accounting for the probability of mortality, and thus integrates both survival and fecundity components. We tested the significance of fixed effects by comparing nested models with likelihood ratio (LR) tests at a significance level of $P = 0.05$.

For each focal species, we estimated the mean absolute fitness of plants in each neighbor identity treatment and tested whether this differed between neighbor identity treatments with a model containing neighbor identity treatment as the only fixed effect and block as a random effect. For phenotypic selection analyses, we modeled fitness as a function of days to emergence, seed mass and neighbor treatment. We included seed mass in phenotypic selection analyses because this trait can influence time to emergence (Simons and Johnston 2000, Susko and Lovett-Doust 2000) as well as directly influence fitness through provisioning effects (Halpern 2005). We standardized seed mass and days to emergence across treatments to a mean of zero and a standard deviation of one. We evaluated only linear trait effects in these analyses because a major goal of this experiment was to explore the associations between measures of community context in neighbor identity treatments and the strength of directional selection on emergence time; associations with more complex fitness functions would be less amenable to formal testing and interpretation with the limited number of neighbor identity treatments in this experiment. Thus, we did not consider quadratic trait effects or cross-products of trait effects.

For all treatments except the control, we evaluated whether neighbor identity was a significant predictor of selection on traits using hurdle models fit with neighbor treatment, trait effects, and trait × treatment interactions, with block treated as a random effect for both model parts. We tested the significance of overall linear trait effects against models in which the interaction between treatment and that trait had been dropped. When emergence time × neighbor identity treatment interactions were significant, we extracted unconditional expected fitness and estimated the strength of directional selection on emergence time from separate models for each neighbor treatment. We also extracted unconditional expected fitness and estimated the strength of directional selection on emergence time in the absence of competition using separate models for control treatments; these reflect hard selection imposed by the general environment under the experimental conditions. To estimate standardized linear selection gradients (β), we relativized unconditional expected fitness to a mean of one within neighbor treatments and carried out ordinary least squares regression with standardized emergence time and seed mass as predictors. We relativized fitness within treatments as is recommended for traits that are under strong soft selection (De Lisle and Svensson 2017).

Where directional selection on emergence time differed significantly between neighbor identity treatments, we investigated potential factors influencing directional selection. We first evaluated whether selection exerted by neighbor identity treatments was consistent in the two focal species by examining the Pearson correlation of the estimated linear selection gradients for *S. pulchra* and *B. diandrus* in each neighbor identity treatment. A significant, positive correlation indicates that neighbor identity treatments exerted consistent selection on each of the two focal species. We did not include the control treatment in this analysis.

To determine whether measures of community context in neighbor identity treatments influenced the strength of directional selection on emergence time, we examined the Pearson correlations of estimated linear selection gradients and each measure of community context that differed significantly between neighbor identity treatments, again excluding the control treatments. We evaluated whether the origin of neighbors (native vs. exotic) influenced directional selection on emergence time in each focal species using hurdle models fit with neighbor origin, trait effects, and trait × neighbor origin interactions as predictor variables, and neighbor identity treatment specified as a random effect for both model parts. We evaluated whether selection on emergence time differed between each mixed species treatment and its constituent single species treatments using hurdle models fit with treatment type (single vs. mixed species), trait effects, and trait × treatment type interactions as predictor variables, and neighbor identity treatment specified as a random effect for both model parts. We note that potential differences in selection between single and mixed species treatments could be driven by density-dependent and density-independent effects because the density of each constituent species in mixed species treatments was one third of their density in single species treatments.

Results

Measures of community context in neighbor identity treatments

We observed significant differences between non-control neighbor identity treatments for days to 50% emergence ($F_{(7)} = 7.60$, $P < 0.001$; Appendix Fig. 2A.2). Neighbor identity treatments also differed significantly in the percentage of PAR intercepted ($F_{(7)} = 8.23$, $P < 0.001$; Appendix Fig. 2A.2), the total aboveground biomass ($F_{(7)} = 15.6$, $P < 0.001$; Appendix Fig. 2A.2), and the density of individuals in plots ($F_{(7)} = 6.06$, $P < 0.001$; Appendix Fig. 2A.2). However, soil VWC did not differ significantly between neighbor identity treatments ($F_{(7)} = 1.25$, $P = 0.29$; Appendix Fig. 2A.2) and was therefore not used as a potential predictor of directional selection in subsequent analyses.

Emergence of focal individuals and trait heritabilities

80.6% of *B. diandrus* seeds emerged in total across the span of 24 days, with a mean time to 50% total emergence in each plot of 7.3 days. 91.3% of *S. pulchra* seeds emerged in total across the span of 20 days, with a mean time to 50% total emergence in each plot of 10.2 days.

Mean days to 50% total emergence in plots for *B. diandrus*, *S. pulchra* and neighbors in each neighbor identity treatment are shown in Appendix Fig. 2A.3. Estimated broad-sense heritabilities were lower in both species for emergence time (*S. pulchra*: $H^2 = 0.07$; *B. diandrus*: $H^2 = 0.31$) than for seed mass (*S. pulchra*: $H^2 = 0.38$; *B. diandrus*: $H^2 = 0.54$).

Influence of the surrounding community on mean fitness

Mean absolute expected fitness, incorporating survival and fecundity, differed significantly between neighbor identity treatments for both *B. diandrus* ($LR_{(14)} = 24.7$, $P = 0.038$) and *S. pulchra* ($LR_{(14)} = 85.5$, $P < 0.001$). Not including the control treatments, mean absolute expected fitness was negatively correlated with total neighbor aboveground biomass in both species (*B. diandrus*: $r = -0.91$, $P = 0.002$; *S. pulchra*: $r = -0.80$, $P = 0.018$; Appendix Table 2A.1 and Appendix Fig. 2A.4), indicating that aboveground neighbor biomass is a proxy for the average intensity of competition experienced by focal plants. Mean absolute expected fitness was not correlated with any other measures of community context in either focal species (Appendix Table 2A.1).

Influence of the surrounding community on selection on emergence time

For the exotic *B. diandrus*, directional selection overall favored earlier emergence in the presence of neighbors ($P = 0.002$; Table 2.2) but the strength of selection varied significantly among competitor identity treatments ($p = 0.048$; Table 2.2; Fig. 2.1). Standardized linear selection gradients for emergence time and their significance in each neighbor identity treatment are shown in Fig. 2.1 (full results of LR tests for each treatment are provided in Appendix Table 2A.2). Seed mass was not a significant overall predictor of fitness for *B. diandrus* ($P = 0.25$; Table 2.2), and this did not differ between neighbor identity treatments ($P = 0.75$; Table 2.2). In the control treatment without neighbors, there was no significant directional selection on emergence time (*P* = 0.94; Appendix Table 2A.2).

For the native *S. pulchra*, directional selection overall favored earlier emergence in the presence of neighbors (*P* < 0.001; Table 2.2), and the strength of selection differed significantly among neighbor identity treatments (*P* < 0.001 Table 2.2; Fig. 2.2). Standardized linear selection gradients for emergence time and their significance in each neighbor identity treatment are shown in Fig. 2.2 (full results of LR tests for each treatment are provided in Appendix Table 2A.2). Directional selection overall favored larger seeds (*P* < 0.001 Table 2.2), but this did not differ significantly among neighbor identity treatments (*P* = 0.46 Table 2.2). In the control treatment, there was no significant directional selection on emergence time (*P* = 0.22; Appendix Table 2A.2).

Table 2.2: Likelihood ratio (LR) tests testing for significant linear selection in neighbor identity treatments. Significant trait × treatment interactions indicate that selection differs between treatments, for which we tested the significance of selection in separate models for each treatment. We tested overall trait effects against models in which we dropped corresponding trait × treatment interactions.

B. diandrus		
Trait	LR Trait	LR Interaction
Emergence time	**$LR_{(2)} = 12.2$** **$P = 0.002$**	**$LR_{(14)} = 23.9$** **$P = 0.048$**
Seed mass	$LR_{(2)} = 2.78$ $P = 0.25$	$LR_{(14)} = 10.2$ $P = 0.75$
S. pulchra		
Trait	LR Trait	LR Interaction
Emergence time	**$LR_{(2)} = 26.7$** **$P < 0.001$**	**$LR_{(14)} = 38.8$** **$P < 0.001$**
Seed mass	**$LR_{(2)} = 22.6$** **$P < 0.001$**	$LR_{(14)} = 13.9$ $P = 0.46$

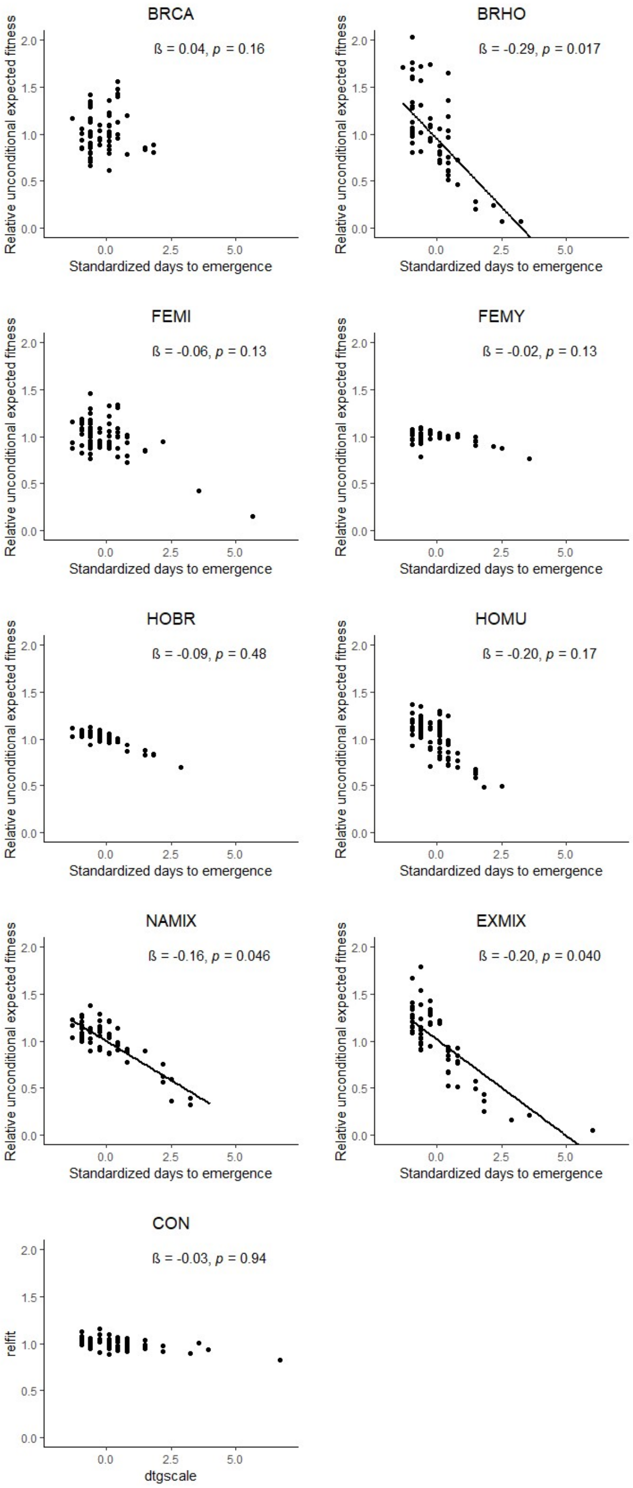

Figure 2.1: Selection on emergence time in *B. diandrus* in response to variation in neighbor identity. We standardized emergence time to a mean of 0 and standard deviation of 1 across caging treatments. We relativized predicted fitness to a mean of one within each caging treatment. Abbreviations for each neighbor identity treatment are listed in Table 2.1.

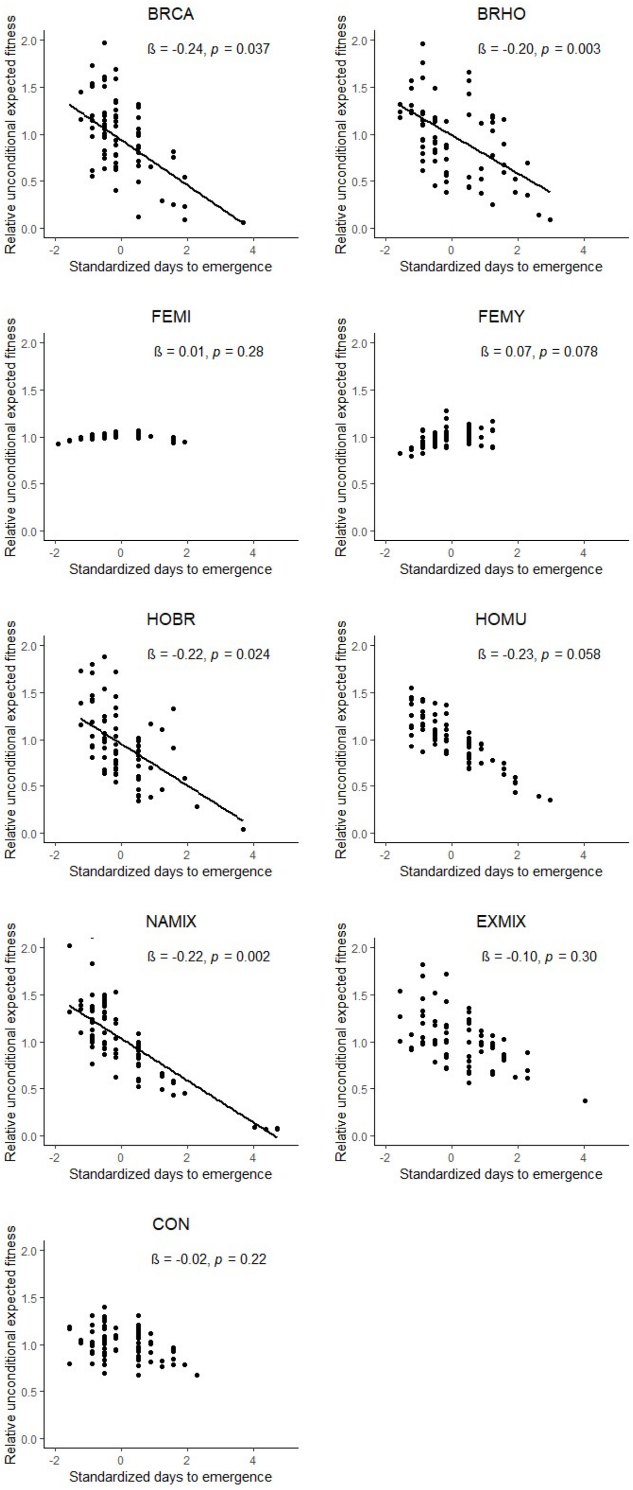

Figure 2.2: Selection on emergence time in *S. pulchra* in response to variation in neighbor identity. We standardized emergence time to a mean of 0 and standard deviation of 1 across caging treatments. We relativized predicted fitness to a mean of one within each caging treatment. Abbreviations for each neighbor identity treatment are listed in Table 2.1.

Mechanisms underlying selection on emergence time

Directional selection on emergence time exerted by neighbor identity treatments was not concordant between the two focal species, as shown by a nonsignificant correlation between estimated linear selection gradients for *B. diandrus* and *S. pulchra* ($r = 0.31$, $P = 0.46$; Fig. 2.3).

Estimated standardized linear selection gradients in *B. diandrus* were not significantly correlated with any measure of community context days to 50% total neighbor emergence ($r = 0.43$, $P = 0.28$; Fig. 2.4A), the percentage of PAR intercepted ($r = -0.01$, $P = 0.98$; Fig. 2.4B), total neighbor aboveground biomass ($r = -0.42$, $P = 0.30$), or neighbor density ($r = -0.08$, $P = 0.85$). In *B. diandrus*, selection for earlier emergence was significantly stronger in the presence of exotic neighbors than native neighbors ($LR_{(2)} = 7.28$, $P = 0.026$, Fig. 2.4C). Selection in *B. diandrus* was not significantly different in the native mix compared to its constituent single species treatments ($LR_{(2)} = 0.53$, $P = 0.77$) or in the exotic mix compared to its constituent single species treatments ($LR_{(2)} = 1.57$, $P = 0.46$).

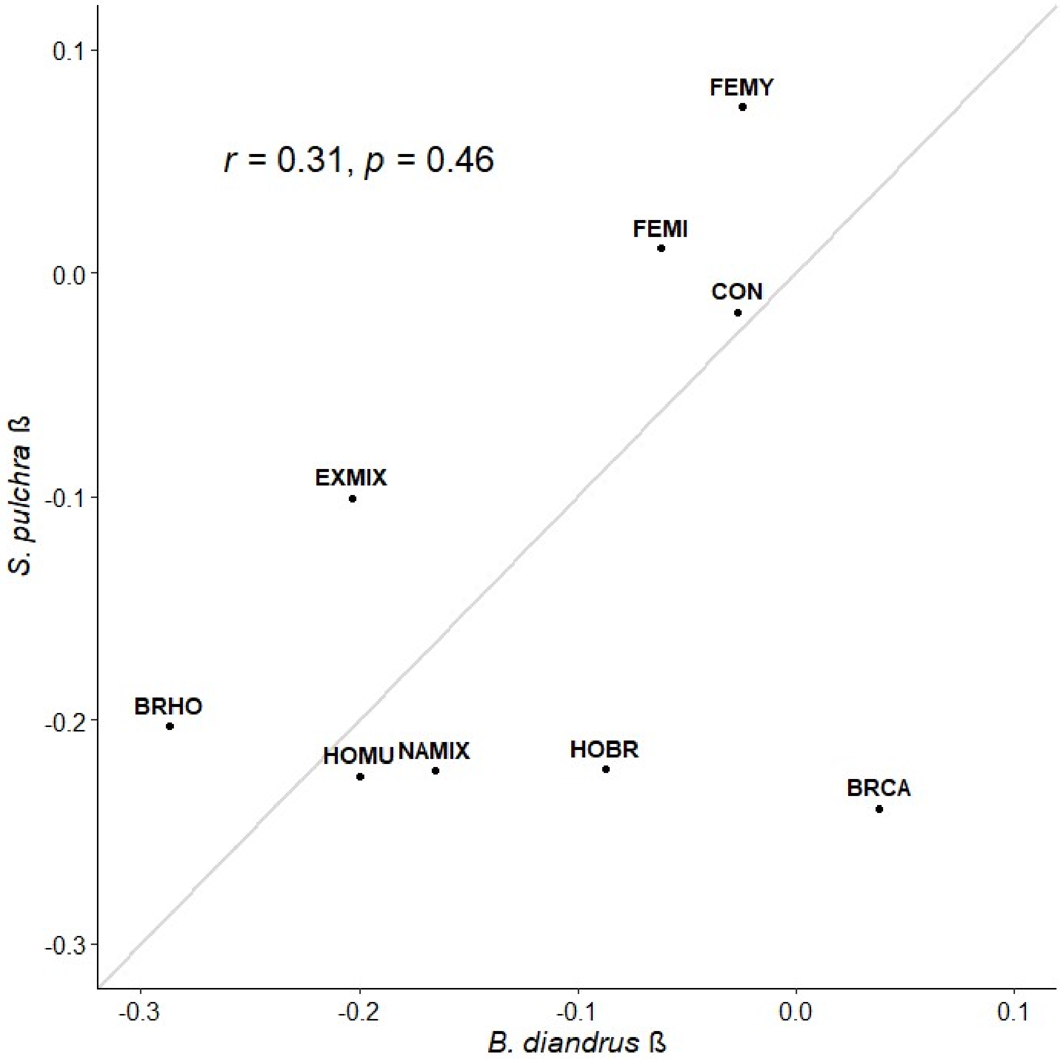

Figure 2.3: Scatter plot showing estimated linear selection gradients for *S. pulchra* and *B. diandrus* in each neighbor identity treatment. Selection gradients in the control treatments are included for reference but were not included when calculating and testing the significance of the Pearson correlation. The grey line represents the identity line on which selection gradients would be the same for both focal species.

Estimated standardized linear selection gradients in *S. pulchra* were weakly correlated with the mean days to 50% total neighbor emergence (r = -0.63, P = 0.095; Fig. 2.5A), with a trend of stronger selection in competition with neighbors that emerged later. Selection gradients were also weakly correlated with the percentage of PAR intercepted (r = -0.68, P = 0.063; Fig. 2.5B), with a trend of stronger directional selection for earlier emergence in neighbor identity treatments that intercepted a greater percentage of PAR. Estimated selection gradients were not significantly correlated with total neighbor aboveground biomass (r = 0.14, P = 0.73) or neighbor density (r = 0.50, P = 0.21). For *S. pulchra*, selection for earlier emergence did not differ significantly between native and exotic neighbor identity treatments ($LR_{(2)}$ = 1.15, P = 0.56, Fig. 2.5C). As with *B. diandrus*, selection in *S. pulchra* was not significantly different in the native mix compared to its constituent single species treatments ($LR_{(2)}$ = 1.11, P = 0.58) or in the exotic mix compared to its constituent single species treatments ($LR_{(2)}$ = 1.45, P = 0.48).

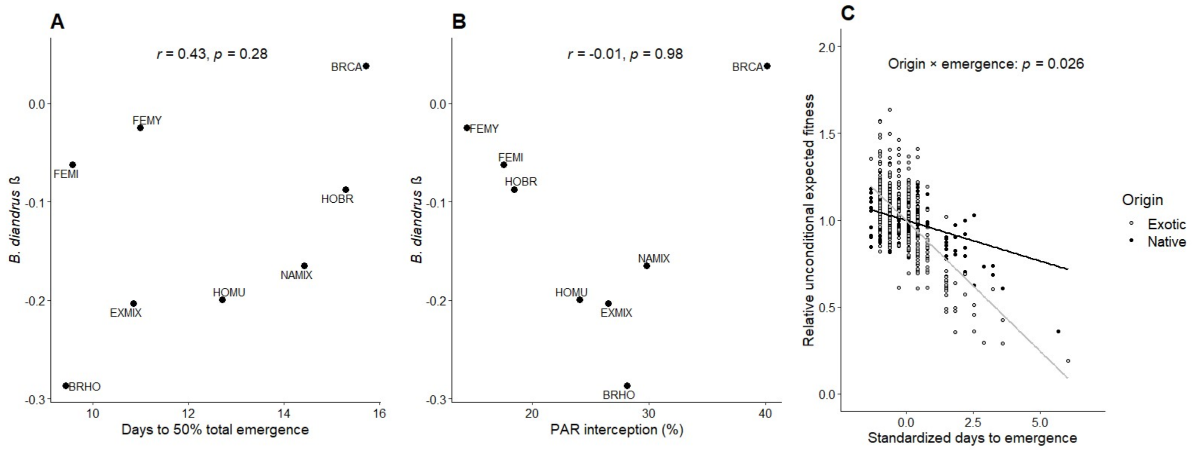

Figure 2.4: Influence of neighbor emergence time (*A*), neighbor PAR interception (*B*), and neighbor origin (*C*) on the strength of directional selection on emergence time in *B. diandrus*. Points in (*A, B*) represent values of estimated standardized linear selection gradients (β) and values for each measure of community context in neighbor identity treatments. We tested for associations between estimated linear selection gradients and each measure of community context by examining Pearson correlations. Points in (*C*) represent relativized unconditional expected fitness for each individual from separate hurdle models for each neighbor origin group. We tested for differences in selection between neighbor origin groups with a hurdle model fit with neighbor origin, trait effects, and trait × neighbor origin interactions as predictor variables, and neighbor identity treatment specified as a random effect for both model parts.

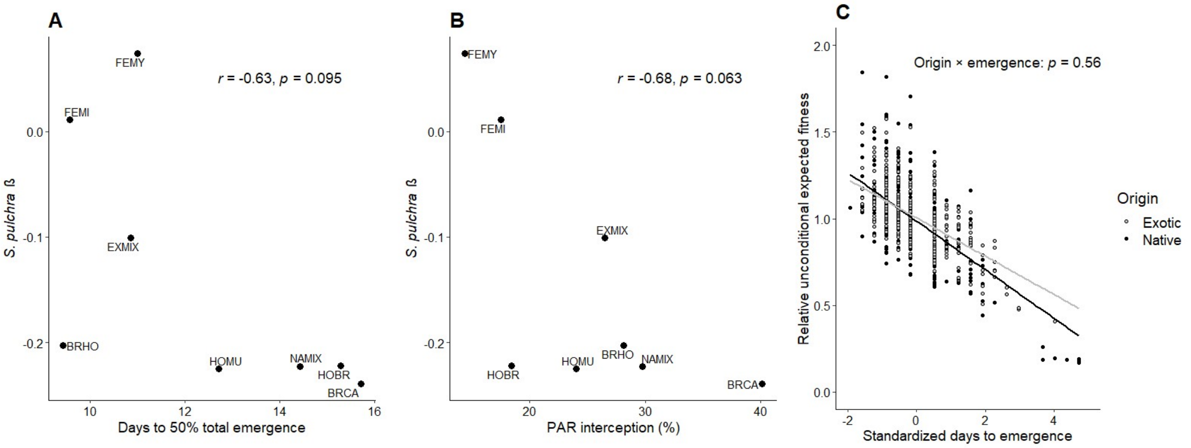

Figure 2.5: Influence of neighbor emergence time (*A*), neighbor PAR interception (*B*), and neighbor origin (*C*) on the strength of directional selection on emergence time in *S. pulchra*. Points in (*A, B*) represent values of estimated standardized linear selection gradients (β) and values for each measure of community context in neighbor identity treatments. We tested for associations between estimated linear selection gradients and each measure of community context by examining Pearson correlations. Points in (*C*) represent relativized unconditional expected fitness for each individual from separate hurdle models for each neighbor origin group. We tested for differences in selection between neighbor origin groups with a hurdle model fit with neighbor origin, trait effects, and trait × neighbor origin interactions as predictor variables, and neighbor identity treatment specified as a random effect for both model parts.

Discussion

It has long been recognized that interactions with neighboring plants are a key factor influencing the fitness effects of variation in emergence time (Ross and Harper 1972). Our results demonstrate clearly that plant community composition shapes selection acting on emergence time in species embedded within. However, the selective effect of competitive neighborhoods was not consistent between *S. pulchra* and *B. diandrus* (Fig. 2.3)*,* suggesting that the evolutionary consequences of interactions with the surrounding community should be evaluated separately for constituent species individually. We found limited support for the hypothesis that light interception and emergence timing — at the community level — influence selection on emergence time in *S. pulchra*; however, neither these nor any other measures of community context were associated with selection in *B. diandrus*. We found that exotic neighbors exerted stronger selection for earlier emergence in the exotic *B. diandrus*, but neighbor origin did not predict the strength of selection for earlier emergence in the native *S. pulchra*. We also found that the presence of multiple species did not intensify selection for earlier emergence in either focal species. Together, our findings demonstrate that community composition can play a key role in determining the strength of selection on emergence time, although further work is necessary to elucidate the underlying mechanisms. Furthermore, shifts in community composition resulting from species invasions or climate change are likely to alter patterns of selection on this important fitness-related trait.

Influence of the surrounding community on selection on emergence time

As expected, earlier emergence was favored by selection across all neighbor identity treatments in both focal species. This finding is consistent with the results of meta-analysis that show earlier emergence tends to enhance fitness (Verdú and Traveset 2005). We are confident that selection favouring earlier emergence in the presence of neighbors is a result of competition because selection on emergence time in the control treatments was not significant (Figs. 2.1 and 2.2), indicating that hard selection imposed by the general environment under the experimental conditions was negligible. Selection favouring earlier emergence resulting from competitive interactions may have been counterbalanced by processes that increase the fitness of late-emerging plants. For example, individuals that emerged earlier may be more impacted by mammalian herbivory due to increased apparency (Waterton and Cleland 2016) and benefit less from any amelioration of abiotic stress provided by earlier-emerging plants (Leverett 2017).

Significant emergence time × neighbor identity treatment interactions in both *S. pulchra* and *B. diandrus* indicated that community composition influenced directional selection on emergence time. This effect was apparent despite neighbor identity treatments containing only grass species and thus representing a fraction of the potential phylogenetic and functional diversity present in many natural communities. The significant effect of neighbor identity on selection on emergence time that we observed is consistent with a previous study by Weinig (2000), which found that selection for earlier emergence in velvetleaf (*Abutilon theophrasti)* was intensified when grown in a diverse community of early-active weed species, as compared to competitive environments with later-active crops. It is well established that variation in the abiotic environment drives variable selection on emergence time over time and space (Kalisz 1986, Donohue 2002, Donohue et al. 2005), and is typically the main focus of studies exploring the causes of substantial variation in emergence time harbored within and among natural populations (reviewed in Cochrane et al. 2015). Our results confirm that variation in community composition is an important, understudied, and potentially widespread selective force contributing to such variation.

Mechanisms underlying selection on emergence time

Estimated linear selection gradients for *S. pulchra* and *B. diandrus* in each neighbor identity treatment were not significantly correlated with one another, indicating that the selective effect of a given community can vary between the species embedded within. However, as we characterized selection for only two focal species in each community, we could not determine the mechanisms underlying these differences.

We characterized several aspects of community context to identify local factors that may influence the strength of selection on emergence time. Soil VWC was highly variable across the experimental site and did not differ significantly between neighbor identity treatments; thus, we were unable to test whether water uptake affected patterns of selection. Higher plant density can intensify selection for earlier emergence (Weis et al. 2015), but the density of emerging neighbors alone was not significantly correlated with the estimated selection gradients in either *B. diandrus* or *S. pulchra*. The potential effects on selection of higher initial population density may have been offset by smaller individual plant sizes (and subsequent mortality) due to self-thinning, and thus population density might not reflect the realized intensity of competition (Schmitt et al. 1987). Total aboveground biomass of the competitors within plots was also not associated with the strength of directional selection in either species. The significant negative correlations between aboveground biomass and mean absolute fitness in both focal species (Appendix Fig. 2A.3) suggest that aboveground biomass reflects the average intensity of competition, but this did not translate to greater differences in relative fitness between earlier and later emerging individuals.

In the native *S. pulchra*, there was a non-significant trend towards stronger selection in treatments that intercepted more light (Fig. 2.5B). In contrast to belowground competition for resources, light competition tends to be highly size-asymmetric, such that larger plants capture disproportionately more light than smaller plants due to the directionality of supply (Schwinning and Weiner 1998). As a result, communities that intercept greater amounts of light might have larger impacts on the environment experienced by late-emerging individuals, leading to stronger priority effects (Vannette and Fukami 2014). Weinig (2000) also found that selection for earlier emergence in velvetleaf (*Abutilon theophrasti*) was strongest in weedy environments that intercepted the most sunlight. While light interception alone was not significantly associated with estimated selection gradients in *B. diandrus*, additional support for a role of light interception in this study comes from the consistently non-significant selection in both focal species when growing with the two species that intercepted the least light, *Festuca microstachys* and *F. myuros*.

We also observed a nonsignificant trend towards stronger selection for earlier emergence in *S. pulchra* in communities that emerged later (Fig. 2.5A). Earlier emergence may have increased relative fitness to a greater extent in later-emerging communities by allowing individuals to emerge before the surrounding community and thus pre-empt resources, which may not have been possible in the earliest-emerging communities. Some of the late-emerging communities that exerted strong selection for earlier emergence in *S. pulchra* (such as the treatments sown with *Bromus carinatus* and *Hordeum brachyantherum*) did not exert significant selection on emergence time in *B. diandrus*. We hypothesize that this occurred because *B. diandrus* emerged on average 3 days earlier than *S. pulchra*, thus even later-emerging individuals likely emerged earlier than the late-emerging community. Another possible explanation for this difference is that, as perennial species that tend to invest more into belowground growth (Vico et al. 2016), late-emerging *B. carinatus* and *H. brachyantherum* have greater niche overlap with the perennial *S. pulchra* than with the annual *B. diandrus* which resulted in stronger priority effects in the former. Regardless of the underlying mechanisms, our results clearly indicate that communities that emerge rapidly do not always result in the stronger selection for earlier emergence. Thus, while early emergence has been identified as a key factor contributing to the competitive dominance of exotic species in California and elsewhere (Reynolds et al. 2001, Deering and Young 2006, Abraham et al. 2009, Wainwright et al. 2012, Wainwright and Cleland 2013, Vaughn and Young 2015, Gioria and Pyšek 2016, Waterton and Cleland 2016), our results suggest that earlier emergence *per se* does not result in stronger selection for earlier emergence in other species embedded within plant communities.

None of the measures of community context in each neighbor identity treatment were strongly associated with the strength of selection for earlier emergence, particularly for *B. diandrus*. One potential reason is that other aspects of community context that we did not measure in this study are important factors influencing selection. We therefore suggest that future studies characterize a wider range of measures of community context. Another potential reason is that selection on emergence time is driven by interactions between multiple aspects of community context, such as light interception and emergence time, as well as the traits of focal species. However, with only eight neighbor identity treatments (excluding the control) and two focal species we were unable to test for such interactions. Given the large sample sizes that generally are required to detect natural selection in the field (Hersch and Phillips 2004), determining how and whether different aspects of community context and focal species traits interact to affect the intensity or direction of selection will likely require much larger-scale experiments.

Numerous studies have shown that exotic plant species can influence the evolutionary trajectories of co-occurring species (e.g. Callaway et al. 2005, Lau 2008, Leger 2008, Lankau 2012, Beans and Roach 2015). We found that exotic neighbors exerted significantly stronger selection for earlier emergence in the exotic *B.* diandrus. This finding is consistent with those of several studies showing that exotic species exert stronger priority effects when arrival time is manipulated experimentally (Dickson et al. 2012, Wilsey et al. 2015, Stuble et al. 2016, Goodale and Wilsey 2018). That exotic neighbors exerted stronger selection for earlier emergence in the exotic annual *B. diandrus*, but not in the native perennial *S. pulchra*, could reflect higher niche overlap between exotic annual neighbors and *B. diandrus*, which would be expected to result in stronger priority effects experienced by late-emerging plants (Vannette and Fukami 2014). Furthermore, if exotic neighbors tend to generate stronger selection for earlier emergence in co-occurring exotics compared to co-occurring natives, this could represent an evolutionary mechanism by which many exotic herbaceous species in California and elsewhere maintain their competitive advantage of earlier emergence (Reynolds et al. 2001, Deering and Young 2006, Abraham et al. 2009, Wainwright et al. 2012, Wainwright and Cleland 2013, Vaughn and Young 2015, Waterton and Cleland 2016). In general, this result suggests that changes in community composition resulting from species invasions could cause chronic shifts in the intensity of selection on emergence time. Furthermore, such changes in patterns of selection may be predictable because exotic species tend to differ from co-occurring natives in a variety of functional traits (Leishman et al. 2007, van Kleunen et al. 2010, Godoy et al. 2011). Therefore, studies that identify the aspects of community context that most strongly determine selection on emergence time (or combinations thereof) are likely to be the most effective approach to predict how species invasions will alter the evolution of this trait.

Diverse plant communities can limit the ability of later-arriving species to establish through complementarity in the use of resources, such as light, as well as greater probabilities of possessing species that strongly suppress establishment (i.e., sampling effects) (Fargione and Tilman 2005, Mwangi et al. 2007, Zhu et al. 2015). However, we found no evidence that the presence of multiple neighbor species resulted in stronger selection for earlier emergence. It is possible that each set of three species in mixed species treatments exhibit low complementarity in resource use, and thus do not result in stronger priority effects on late-emerging plants. Alternatively, each set of three species may have indeed exhibited complementarity in resource use, but for resources that did not generate significantly stronger priority effects. Furthermore, the strength of selection on emergence time can increase with plant density (Weis et al. 2015); thus, the effects of complementarity in resource use on the strength of selection for earlier emergence may have been offset by the effects of reduced densities of each constituent neighbor species in mixed species treatments. However, given the limited number of mixed species treatments examined here, we were unable to explore more broadly the potential for the diversity of neighboring communities to influence selection on emergence time in competition. We suggest that studies that test the mechanisms by which functional diversity or species diversity influences resistance to invasion by later-arriving species (e.g. Fargione and Tilman 2005, Mwangi et al. 2007, Zhu et al. 2015) will be useful for informing hypotheses regarding how species are likely to interact to determine selection on emergence time.

Implications for evolutionary adaptation to climate change

Adaptive evolution in the timing of emergence is considered to be an important component of *in situ* responses to climate change that will promote long-term species persistence (Walck et al. 2011, Cochrane et al. 2015). Most studies that aim to predict how plant populations will adaptively evolve and persist under climate change have focused on the direct effects of climate warming or drought (Etterson and Shaw 2001, Franks et al. 2007, Anderson et al. 2012, Dickman et al. 2019), but rarely consider the potential for plant-plant interactions to mediate adaptive evolutionary responses. The composition of plant communities across the globe is changing directly as a result of climate change, with plant species being lost from communities or moving into new areas (Harrison et al. 2015, Kopp and Cleland 2015, Liu et al. 2018). The results presented here demonstrate that such changes in the taxonomic composition of a species' local competitors resulting from climate change are likely affect the strength of phenotypic selection on emergence time. Such changes in the intensity of selection on emergence timing could potentially promote or impede adaptation to climate change depending on whether they are concordant with or antagonistic to long-term changes in selection due to climate change. Furthermore, if such changes in the intensity of selection cause the mean emergence time of select native or exotic taxa to evolve, then the nature of antagonistic inter-specific interactions are likely to change as well (Dickson et al. 2012, Cleland et al. 2015, Vaughn and Young 2015, Wilsey et al. 2015, Stuble et al. 2016, Goodale and Wilsey 2018), potentially resulting in cascading effects on the relative abundances and persistence of co-occurring species.

Acknowledgements

This work was supported by an Institute for the Study of Ecological Effects of Climate Impacts (ISEECI) Graduate Student Researcher Fellowship and Jeanne M. Messier Memorial Fellowship to J.W. We thank members of the Cleland lab for help in the field and lab. We also thank Jill Anderson, Susana Wadgymar and Marcus Warwell for helpful advice with statistical analyses.

Chapter 2, in full, is currently being prepared for submission for publication of the material. Waterton, J., Mazer, S.J., and E.E. Cleland. The dissertation author is the primary investigator and author of this paper.

Appendix 2A

Table 2A.1: Pearson correlations of mean absolute expected fitness and each measure of community context that differed significantly between neighbor identity treatments.

	Focal species	
Measure of community context	*B. diandrus*	*S. pulchra*
Total aboveground biomass	***r* = -0.91** ***P* = 0.002**	***r* = -0.80** ***P* = 0.018**
Days to 50% total emergence	*r* = 0.39 *P* = 0.35	*r* = 0.49 *P* = 0.22
PAR interception (%)	*r* = -0.32 *P* = 0.44	*r* = -0.01 *P* = 0.98
Plant density (/m^2)	*r* = -0.23 *P* = 0.58	*r* = -0.44 *P* = 0.27

Table 2A.2: Likelihood ratio (LR) tests testing for significant directional selection on emergence timing in each neighbor identity treatment.

	Focal species	
Neighbor identity treatment	*B. diandrus*	*S. pulchra*
Bromus carinatus	$LR_{(2)}$ = 3.67 *P* = 0.16	**$LR_{(2)}$ = 6.58** ***P* = 0.037**
Festuca microstachys	$LR_{(2)}$ = 4.02 *P* = 0.13	$LR_{(2)}$ = 2.57 *P* = 0.28
Hordeum brachyantherum	$LR_{(2)}$ = 1.47 *P* = 0.48	**$LR_{(2)}$ = 7.44** ***P* = 0.024**
Native mix	**$LR_{(2)}$ = 6.14** ***P* = 0.046**	**$LR_{(2)}$ = 12.0** ***P* = 0.002**
Bromus hordeaceus	**$LR_{(2)}$ = 8.15** ***P* = 0.017**	**$LR_{(2)}$ = 11.4** ***P* = 0.003**
Festuca myuros	$LR_{(2)}$ = 4.12 *P* = 0.13	$LR_{(2)}$ = 5.11 *P* = 0.078
Hordeum murinum	$LR_{(2)}$ = 3.52 *P* = 0.17	$LR_{(2)}$ = 5.69 *P* = 0.058
Exotic mix	**$LR_{(2)}$ = 6.46** ***P* = 0.040**	$LR_{(2)}$ = 2.40 *P* = 0.30
Control	$LR_{(2)}$ = 0.12 *P* = 0.94	$LR_{(2)}$ = 3.01 *P* = 0.22

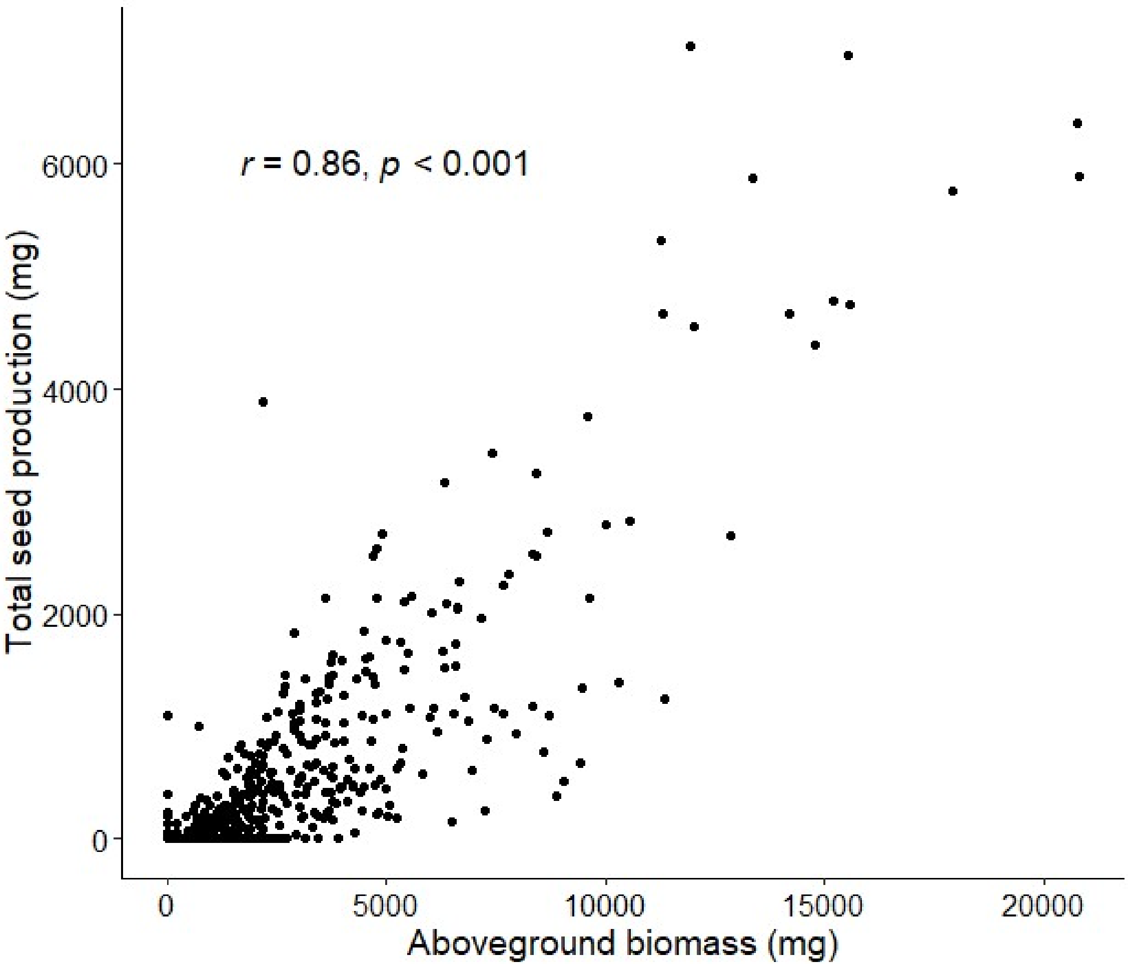

Figure 2A.1: Scatterplot of *S. pulchra* aboveground biomass and total seed production across both growing seasons.

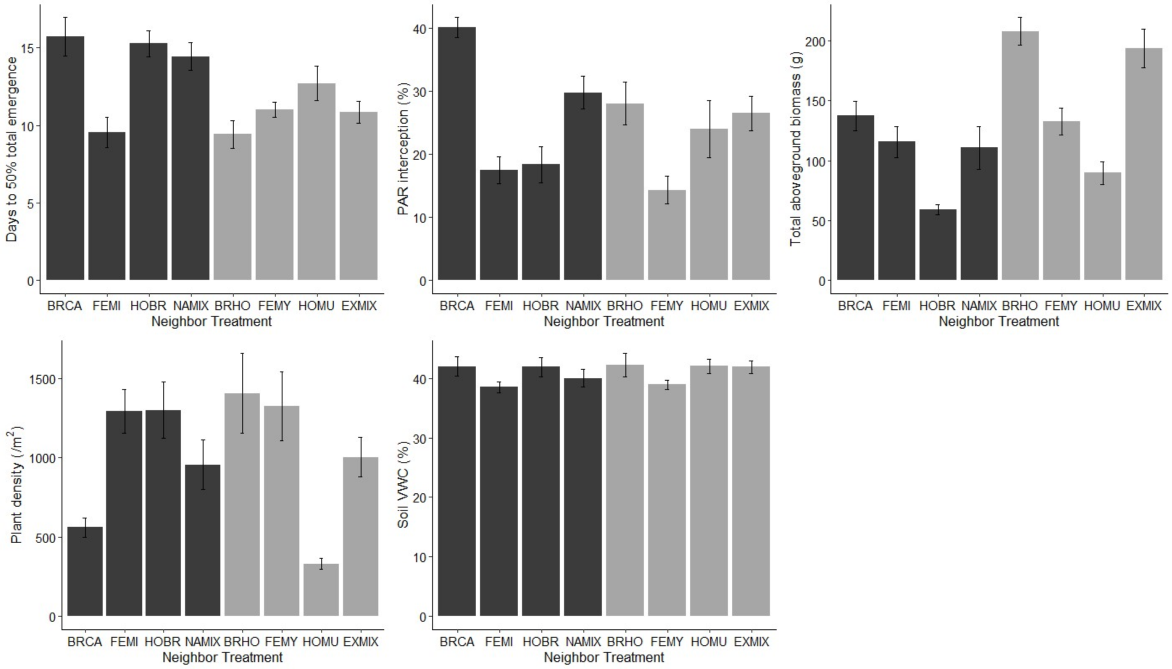

Figure 2A.2: Bar plots of measures of community context in neighbor identity treatments. Dark grey and light grey bars represent treatments with native and exotic species, respectively. Error bars indicate the standard error, where *n* = 7, the number of plots for each neighbor identity treatment. Abbreviations for each neighbor identity treatment are listed in Table 2.1.

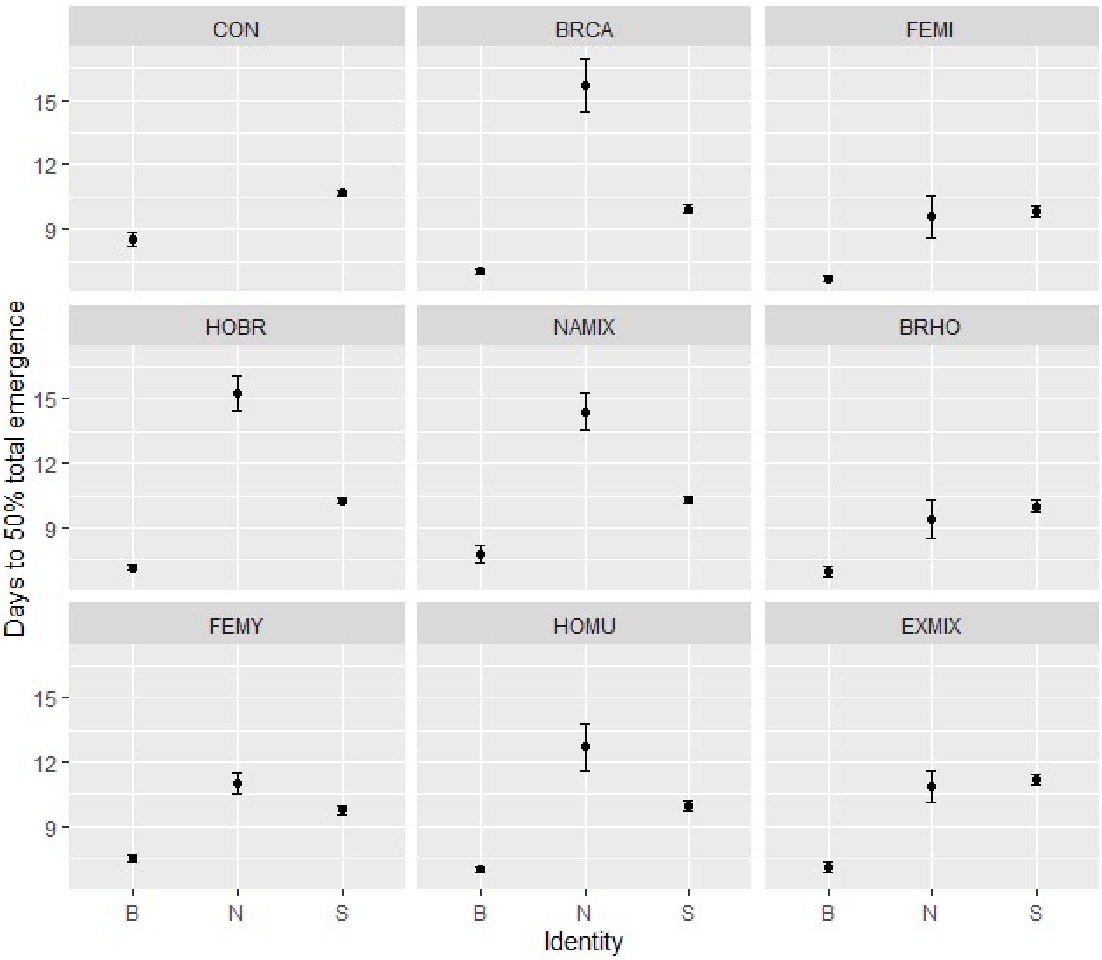

Figure 2A.3: Mean days to 50% total emergence in plots for *S. pulchra* (S), *B. diandrus* (B), and neighbors (N) in each neighbor identity treatment. Error bars represent the standard error, where *n* = 7 for neighbors and *n* = 63 for *S. pulchra* and *B. diandrus.* Abbreviations for each neighbor identity treatment are listed in Table 2.1.

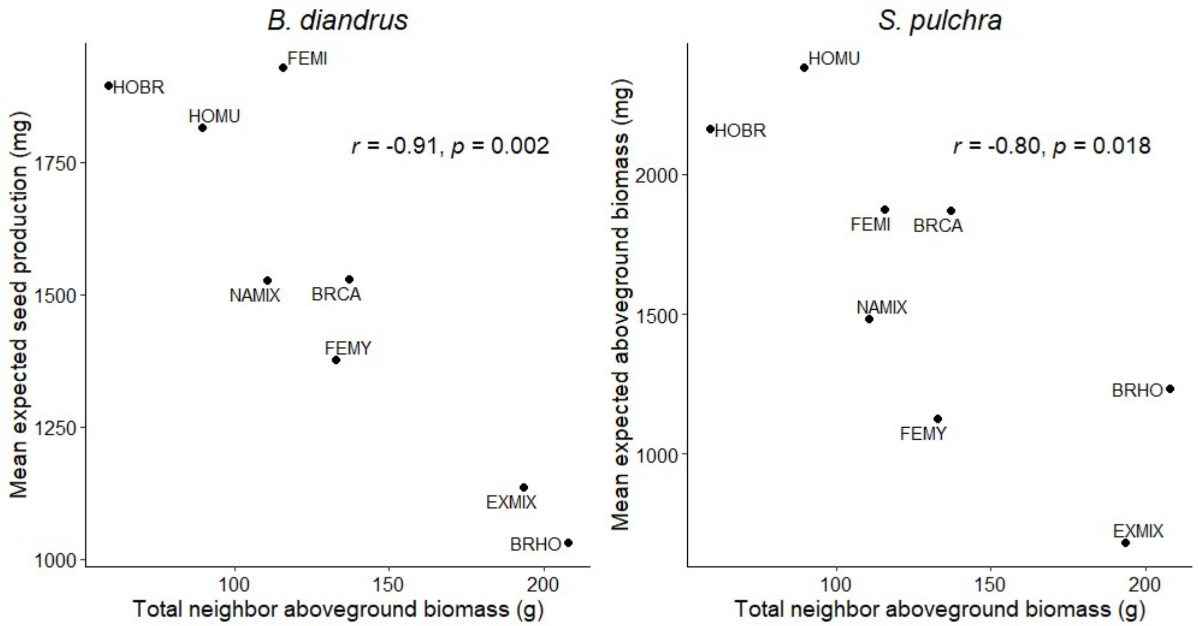

Figure 2A.4: Scatter plots of mean absolute fitness (total seed production for *B. diandrus* and aboveground biomass for *S. pulchra*) and total neighbor aboveground biomass in plots.

References

Abraham, J. K., J. D. Corbin, and C. M. D'Antonio. 2009. California native and exotic perennial grasses differ in their response to soil nitrogen, exotic annual grass density, and order of emergence. Plant Ecology **201**:445-456.

Anderson, J. T., D. W. Inouye, A. M. McKinney, R. I. Colautti, and T. Mitchell-Olds. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proc Biol Sci **279**:3843-3852.

Baldwin, B. G., D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken, editors. 2012. The Jepson manual: vascular plants of California. 2nd edition. University of California Press, Berkeley.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software **67**:1-48.

Beans, C. M., and D. A. Roach. 2015. An invasive plant alters phenotypic selection on the vegetative growth of a native congener. American Journal of Botany **102**:217–224.

Callaway, R. M., W. M. Ridenour, T. Laboski, T. Weir, and J. M. Vivanco. 2005. Natural selection for resistance to the allelopathic effects of invasive plants. Journal of Ecology **93**.

Cleland, E. E., E. Esch, and J. McKinney. 2015. Priority effects vary with species identity and origin in an experiment varying the timing of seed arrival. Oikos **124**:33-40.

Cochrane, A., C. J. Yates, G. L. Hoyle, and A. B. Nicotra. 2015. Will among-population variation in seed traits improve the chance of species persistence under climate change? Global Ecology and Biogeography **24**:12-24.

De Lisle, S. P., and E. I. Svensson. 2017. On the standardization of fitness and traits in comparative studies of phenotypic selection. Evolution **71**:2313-2326.

Deering, R. H., and T. P. Young. 2006. Germination speeds of exotic annual and native perennial grasses in California and the potential benefits of seed priming for grassland restoration. Grasslands **16**:14-16.

Dickman, E. E., L. K. Pennington, S. J. Franks, and J. P. Sexton. 2019. Evidence for adaptive responses to historic drought across a native plant species range. Evolutionary Applications.

Dickson, T. L., J. L. Hopwood, and B. J. Wilsey. 2012. Do priority effects benefit invasive plants more than native plants? An experiment with six grassland species. Biological Invasions **14**:2617-2624.

Donohue, K. 2002. Germination timing influences natural selection on life-history characters in Arabidopsis thaliana. Ecology **83**:1006-1016.

Donohue, K., L. Dorn, C. Griffith, E. Kim, A. Aguilera, C. R. Polisetty, and J. Schmitt. 2005. The evolutionary ecology of seed germination of Arabidopsis thaliana: variable natural selection on germination timing. Evolution **59**:758.

Etterson, J. R., and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. Science **294**:151-154.

Fargione, J. E., and D. Tilman. 2005. Diversity decreases invasion via both sampling and complementarity effects. Ecology Letters **8**:604-611.

Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences USA **104**:1278-1282.

Funk, J. L., R. J. Standish, W. D. Stock, and F. Valladares. 2016. Plant functional traits of dominant native and invasive species in mediterranean- climate ecosystems. Ecology **97**:75-83.

Gioria, M., and P. Pyšek. 2016. Early bird catches the worm: germination as a critical step in plant invasion. Biological Invasions **19**:1055-1080.

Gioria, M., P. Pysek, and B. A. Osborne. 2018. Timing is everything: does early and late germination favor invasions by herbaceous alien plants? Journal of Plant Ecology **11**:4-16.

Godoy, O., F. Valladares, and P. Castro-Diez. 2011. Multispecies comparison reveals that invasive and native plants differ in their traits but not in their plasticity. Functional Ecology **25**:1248-1259.

Goodale, K. M., and B. J. Wilsey. 2018. Priority effects are affected by precipitation variability and are stronger in exotic than native grassland species. Plant Ecology **219**:429-439.

Halpern, S. L. 2005. Sources and consequences of seed size variation in Lupinus perennis (Fabaceae): Adaptive and non-adaptive hypotheses. American Journal of Botany **92**:205-213.

Harrison, S. P., E. S. Gornish, and S. Copeland. 2015. Climate-driven diversity loss in a grassland community. Proceedings of the National Academy of Sciences **112**:8672–8677.

Heady, H. F. 1977. Valley grassland. Pages 491–514 *in* M. G. Barbour and J. Major, editors. Terrestrial vegetation in California. Wiley, New York.

Hersch, E. I., and P. C. Phillips. 2004. Power and potential bias in field studies of natural selection. Evolution **58**:479-485.

Kalisz, S. 1986. Variable selection on the timing of germination in Collinsia verna (Scrophulariaceae). Evolution **40**:479.

Kon, K. F., and W. M. Blacklow. 1990. Polymorphism, outcrossing and polyploidy in *Bromus diandrus* and *B. rigidus* Australian Journal of Botany **38**:609–618.

Kopp, C. W., and E. E. Cleland. 2015. A range-expanding shrub species alters plant phenological response to experimental warming. PLoS One **10**:e0139029.

Kuebbing, S. E., M. A. Nuñez, and D. Simberloff. 2013. Current mismatch between research and conservation efforts: The need to study co-occurring invasive plant species. Biological Conservation **160**:121-129.

Lankau, R. A. 2012. Coevolution between invasive and native plants driven by chemical competition and soil biota. Proceedings of the National Academy of Sciences USA **109**:11240–11245.

Larson, S. R., E. Cartier, C. L. McCracken, and D. Dyer. 2001. Mode of reproduction and amplified fragment length polymorphism variation in purple needlegrass (Nassella pulchra): utilization of natural germplasm sources. Molecular Ecology **10**:1165-1177.

Lau, J. A. 2008. Beyond the ecological: Biological invasions alter natural selection on a native plant species. Ecology **89**:1023–1031.

Lavorel, S., and E. Garnier. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional Ecology **16**:545-556.

Leger, E. A. 2008. The adaptive value of remnant native plants in invaded communities: an example from the Great Basin. Ecological Applications **18**:1226-1235.

Leishman, M. R., T. Haslehurst, A. Ares, and Z. Baruch. 2007. Leaf trait relationships of native and invasive plants: community- and global-scale comparisons. New Phytologist **176**:635-643.

Leverett, L. D. 2017. Germination phenology determines the propensity for facilitation and competition. Ecology **98**:2437–2446.

Liu, H., Z. Mi, L. Lin, Y. Wang, Z. Zhang, F. Zhang, H. Wang, L. Liu, B. Zhu, G. Cao, X. Zhao, N. J. Sanders, A. T. Classen, P. B. Reich, and J. S. He. 2018. Shifting plant species composition in response to climate change stabilizes grassland primary production. Proc Natl Acad Sci U S A **115**:4051-4056.

Mwangi, P. N., M. Schmitz, C. Scherber, C. Roscher, J. Schumacher, M. Scherer-Lorenzen, W. W. Weisser, and B. Schmid. 2007. Niche pre-emption increases with species richness in experimental plant communities. Journal of Ecology **95**:65-78.

Post, D. M., and E. P. Palkovacs. 2009. Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. Philos Trans R Soc Lond B Biol Sci **364**:1629-1640.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raven, P. H., and D. I. Axelrod. 1978. Origin and relationships of the California flora. University of California Press, Berkeley.

Reynolds, S. A., J. D. Corbin, and C. M. D'Antonio. 2001. The effects of litter and temperature on the germination of native and exotic grasses in a coastal California grassland. Madroño **48**:230-235.

Rizopoulos, D. 2019. Generalized Linear Mixed Models using Adaptive Gaussian Quadrature [R package version 0.5-1].

Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. Annual Review of Ecology, Evolution, and Systematics **18**:209-235.

Ross, M. A., and J. L. Harper. 1972. Occupation of biological space during seedling establishment. Journal of Ecology **60**:77-88.

Schmitt, J., J. Eccleston, and D. W. Ehrhardt. 1987. Dominance and suppression, size-dependent growth and self-thinning in a natural Impatiens capensis population. Journal of Ecology **75**:651-665.

Schwinning, S., and J. Weiner. 1998. Mechanisms determining the degree of size asymmetry in competition among plants. Oecologia **113**:447–455.

Seabloom, E. W., W. S. Harpole, O. J. Reichman, and D. Tilman. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. Proceedings of the National Academy of Sciences USA **100**:13384-13389.

Simons, A. M., and M. O. Johnston. 2000. Variation in seed traits of Lobelia inflata (Campanulaceae): sources and fitness consequences. American Journal of Botany **87**:124-132.

Skálová, H., L. Moravcová, and P. Pyšek. 2011. Germination dynamics and seedling frost resistance of invasive and native Impatiens species reflect local climatic conditions. Perspectives in Plant Ecology, Evolution and Systematics **13**:173–180.

Stromberg, M. R., and J. R. Griffin. 1996. Long-term patterns in coastal California grasslands in relation to cultivation, gophers, and grazing. Ecological Applications **6**:1189–1211.

Stuble, K. L., L. Souza, and M. Avolio. 2016. Priority effects: natives, but not exotics, pay to arrive late. Journal of Ecology **104**:987-993.

Susko, D. J., and L. Lovett-Doust. 2000. Patterns of seed mass variation and their effects on seedling traits in Alliaria petiolata (Brassicaceae). American Journal of Botany **87**:56-66.

Thorpe, A. S., E. T. Aschehoug, D. Z. Atwater, and R. M. Callaway. 2011. Interactions among plants and evolution. Journal of Ecology **99**:729–740.

van Kleunen, M., W. Dawson, F. Essl, J. Pergl, M. Winter, E. Weber, H. Kreft, P. Weigelt, J. Kartesz, M. Nishino, L. A. Antonova, J. F. Barcelona, F. J. Cabezas, D. Cardenas, J. Cardenas-Toro, N. Castano, E. Chacon, C. Chatelain, A. L. Ebel, E. Figueiredo, N. Fuentes, Q. J. Groom, L. Henderson, Inderjit, A. Kupriyanov, S. Masciadri, J. Meerman, O. Morozova, D. Moser, D. L. Nickrent, A. Patzelt, P. B. Pelser, M. P. Baptiste, M. Poopath, M. Schulze, H. Seebens, W. S. Shu, J. Thomas, M. Velayos, J. J. Wieringa, and P. Pysek. 2015. Global exchange and accumulation of non-native plants. Nature **525**:100-103.

van Kleunen, M., E. Weber, and M. Fischer. 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. Ecology Letters **13**:235-245.

Vannette, R. L., and T. Fukami. 2014. Historical contingency in species interactions: towards niche-based predictions. Ecology Letters **17**:115-124.

Vaughn, K. J., and T. P. Young. 2015. Short-term priority over exotic annuals increases the initial density and longer-term cover of native perennial grasses. Ecological Applications **25**:791-799.

Verdú, M., and A. Traveset. 2005. Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. Ecology **86**:1385–1394.

Vico, G., S. Manzoni, L. Nkurunziza, K. Murphy, and M. Weih. 2016. Trade-offs between seed output and life span - a quantitative comparison of traits between annual and perennial congeneric species. New Phytol **209**:104-114.

Wainwright, C. E., and E. E. Cleland. 2013. Exotic species display greater germination plasticity and higher germination rates than native species across multiple cues. Biological Invasions **15**:2253-2264.

Wainwright, C. E., E. M. Wolkovich, and E. E. Cleland. 2012. Seasonal priority effects: implications for invasion and restoration in a semi-arid system. Journal of Applied Ecology **49**:234-241.

Walck, J. L., S. N. Hidayati, K. W. Dixon, K. Thompson, and P. Poschlod. 2011. Climate change and plant regeneration from seed. Global Change Biology **17**:2145–2161.

Waterton, J., and E. E. Cleland. 2016. Trade-off between early emergence and herbivore susceptibility mediates exotic success in an experimental California plant community. Ecology and Evolution **6**:8942-8953.

Weinig, C. 2000. Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. Evolution **54**:124-136.

Weis, A. E., K. M. Turner, B. Petro, E. J. Austen, and S. M. Wadgymar. 2015. Hard and soft selection on phenology through seasonal shifts in the general and social environments: A study on plant emergence time. Evolution **69**:1361-1374.

Wilsey, B. J., K. Barber, and L. M. Martin. 2015. Exotic grassland species have stronger priority effects than natives regardless of whether they are cultivated or wild genotypes. New Phytologist **205**:928-937.

Zhu, D. H., P. Wang, W. Z. Zhang, Y. Yuan, B. Li, and J. Wang. 2015. Sampling and complementarity effects of plant diversity on resource use increases the invasion resistance of communities. PLoS One **10**:e0141559.

CHAPTER 3

Mammalian herbivory weakens directional selection for earlier emergence in competition

Abstract

The timing of seedling emergence is strongly linked with fitness because it determines the biotic and abiotic environment experienced by plants following dispersal. Hence emergence timing is expected to be under strong selection to shift in concert with climate change. Experiments and observations consistently find that earlier emerging plants have a competitive advantage over those emerging later. However, emergence timing varies substantially within and among plant populations, making it important to understand the selective forces maintaining this variation. In seasonal herbaceous communities, mammalian herbivores may maintain variation in emergence timing by weakening directional selection for earlier emergence in competitive environments. To investigate this, we carried out phenotypic selection analyses on emergence timing in two California grass species, the native *Stipa pulchra* and exotic *Bromus diandrus*, growing in close competition with and without mammalian herbivore exclusion. Mammalian herbivores consistently weakened directional selection for earlier emergence independently of reductions in mean fitness. Our results demonstrate that mammalian herbivores play an underappreciated role in shaping emergence timing in plant populations, with implications for the potential of species to adapt to current and future global changes.

Introduction

Phenological traits are a critical component of adaptation to seasonal environments (Forrest and Miller-Rushing 2010). The timing of seedling emergence is the earliest phenological trait expressed within a growing season; this determines the biotic and abiotic environment experienced by plants, influencing both fitness and patterns of selection on traits expressed later in development (Donohue 2002). As with other phenological traits, such as flowering phenology (Franks et al. 2007, Anderson et al. 2012), emergence time is expected to evolve with climate change (Walck et al. 2011, Cochrane et al. 2015), and such shifts in emergence timing have already been observed (Dickman et al. 2019). Earlier emergence within growing seasons is consistently associated with higher fitness (Ross and Harper 1972, Verdú and Traveset 2005) because it allows longer periods of resource uptake as well as the ability to pre-empt resources and suppress later-arriving individuals (priority effects) (Vannette and Fukami 2014). Despite this competitive advantage of early emergence, emergence timing varies substantially within and among populations (Donohue et al. 2010, Cochrane et al. 2015), calling for a deeper understanding of the selective forces maintaining such variation and thus mediating evolutionary responses to global changes.

In seasonal herbaceous communities, grazing by mammalian herbivores might weaken directional selection for earlier emergence within growing seasons through several potential mechanisms. Firstly, plants that emerge earlier will be exposed to herbivores for longer periods, whereas later-emerging individuals may escape attack by remaining as seeds in the soil. Secondly, the size advantage gained from earlier emergence may result in increased apparency to herbivores (Hulme 1994, Louthan et al. 2014). Thirdly, any grazing activity that reduces competing plants to a similar size is likely to disproportionately impact larger individuals that emerged earlier, thus acting as a competitive release for late-emerging individuals. Across species, earlier emergence is associated with greater susceptibility to mammalian herbivores (Waterton and Cleland 2016), but whether a similar process weakens directional selection within species is unknown.

To investigate this, we carried out a field experiment in which we characterized phenotypic selection on emergence timing in two California grasses, the native perennial *Stipa pulchra* (Hitchc.) Barkworth and the exotic annual *Bromus diandrus* (Roth), growing in close competition with and without the exclusion of mammalian herbivores.

Materials and methods

Study site

We conducted the experiment between February and June 2018 on an experimental field at the University of California San Diego Biological Field Station (32.885° N, 117.230° W). The site is flat, regularly tilled, and is classified as having sandy clay loam soil. The climate is Mediterranean, with most precipitation typically falling between November and May, during which the majority of seedling emergence occurs. The site is typically dominated by exotic annual species including *Hordeum murinum* (Poaceae), *Erodium cicutarium* (Geraniaceae), and *Malva parviflora* (Malvaceae). Generalist mammalian herbivores documented at the site include the brush rabbit (*Sylvilagus bachmani*), the desert cottontail (*Sylvilagus audubonii*), and California ground squirrels (*Otospermophilus beecheyi*). No data were available on the density of herbivore species during the experiment or at other times.

Study species

Stipa pulchra (purple needlegrass) is a perennial bunchgrass native to California that is found in woodland, chaparral, and grassland from Baja California to northern California (Baldwin et al. 2012). *S. pulchra* typically do not reproduce in their first growing season, therefore reproductive output could not be assessed in the duration of this experiment. However, aboveground biomass of *S. pulchra* is positively correlated with probability of survival through summer drought (Allen 1995) and seed production after two growing seasons (J. Waterton, unpublished data); therefore, we used end-of-season aboveground biomass as a measure of fitness.

Bromus diandrus is an annual grass native to Eurasia that has become naturalized in California since European settlement (Jackson 1985). This species is found in many habitats across California, and is particularly dominant in disturbed areas, and such as abandoned agricultural fields (Stromberg and Griffin 1996). *B. diandrus* is largely self-fertilizing, with reported outcrossing frequencies <1% (Kon and Blacklow 1990). As an annual species, we used the total mass of seeds produced as a measure of fitness.

Herbivore exclusion experiment

We used field collected seeds, with *B. diandrus* sourced from Elliott Chaparral Reserve (32.889° N, 117.091° W) and *S. pulchra* sourced from Sedgwick Reserve (34.692° N 120.043° W). To capture variation in emergence timing occurring within populations of each focal species, we used seeds collected from 16 maternal plants (hereon referred to as maternal lines) spaced at least 5m apart in source populations. We collected seeds in April 2015 and stored them at 4° C until use.

For each maternal line, we selected 32 seeds that outwardly appeared viable and randomly assigned half to each of two caging treatments: “herbivory” or “exclusion”. This resulted in 256 seeds for each focal species in each caging treatment (1024 seeds in total). We weighed seeds individually to account for effects of maternal provisioning which can influence emergence and subsequent growth (Roach and Wulff 1987).

In January 2018, we established sixteen 85 cm × 50 cm plots, eight for each caging treatment, arranged in rows of four, with 1 m spacing between adjacent plots. We watered and tilled plots to germinate and remove the standing seedbank. We placed covered cages made from 6.3- mm hardware cloth around each plot and cut 30cm × 30cm openings in the two shorter sides of cages in the herbivory treatment. Cages in both treatments intercepted approximately 27% of photosynthetically active radiation (PAR), as measured with an AccuPAR LP- 80 PAR Ceptometer (Decagon Devices Inc., Washington, USA). We arranged plots so that caging treatments alternated along each row to minimize potential spatial effects on plant growth and rates of herbivory. Within plots, we established two separate 4 × 8 planting grids, made from 1.25 cm × 1. 25 cm hardware cloth, placed 20 cm apart in the center of each plot.

On 1 February 2018, we planted 32 seeds of each species (two seeds per maternal line) separately into each planting grid in each plot. To account for potential positional effects within grids, we generated eight random planting plans, each of which we used in two adjacent plots belonging to each caging treatment. Immediately after planting all seeds, we applied 1.5 l of water evenly across plots to initiate germination. We applied this same volume of water daily until the first rain event after planting on 21 February. Following this, we applied 2.5 l of water to all plots each Wednesday and Friday in weeks without major forecasted rain events. We monitored plots daily for emergence of focal individuals. We continually removed weeds from plots wherever possible without disturbing focal individuals. We stopped applying water after 9 May, by which point all *B. diandrus* individuals that had flowered had begun to senesce. As expected, no *S. pulchra* individuals flowered during the experiment. We harvested aboveground biomass of *S. pulchra* on 7 June. We harvested *B. diandrus* seeds on 13 June once seeds had reached maturity but were still firmly attached to culms. We dried samples for 3 days at 40°C before weighing to the nearest milligram.

Statistical analyses

We conducted all statistical analyses using R version 3.5.2 (R Core Team 2018). We estimated broad-sense heritabilities of seed size and emergence time as the variance associated with maternal line (V_G) divided by the total phenotypic variance (V_P). We extracted variance components using restricted maximum likelihood (REML) models fit using the package *lme4* (Bates et al. 2015). For seed size, we included maternal line as a random effect. For emergence time, we included both block and maternal line as random effects.

We analyzed fitness using generalized linear mixed effects models (GLMMs) fit using the *mixed_model* function in the package *GLMMadaptive* (Rizopoulos 2019). Seed production of *B. diandrus* was zero-inflated (due to mortality) and over-dispersed; therefore, we analyzed fitness using hurdle negative binomial mixed effects models that integrate both survival and fecundity fitness components. These models proceed in two parts, specifying a logistic regression for generating zero values (whether or not seeds were harvested) and a negative binomial regression for non-zero values (seed mass given that any seeds were harvested). In all models, we specified block as a random effect for both model parts. For *S. pulchra*, mortality by the time of harvest was low (2.2% across both caging treatments), therefore in this species we modelled zero values as being generated by the same process as non-zero values. Aboveground biomass of *S. pulchra* was over-dispersed and therefore we analyzed fitness using negative binomial mixed effects models, with block specified as a random effect in all models. We tested the significance of fixed effects by comparing nested models with likelihood ratio (LR) tests at a significance level of $P = 0.05$.

We estimated mean absolute fitness and tested whether it differed between caging treatments with a model containing caging treatment as the only fixed effect. For phenotypic selection analyses, we analyzed fitness as a function of days to emergence, seed size and caging treatment. We included seed size in selection analyses because this trait influences emergence time (Simons and Johnston 2000, Susko and Lovett-Doust 2000) as well as fitness directly through provisioning effects (Halpern 2005). We standardized seed size and days to emergence across treatments to a mean of zero and a standard deviation of one. We included linear effects of traits to test for directional selection, quadratic effects of traits to test for curvature in fitness surfaces, and interactions between trait effects (linear and quadratic) and caging treatment to test for differences in selection due to herbivory. We tested for directional selection and interactions with caging treatment in models containing linear trait effects only. We tested for curvature in fitness surfaces and interactions with caging treatment in models containing both linear and quadratic effects (Lande and Arnold 1983). When testing the significance of quadratic trait effects and their interactions with treatment, we dropped nonsignificant linear seed size × treatment interactions from models because we had no *a priori* hypothesis of how herbivory would influence directional selection on this trait (including this term did not qualitatively change the results). We tested the significance of overall trait effects against models in which the corresponding trait × treatment interactions were dropped. When trait × caging treatment interactions were significant, we tested the significance of trait effects in separate models for each treatment. We extracted predicted fitness from models that included only significant effects of traits and trait × treatment interactions. We relativized predicted fitness to a mean of one within caging treatments and used ordinary least squares regression to extract standardized linear selection gradients (β) and nonlinear selection gradients (γ). We relativized fitness within treatments as is recommended for traits such as emergence time that are under strong soft selection, where fitness associated with trait values is highly dependent on other trait values in the population (Weis et al. 2015, De Lisle and Svensson 2017). We calculated linear and nonlinear selection gradients across caging treatments when corresponding trait × treatment interactions were nonsignificant. Nonlinear selection gradients were calculated by doubling quadratic regression coefficients and their standard errors (Stinchcombe et al. 2008).

Results

495 *S. pulchra* and 475 *B. diandrus* individuals emerged over 8 and 11 days, respectively. Estimated broad-sense heritabilities were substantial in both species for emergence time (*S. pulchra*: $H^2 = 0.19$; *B. diandrus*: $H^2 = 0.34$) and seed size (*S. pulchra*: $H^2 = 0.49$; *B. diandrus*: $H^2 = 0.55$). The first observed herbivore damage occurred on the final day in which seedlings emerged (three *B. diandrus* seedlings emerged). Herbivores significantly lowered mean absolute fitness in both species; fitness was reduced by 19% in *S. pulchra* (likelihood ratio $(LR)_{(1)} = 6.03$, $P = 0.01$) and by 90% in *B. diandrus* ($LR_{(2)} = 236.3$, $P < 0.001$).

In the native *S. pulchra*, directional selection for earlier emergence was significantly weaker when herbivores were present (Emergence × Caging: $LR_{(1)} = 9.47$, $P = 0.002$; Fig. 3.1), but still favored earlier emergence in each treatment ($\text{Emergence}_{[\text{Exclusion}]}$: $LR_{(1)} = 25.4$, $P < 0.001$; $\text{Emergence}_{[\text{Herbivory}]}$: $LR_{(1)} = 21.2$, $P < 0.001$; Fig. 3.1). Directional selection for earlier emergence displayed consistent curvilinearity across caging treatments (Emergence^2: $LR_{(1)} = 8.03$, $P = 0.005$; Emergence^2 × Caging: $LR_{(1)} = 2.07$, $P = 0.15$; Fig. 3.1). In the exotic *B. diandrus*, directional selection for earlier emergence was significantly weaker when herbivores were present (Emergence × Caging: $LR_{(2)} = 11.8$, $P = 0.003$; Fig. 3.1), but favored earlier emergence in each treatment ($\text{Emergence}_{[\text{Exclusion}]}$: $LR_{(2)} = 68.9$, $P < 0.001$; $\text{Emergence}_{[\text{Herbivory}]}$: $LR_{(2)} = 22.5$, $P < 0.001$). Selection favored larger seeds with no significant differences between caging treatments in both *S. pulchra* (Seed Size: $LR_{(1)} = 23.2$, $P < 0.001$; Seed Size × Caging:

LR$_{(1)}$ = 1.35, P = 0.25; Appendix Fig. 3A.1) and *B. diandrus* (Seed Size: LR$_{(2)}$ = 13.4, P = 0.001; Seed Size × Caging: LR$_{(2)}$ = 3.38, P = 0.18; Appendix Fig. 3A.1). The significance of all linear and quadratic trait effects and their interactions with caging treatment are given in Appendix Table 3A.1. Standardized linear selection gradients (β) and nonlinear selection gradients (**γ**) are given in Table 3.1.

Table 3.1: Standardized selection gradients for emergence time and seed size in caging treatments. We calculated selection gradients for statistically significant selection only. Asterisks indicate selection that differed significantly between caging treatments; otherwise we calculated selection gradients across caging treatments. We doubled quadratic regression coefficients and standard errors to calculate nonlinear selection gradients (Stinchcombe et al. 2008).

Species	Trait	β		γ	
		Exclusion	Herbivory	Exclusion	Herbivory
S. pulchra	Emergence time	-0.33 (±0.007) *	-0.10 (±0.008) *	-0.04 (±0.004)	-0.04 (±0.004)
	Seed size	0.20 (±0.004)	0.20 (±0.004)	-0.10 (±0.005)	-0.10 (±0.005)
B. diandrus	Emergence time	-0.44 (±0.01) *	-0.29 (±0.02) *	--	--
	Seed size	0.24 (±0.01)	0.24 (±0.01)	--	--

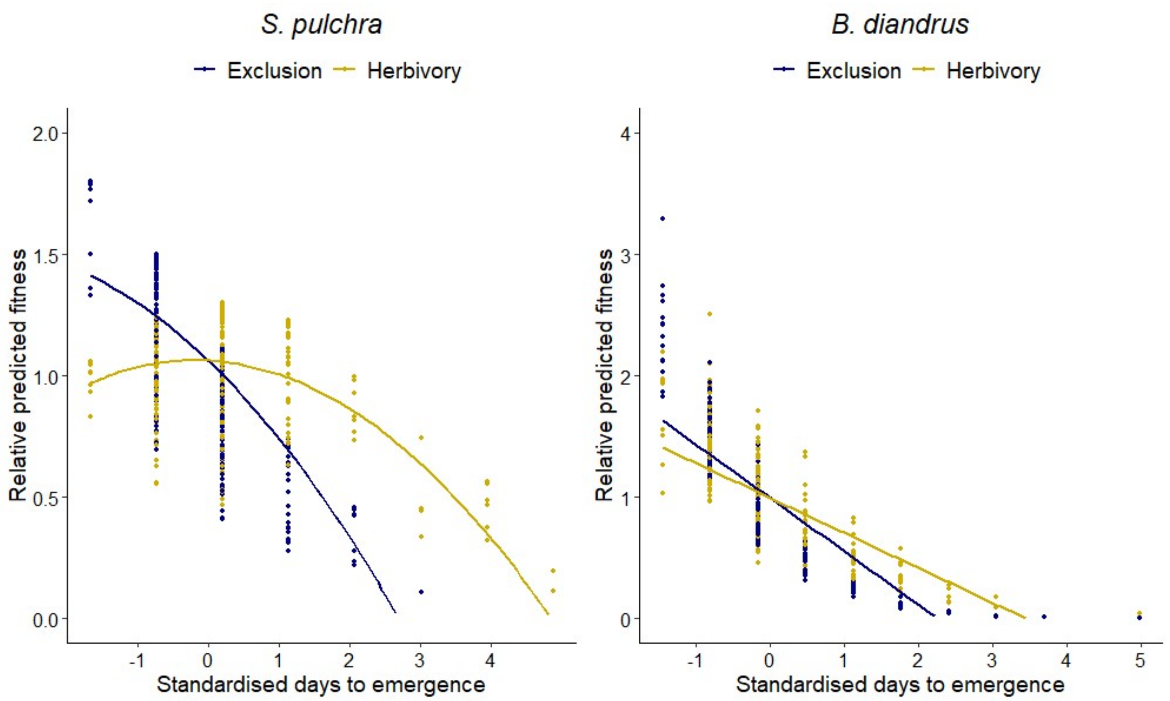

Figure 3.1: Phenotypic selection on emergence time in caging treatments. We standardized emergence time to a mean of 0 and standard deviation of 1 across caging treatments. We relativized predicted fitness to a mean of one within each caging treatment. In both species, directional selection for earlier emergence was weaker in the herbivory treatment, as indicated by significant linear emergence time × caging treatment interactions (*S. pulchra*: $LR_{(1)} = 9.47$, $P = 0.002$, $n = 495$ across treatments; *B. diandrus*: $LR_{(2)} = 11.8$, $P = 0.003$, $n = 475$ across treatments). Fitness curves for *S. pulchra* depict linear and nonlinear selection gradients from models that included significant linear and nonlinear trait effects. For *B. diandrus*, nonlinear selection was not statistically significant and thus fitness curves depict linear selection gradients from models with significant linear trait effects.

Discussion

Despite the consistent positive effect on fitness of earlier emergence in competitive environments (Verdú and Traveset 2005), within-season emergence timing varies considerably within and among plant populations (Donohue et al. 2010, Cochrane et al. 2015). It is well established that the abiotic environment drives variable selection on emergence timing over time and space (Kalisz 1986, Donohue 2002, Donohue et al. 2005). In this study, we demonstrate that mammalian herbivores weaken directional selection for earlier emergence in the field. Biotic interactions with pollinators and herbivores are well known to influence selection on flowering phenology (Brody 1997, Pilson 2000, Wilcox Wright and Meagher 2003, Kawagoe and Kudoh 2010, Fukano et al. 2013), but to our knowledge this is the first study to show that herbivores directly alter selection on emergence timing.

Mammalian herbivores consistently weakened directional selection for earlier emergence, despite our focal species representing distinct functional groups that differ with respect to origin and life history strategy. This selective effect of mammalian herbivory likely contributes to results of meta-analysis showing lower fitness advantages associated with early emergence in field vs. controlled conditions (Verdú and Traveset 2005). Weaker directional selection for earlier emergence was apparent when relativizing estimated fitness within caging treatments, and therefore was not only a consequence of lower variation in absolute fitness due to herbivory. Grazing can also increase variation in fitness among individuals through the competitive release of surviving plants (Crawley and Weiner 1991); however, unless earlier emergence also consistently increases survival in response to herbivory, we expect that such grazing effects will weaken the association between emergence time and fitness.

Of the three mechanisms we propose by which grazing might weaken selection for earlier emergence, the first — escaping damage by remaining in the soil — did not contribute substantially to observed results because the first instances of herbivore damage occurred after almost every individual had emerged. However, we expect this mechanism will be more important in populations where emergence is spread over longer periods, resulting in comparatively longer exposure to herbivores in the earliest emerging individuals. Because we could only determine the date of first damage for each plant, we were unable to determine the extent to which, across the season, weaker selection for earlier emergence was driven by the selective targeting of larger individuals as opposed to simply greater relative impacts of grazing on such plants. The contribution of each mechanism to weakening selection for earlier emergence will likely depend on plant species and herbivore feeding behaviors (Searle and Shipley 2008).

The weakening of selection for earlier emergence by mammalian herbivores has implications for the potential of species in seasonal herbaceous communities to adapt to current and future global change. As shown here, despite having direct negative impacts on plant fitness, mammalian herbivory is a selective force capable of maintaining genetic variation in emergence time. Genetic variation in phenological traits, including emergence time, will be a key factor determining the potential for *in situ* adaptive evolution in response to anthropogenic climate change (Walck et al. 2011, Cochrane et al. 2015, Sheth and Angert 2016). However, many species are rapidly evolving to advance their seasonal phenologies with climate change; for example, earlier flowering has evolved in concert with rising temperatures (Anderson et al. 2012), and populations have shifted towards earlier emergence in response to drought (Dickman et al. 2019). In such cases, the selective effect of mammalian herbivory could impede adaptive responses, with negative impacts on long term species persistence in the face of global changes.

Acknowledgements

This work was supported by an Institute for the Study of Ecological Effects of Climate Impacts (ISEECI) Graduate Student Researcher Fellowship and Jeanne M. Messier Memorial Fellowship to J.W. We thank A. Cugini, J. Gaudio, C. Hua and G. Bair for help in the field and lab. We also thank J. Anderson, S. Wadgymar and M. Warwell for helpful advice with statistical analyses.

Chapter 3, in full, has been submitted for publication of the material. Waterton, J., and E.E. Cleland. The dissertation author is the primary investigator and author of this paper.

Appendix 3A

Table 3A.1: Likelihood ratio (LR) tests testing for significant linear and nonlinear selection in caging treatments. Significant trait × treatment interactions indicate that selection differs between treatments, for which we tested the significance of selection in separate models for each treatment. Overall trait effects were tested against models in which the corresponding trait × treatment interactions were dropped. We dropped nonsignificant linear seed size × caging treatment interactions from models testing the significance of quadratic trait effects and their interactions with treatment.

S. pulchra					
Trait	Trait effect	LR Trait	LR Interaction	LR Exclusion	LR Herbivory
Emergence time	Linear	**$LR_{(1)} = 20.3$** **$P < 0.001$**	**$LR_{(1)} = 9.47$** **$P = 0.002$**	**$LR_{(1)} = 25.4$** **$P < 0.001$**	**$LR_{(1)} = 21.2$** **$P < 0.001$**
	Quadratic	**$LR_{(1)} = 8.03$** **$P = 0.005$**	$LR_{(1)} = 2.07$ $P = 0.15$	--	--
Seed size	Linear	**$LR_{(1)} = 23.2$** **$P < 0.001$**	$LR_{(1)} = 1.35$ $P = 0.25$	--	--
	Quadratic	**$LR_{(1)} = 8.84$** **$P < 0.003$**	$LR_{(1)} = 1.91$ $P = 0.17$	--	--
B. diandrus					
Trait	Trait effect	LR Trait	LR Interaction	LR Exclusion	LR Herbivory
Emergence time	Linear	**$LR_{(2)} = 70.5$** **$P < 0.001$**	**$LR_{(2)} = 11.8$** **$P = 0.003$**	**$LR_{(2)} = 68.9$** **$P < 0.001$**	**$LR_{(2)} = 22.5$** **$P < 0.001$**
	Quadratic	$LR_{(2)} = 2.72$ $P = 0.26$	$LR_{(2)} = 1.2$ $P = 0.55$	--	--
Seed size	Linear	**$LR_{(2)} = 13.4$** **$P = 0.001$**	$LR_{(2)} = 3.38$ $P = 0.18$	--	--
	Quadratic	$LR_{(2)} = 0.2$ $P = 0.91$	$LR_{(2)} = 0.56$ $P = 0.75$	--	--

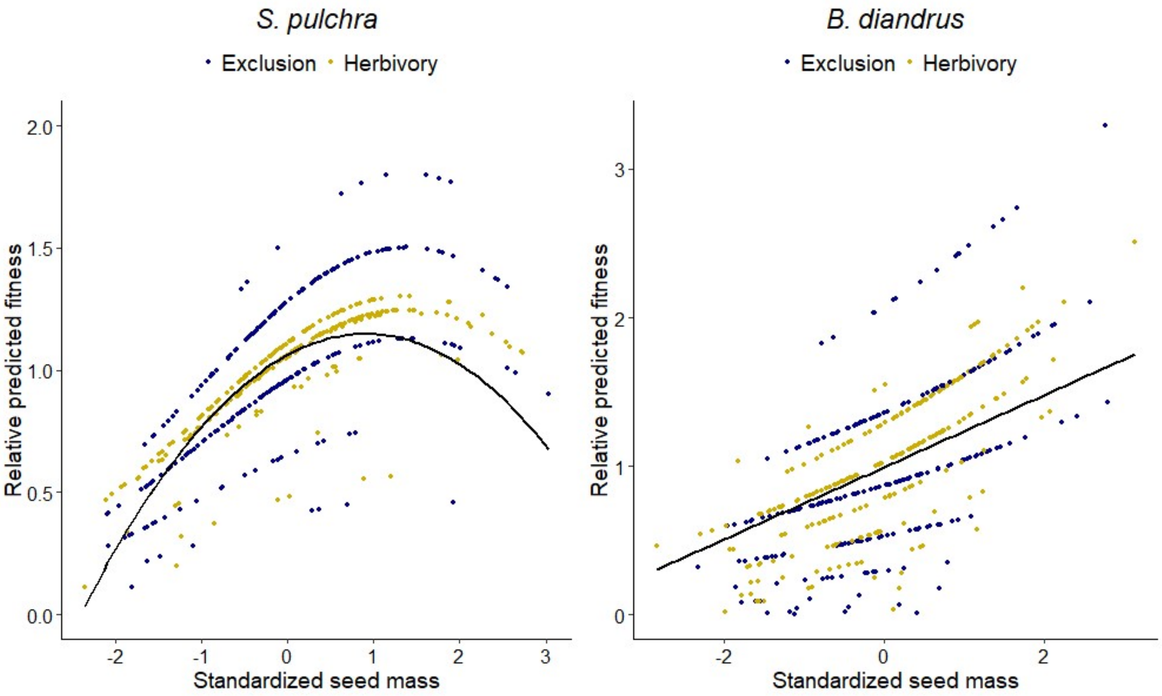

Figure 3A.1: Phenotypic selection on seed size in caging treatments. Seed mass was standardized to a mean of 0 and standard deviation of 1 across caging treatments. Predicted fitness was relativized to a mean of one within each caging treatment. In both species, directional selection did not differ between caging treatments, as indicated by nonsignificant linear seed size × caging treatment interactions (*S. pulchra*: $\mathrm{LR}_{(1)} = 1.35$, $P = 0.25$, $n = 495$ across treatments; *B. diandrus*: $\mathrm{LR}_{(2)} = 3.38$, $P = 0.18$, $n = 475$ across treatments); therefore, we calculated selection gradients across caging treatments. The fitness curve for *S. pulchra* depicts linear and nonlinear selection gradients from models that included significant linear and nonlinear trait effects. For *B. diandrus*, nonlinear selection was not statistically significant and thus the fitness curve depicts the linear selection gradient from a model with only significant linear trait effects.

References

Allen, E. B. 1995. Restoration ecology: limits and possibilities in arid and semiarid lands. Pages 7-15 *in* B. A. Roundy, E. D. McArthur, J. S. Haley, and D. K. Mann, editors. Proceedings: wildland shrub and arid land restoration symposium. Intermountain Research Station, Forest Service, U.S. Department of Agriculture Las Vegas, NV.

Anderson, J. T., D. W. Inouye, A. M. McKinney, R. I. Colautti, and T. Mitchell-Olds. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proc Biol Sci **279**:3843-3852.

Baldwin, B. G., D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken, editors. 2012. The Jepson manual: vascular plants of California. 2nd edition. University of California Press, Berkeley.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software **67**:1-48.

Brody, A. K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. Ecology **78**:1624-1631.

Cochrane, A., C. J. Yates, G. L. Hoyle, and A. B. Nicotra. 2015. Will among-population variation in seed traits improve the chance of species persistence under climate change? Global Ecology and Biogeography **24**:12-24.

Crawley, M. J., and J. Weiner. 1991. Plant size variation and vertebrate herbivory: winter wheat grazed by rabbits. Journal of Applied Ecology **28**:154-172.

De Lisle, S. P., and E. I. Svensson. 2017. On the standardization of fitness and traits in comparative studies of phenotypic selection. Evolution **71**:2313-2326.

Dickman, E. E., L. K. Pennington, S. J. Franks, and J. P. Sexton. 2019. Evidence for adaptive responses to historic drought across a native plant species range. Evolutionary Applications.

Donohue, K. 2002. Germination timing influences natural selection on life-history characters in Arabidopsis thaliana. Ecology **83**:1006-1016.

Donohue, K., L. Dorn, C. Griffith, E. Kim, A. Aguilera, C. R. Polisetty, and J. Schmitt. 2005. The evolutionary ecology of seed germination of Arabidopsis thaliana: variable natural selection on germination timing. Evolution **59**:758.

Donohue, K., R. Rubio de Casas, L. Burghardt, K. Kovach, and C. G. Willis. 2010. Germination, postgermination adaptation, and species ecological ranges. Annual Review of Ecology, Evolution, and Systematics **41**:293–319.

Forrest, J., and A. J. Miller-Rushing. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. Philos Trans R Soc Lond B Biol Sci **365**:3101-3112.

Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences USA **104**:1278-1282.

Fukano, Y., K. Tanaka, and T. Yahara. 2013. Directional selection for early flowering is imposed by a re-associated herbivore - but no evidence of directional evolution. Basic and Applied Ecology **14**:387-395.

Halpern, S. L. 2005. Sources and consequences of seed size variation in Lupinus perennis (Fabaceae): Adaptive and non-adaptive hypotheses. American Journal of Botany **92**:205-213.

Hulme, P. E. 1994. Seedling herbivory in grassland: relative impact of vertebrate and invertebrate herbivores. Journal of Ecology **82**:873-880.

Kalisz, S. 1986. Variable selection on the timing of germination in Collinsia verna (Scrophulariaceae). Evolution **40**:479.

Kawagoe, T., and H. Kudoh. 2010. Escape from floral herbivory by early flowering in Arabidopsis halleri subsp. gemmifera. Oecologia **164**:713-720.

Kon, K. F., and W. M. Blacklow. 1990. Polymorphism, outcrossing and polyploidy in *Bromus diandrus* and *B. rigidus* Australian Journal of Botany **38**:609–618.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution **37**:1210-1226.

Louthan, A. M., D. F. Doak, J. R. Goheen, T. M. Palmer, and R. M. Pringle. 2014. Mechanisms of plant-plant interactions: concealment from herbivores is more important than abiotic-stress mediation in an African savannah. Proc Biol Sci **281**:20132647.

Pilson, D. 2000. Herbivory and natural selection on flowering phenology in wild sunflower, Helianthus annuus. Oecologia **122**:72-82.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rizopoulos, D. 2019. Generalized Linear Mixed Models using Adaptive Gaussian Quadrature [R package version 0.5-1].

Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. Annual Review of Ecology, Evolution, and Systematics **18**:209-235.

Ross, M. A., and J. L. Harper. 1972. Occupation of biological space during seedling establishment. Journal of Ecology **60**:77-88.

Searle, K. R., and L. A. Shipley. 2008. The comparative feeding behaviour of large browsing and grazing herbivores. Pages 117-148 *in* I. J. Gordon and H. H. T. Prins, editors. The Ecology of Browsing and Grazing. Springer, Berlin and Heidelberg.

Sheth, S. N., and A. L. Angert. 2016. Artificial selection reveals high genetic variation in phenology at the trailing edge of a species range. American Naturalist **187**:182-193.

Simons, A. M., and M. O. Johnston. 2000. Variation in seed traits of Lobelia inflata (Campanulaceae): sources and fitness consequences. American Journal of Botany **87**:124-132.

Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? Evolution **62**:2435-2440.

Stromberg, M. R., and J. R. Griffin. 1996. Long-term patterns in coastal California grasslands in relation to cultivation, gophers, and grazing. Ecological Applications **6**:1189–1211.

Susko, D. J., and L. Lovett-Doust. 2000. Patterns of seed mass variation and their effects on seedling traits in Alliaria petiolata (Brassicaceae). American Journal of Botany **87**:56-66.

Vannette, R. L., and T. Fukami. 2014. Historical contingency in species interactions: towards niche-based predictions. Ecology Letters **17**:115-124.

Verdú, M., and A. Traveset. 2005. Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. Ecology **86**:1385–1394.

Walck, J. L., S. N. Hidayati, K. W. Dixon, K. Thompson, and P. Poschlod. 2011. Climate change and plant regeneration from seed. Global Change Biology **17**:2145–2161.

Waterton, J., and E. E. Cleland. 2016. Trade-off between early emergence and herbivore susceptibility mediates exotic success in an experimental California plant community. Ecology and Evolution **6**:8942-8953.

Weis, A. E., K. M. Turner, B. Petro, E. J. Austen, and S. M. Wadgymar. 2015. Hard and soft selection on phenology through seasonal shifts in the general and social environments: A study on plant emergence time. Evolution **69**:1361-1374.

Wilcox Wright, J., and T. R. Meagher. 2003. Pollination and seed predation drive flowering phenology in Silene latifolia (Caryophyllaceae). Ecology **84**:2062-2073.

CHAPTER 4

Trade-off between early emergence and herbivore susceptibility mediates exotic success in an experimental California plant community

Abstract

Ecological trade-offs are fundamental to theory in community ecology; critical for understanding species co-existence in diverse plant communities, as well as the evolution of diverse life-history strategies. Invasions by exotic species can provide insights into the importance of trade-offs in community assembly, because the ecological strategies of invading species often differ from those present in the native species pool. Exotic annual species have invaded many Mediterranean climate areas around the globe, and often germinate and emerge earlier in the growing season than native species. Early-season growth can enable exotic annual species to pre-empt space and resources, competitively suppressing later-emerging native species; however, early-emerging individuals may also be more apparent to herbivores. This suggests a potential trade-off between seasonal phenology and susceptibility to herbivory. To evaluate this hypothesis, we monitored the emergence and growth of 12 focal species (six each native and exotic) in monoculture and polyculture, whilst experimentally excluding generalist herbivores both early and later in the growing season. Consistent with past studies, the exotic species emerged earlier than native species. Regardless of species origin, earlier emerging species achieved greater biomass by the end of the experiment, but were more negatively impacted by herbivory, particularly in the early part of the growing season. This greater impact of early-season herbivory on early-active species lead to a reduction in the competitive advantage of exotic species growing in polyculture and improved the performance of later-emerging natives. Such a trade-off between early growth and susceptibility to herbivores could

be an important force in community assembly in seasonal herbaceous-dominated ecosystems. These results also show how herbivore exclusion favours early-active exotic species in this system, with important implications for management in many areas invaded by early-active exotic species.

Introduction

Ecosystems with Mediterranean-type climates harbor exceptional plant diversity, and are among the most at risk to biodiversity loss in the coming decades due to multiple factors including changes in climate and land-use, as well as invasion by exotic species (Sala et al. 2000, Underwood et al. 2009). Exotic species often differ from native species in suites of functional traits related to strategies for resource capture (Leishman et al. 2007, van Kleunen et al. 2010, Godoy et al. 2011). In the Mediterranean-climate regions of California and southwestern Australia, where the onset of the growing season is initiated by the start of winter rains, exotic annual species have been observed to germinate faster and earlier than native species (Pérez-Fernández et al. 2000, Reynolds et al. 2001, Deering and Young 2006, Abraham et al. 2009, Wainwright et al. 2012, Wainwright and Cleland 2013). Early emergence allows exotic species to pre-empt resources and competitively suppress the growth of later-active natives, a type of seasonal priority effect (Wainwright et al. 2012). Priority effects mediated by such differences in arrival time have been shown to exert strong effects on community structure and function (Fukami 2015). This is demonstrated by experiments in which the arrival time of late-germinating native species is experimentally advanced, leading to competitive suppression of later planted exotic species (Grman and Suding 2010, Cleland et al. 2015), with these effects sometimes persisting over multiple years (Vaughn and Young 2015). The results of such experiments raise an important question: why do certain species, including many natives, display later emergence within the growing season despite the competitive advantage associated with earlier activity? Selection for early phenology has been hypothesized to be limited by exposure to stressful abiotic conditions before the onset of consistent growing season conditions (e.g. Anderson et al. 2012, Augspurger 2013), and could also expose early-active individuals to a greater risk of herbivory (Hanley 1998).

Exclusion experiments have shown that herbivores, particularly small mammals, exert strong control over plant productivity in herbaceous communities in Mediterranean-climate regions (Peters 2007). In these ecosystems, there is often little herbaceous vegetation suitable for consumption prior to the arrival of seasonal rains, meaning individuals that emerge first may initially represent the only herbaceous vegetation available to consumers, and are also likely to be highly apparent and accessible in the landscape (Wainwright et al. 2012). Furthermore, individuals that emerge earlier generally grow to be larger, through longer growing time and seasonal priority effects, and as a result may be selectively targeted even after the initial period following emergence (Hulme 1994). In contrast, seedlings emerging later might benefit from association with larger established neighbors if this makes them less apparent and accessible, a form of associational resistance (Underwood et al. 2014).

Herbivory during the earliest period of the growing season is likely to be particularly impactful for newly emerging individuals because seedlings tend to have weak structural defenses, low concentrations of some defense compounds, and are often of greater nutritional quality than mature plants (Quintero et al. 2014). Seedlings are also small relative to the size of consumers; hence herbivory is often fatal (Fenner and Thompson 2005). For example, mortality in six California grassland species exposed to gastropods has been shown to be greater in the first 3 weeks following emergence than the following 2 months combined (Cleland et al. 2006). For seedlings that survive attack, stored reserves and photosynthetic capacity are lower in these early ontogenetic stages; as such, tolerance to herbivory, the ability to regrow and reproduce following damage, is generally low (Strauss and Agrawal 1999, Boege and Marquis 2005). Therefore, any trade-off between emergence time and susceptibility to herbivores is likely to be most apparent when herbivores are active in the earliest period of the growing season. Furthermore, such large impacts of herbivory in the early-season may lead to large effects on community-level productivity; however, such effects of the timing of herbivory within a growing season have largely been overlooked (but see Sullivan and Howe 2009).

Release from specialist enemies or avoidance by generalist herbivores in introduced ranges are two mechanisms often hypothesized to promote invasion by exotic species (i.e. the enemy release hypothesis; Keane and Crawley 2002). However, generalist mammalian herbivory has been shown in many cases to slow the invasion of exotic herbaceous species into Mediterranean-climate regions (Rice 1987, Vilà and D'Antonio 1998, Lambrinos 2006, Peters 2007, Cushman et al. 2011, Wainwright et al. 2012). Evolutionary *naivety* due to a lack of coevolution with herbivores is often cited as an explanation for such high impacts of herbivory on exotic species (Colautti et al. 2004, Parker et al. 2006), yet our understanding of the particular plant traits underlying herbivore preference for exotic species is limited. If earlier emergence *per se* leads to greater overall susceptibility to herbivores, this could provide a mechanistic explanation for why many exotic species appear to be heavily impacted by generalist herbivores.

Previous studies investigating the mechanisms underlying the impacts of generalist herbivores on community assembly have largely focused on the role of differences in plant defense. In such studies, germination of focal species is often staggered to ensure synchronous emergence (Burt-Smith et al. 2003) or plants are presented to herbivores simultaneously (Hanley and Sykes 2009, Kempel et al. 2015). However, such studies are unlikely to represent the action of herbivores in nature when emergence time varies greatly between species. Furthermore, much of our understanding of herbivore impacts on community assembly comes from studies on invertebrates (reviewed in Barton and Hanley 2013); however, mammals consume a higher proportion of tissue of plants they encounter (Hulme 1994) and can exert at least as great an impact on herbaceous production in Mediterranean systems than invertebrate herbivores (e.g. Peters 2007).

We tested two hypotheses regarding the relationship between emergence timing and mammalian herbivory on species performance: (1) earlier emerging species are more susceptible to herbivores, and this susceptibility is greatest early in the growing season, and (2) in polyculture, the competitive advantage of early-arriving exotic species is reduced by herbivory, and this effect is greatest early in the growing season.

Materials and methods

Study site

The experiment was conducted at the University of California San Diego Biology Field Station from November 2014 to January 2015. This site is characterized by a flat, regularly tilled experimental field with sandy clay loam soil. Prior to planting of focal species, the experimental site was dominated by exotic species including *Hordeum murinum* (Poaceae), *Erodium cicutarium* (Geraniaceae) and *Malva parviflora* (Malvaceae). The site is inhabited by two native generalist mammalian herbivores: the brush rabbit (*Sylvilagus bachmani*), and desert cottontail (*Sylvilagus audubonii*). However, no data is available on the density of these two herbivore species during the experiment or at other times.

Experimental Design

We used 12 focal species (Table 4.1), six native and six exotic, to test our two hypotheses. These locally common native and exotic species were taxonomically balanced at the family level, with six species in the families Fabaceae and Poaceae. We used an additional two species, *Rumex crispus* and *R. salicifolius* (Polygonaceae); however, these failed to emerge and thus we excluded them from all analysis. We obtained seeds from a commercial supplier (S&S Seeds, Carpinteria, California USA). We did not observe any of the focal species at the site before starting the experiment, ensuring that all individuals measured did not emerge from an existing seedbank.

We grew plants under one of three herbivore treatments: “Early exclusion”, “Late exclusion”, and “No exclusion”. We constructed herbivore exclosures using 6.3 mm hardware cloth, designed to exclude all vertebrate, but not invertebrate herbivores. We measured of photosynthetically active radiation (PAR) inside and outside exclosures with an AccuPAR LP-80 PAR Ceptometer (Decagon Devices Inc., Washington, USA); shading by exclosures reduced PAR by approximately 30% (Appendix Table 4A.1). We excluded herbivores for the first half of the experiment in the early exclusion treatment, the second half of the experiment in the late exclusion treatment, and allowed herbivore access for the entire duration in the no exclusion treatment.

Table 4.1: Focal species used in the experiment, along with abbreviations used in figures, origin (with reference to California), family, and life history.

Scientific Name	Abbreviation	Origin	Family	Life History
Acmispon americanus	ACM AM	Native	Fabaceae	Annual
Lupinus bicolor	LUP BIC	Native	Fabaceae	Annual
Trifolium willdenovii	TRI WIL	Native	Fabaceae	Annual
Bromus carinatus	BRO CAR	Native	Poaceae	Perennial
Festuca microstachys	FES MIC	Native	Poaceae	Annual
Festuca rubra	FES RUB	Native	Poaceae	Perennial
Medicago polymorpha	MED POL	Exotic	Fabaceae	Annual
Trifolium hirtum	TRI HIR	Exotic	Fabaceae	Annual
Vicia villosa	VIC VIL	Exotic	Fabaceae	Annual
Bromus hordeaceus	BRO HOR	Exotic	Poaceae	Annual
Festuca myuros	FES MY	Exotic	Poaceae	Annual
Festuca perennis	FES PER	Exotic	Poaceae	Annual/Perennial

We grew each species in both monocultures and in polycultures containing all 12 species. We assigned eight plots to each herbivore exclusion treatment, with each plot containing 12 monoculture subplots and one polyculture subplot (each subplot measured 15 cm x 15 cm with 2.5 cm between adjacent subplots), for a total of 288 monoculture and 24 polyculture subplots (Fig. 4.1). When in place, single herbivore exclosures covered entire plots. To account for potential effects of subplot location within plots, we used eight planting plans that mixed the edge versus interior positions of subplots within plots for each exclusion treatment. We spaced plots 0.8 m apart in a 3 x 8 grid, with one plot of each exclusion treatment in every row of three (Fig. 4.1). We arranged rows at different distances from nearby greenhouses (which might potentially affect herbivore activity), so we refer to these hereafter as "blocks" for statistical analyses. We planted monoculture subplots with seeds to achieve 14 individuals, and planted polyculture with seeds to achieve two individuals of each species (Appendix Table 4A.2 contains emergence rates and numbers of seeds planted for each focal species). Whilst these densities are lower than those previously reported in California grasslands (Heady 1958, Bartolome 1979), we used these densities to ensure we could accurately monitor the emergence of individual plants. We conducted greenhouse emergence trials prior to the start of the experiment to estimate the number of seeds required to produce the targeted number of individuals in monoculture and polyculture, and to aid identification of seedlings in the field.

We watered and raked the experimental area daily for 2 weeks before the start of the experiment to emerge and remove the standing seed bank. We planted seeds of focal species on 3 November, 2 days after the first winter rain event. To stimulate emergence, we applied 1 L of water evenly across each plot on a daily basis from 8 November until the next rain event on 20 November. We removed weeds carefully by hand throughout the experiment. We monitored the number of surviving seedings and cumulative total number of seedlings daily in monoculture subplots beginning 9 November when we observed the first emerging seedling; we designated this day as Day 1 of the experiment. Due to the difficulty of seedling identification, we did not monitor emergence in polyculture subplots.

We recorded newly emerging individuals for 35 days (until 13 December), at which point we transferred exclosures from early exclusion treatments to late exclusion treatments. Beginning 16 January, 35 days after transferring exclosures and 70 days after we observed the first emergence event, we counted all remaining individuals and harvested aboveground biomass. The duration of the experiment matched the length of time between widespread emergence and peak biomass observed in local herbaceous dominated vegetation communities, estimated at 2.5

months (see Figure 22.5 in Cleland et al. 2016). No plants of any species had begun flowering at the time of harvest. To confirm that the majority of emergence had occurred by the midway point of the experiment, we calculated for each species the difference in the mean number of individuals harvested in each subplot after 70 days and the mean cumulative total of emerging plants observed after 35 days (regardless of mortality). This value was below one for all species, confirming that most emergence had occurred by this point.

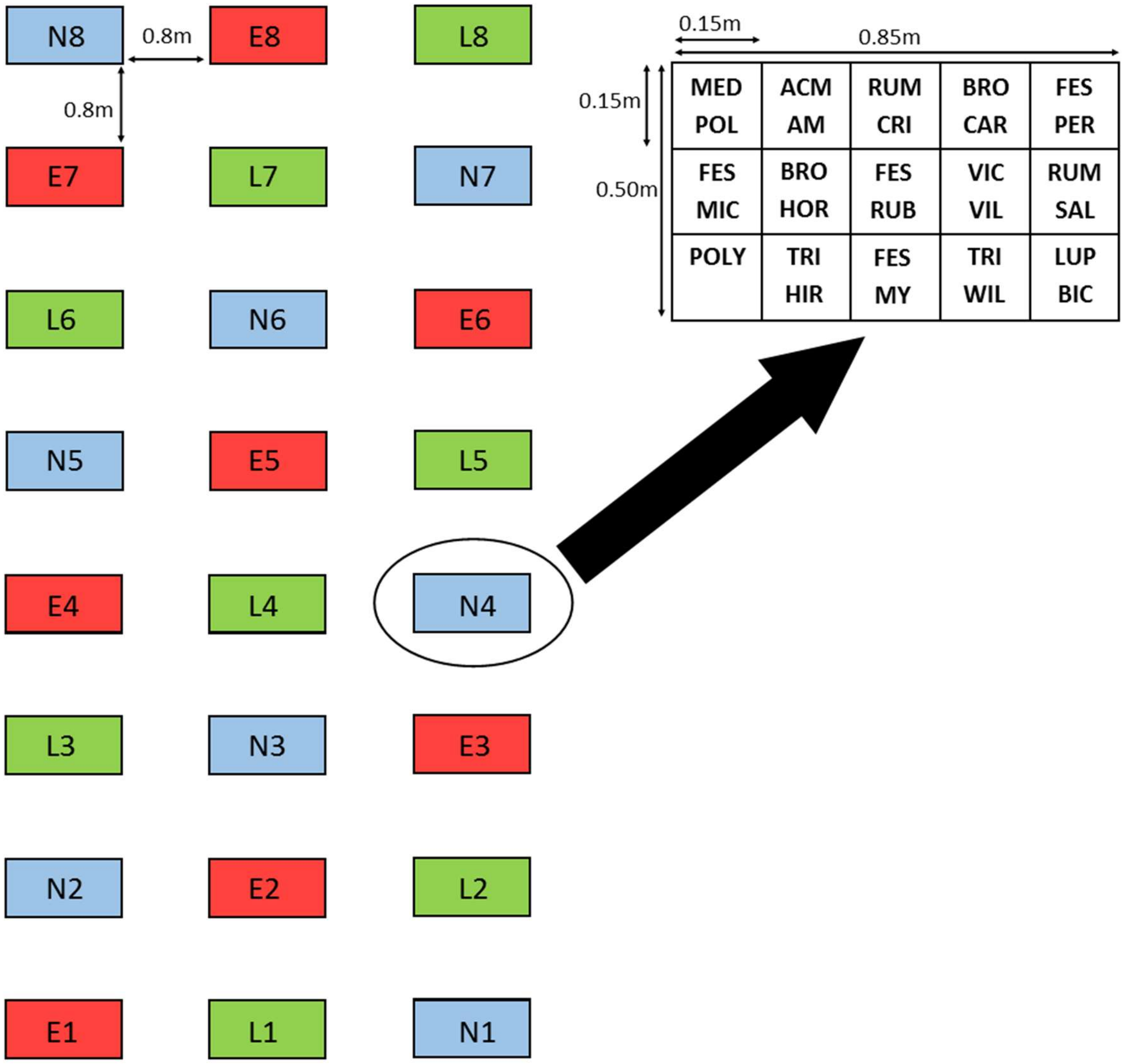

Figure 4.1: Experimental design showing the arrangement of 24 plots (left) and example arrangement of subplots within a single plot (above). One plot in each of early, late and no exclusion treatments (red, green and blue rectangles, respectively) is represented in every block of three plots. Within plots, one polyculture subplot ('POLY') and 14 monoculture subplots (see Table 4.1 for species abbreviations) are arranged in a 3 x 5 grid. Two monoculture subplots were planted with *Rumex* spp. ('RUM CRI' and 'RUM SAL'); these failed to emerge and were removed from all analysis.

We calculated emergence time as the number of days taken to reach 50% of total emergence after 35 days. We used the number of individual plants at time of harvest as a measure of density in monoculture and polyculture subplots. We dried harvested plants at 40°C for 7 days, and then recorded the total biomass in monoculture subplots and the biomass of each species in polyculture subplots.

To assess the performance of focal species in polyculture under each exclusion treatment (hereon referred to as 'relative performance in polyculture' [RPP]), we calculated the proportional deviation of a species' yield in polyculture from its yield in monoculture in the same exclusion treatment (*sensu* Loreau 1998). We used the mean biomass of individual plants of a species in monoculture and polyculture, pooled across all eight plots within exclusion treatments, as measures of yield to calculate the RPP, as given by the following equation.

$$RPP = \frac{P - M}{M}$$

Where P and M represent the mean biomass per individual of a species in a particular exclusion treatment in polyculture and monoculture, respectively. A RPP value of 0 indicates no difference in performance (individual size) between monoculture and polyculture for a species in a particular exclusion treatment. A RPP value below 0 indicates plants grown in polyculture were on average smaller than those in monoculture, whilst a RPP value greater than 0 indicates plants grown in polyculture were on average larger than those in monoculture.

Statistical analyses

We conducted all statistical analyses using R version 3.2.3 (R Core Team 2015). We specified linear mixed-effects models with the *lme* function in the package *nlme* (Pinheiro et al. 2015). We specified linear fixed-effect models using the *lm* function in the package *stats.* We evaluated the significance of all factors using Type II tests with the *Anova* function in the *car* package (Fox and Weisberg 2011), calculating Wald chi-square statistics for models in *lme* and F ratio statistics for models in *lm*. We carried out post hoc Tukey tests of main effects using the *glht* function in the package *multcomp* (Hothorn et al. 2008), with main effects averaged over covariates.

We excluded from all analysis four monoculture subplots where no individuals emerged. Furthermore, one *Bromus carinatus* subplot had more than twice the biomass of the next most productive subplot, and so we removed as an outlier for analyses of biomass and performance in polyculture.

To analyze how emergence time varied with origin, herbivore exclusion treatment and block, we fit a linear mixed-effects model with species nested in origin treated as a random effect. The block effect was non-significant (Appendix Table 4A.3), and so we removed this term from the final model. We fit a second mixed-effects model to analyze how density in monoculture subplots varied with origin, exclusion treatment and block; species nested in origin was treated as a random effect. Again, there was no significant effect of block (Appendix Table 4A.4), and so we removed this term from the final model.

To analyze how total biomass in monoculture varied with origin, exclusion treatment, emergence time (and their interactions), as well as plant density and block, we used a linear mixed-effects model with species nested in origin as a random effect. We observed no significant effect of block (Appendix Table 4A.5), therefore we removed this term from the final model. We fit a linear model to analyze how total biomass in polyculture subplots varied with exclusion treatment, density and block; because polyculture subplots contained all species, there were no random effects in this model. We observed no significant effect of block (Appendix Table 4A.6), therefore we removed this term from the final model.

To analyze how the relative performance of focal species in polyculture varied with origin, exclusion treatment, and emergence time (and their interactions), we used a linear mixed-effect model with species nested in origin as a random effect. Because we did not monitor emergence in polyculture subplots, we used the mean emergence times of species across all exclusion treatments in monoculture; this approach was justified as exclosures did not influence time to emergence for any species (Appendix Table 4A.7). We pooled data from all plots in exclusion treatments to calculate mean individual biomass, therefore we did not include block in the model.

To confirm that potential differences in mean biomass were driven by herbivory effects rather than differences in density between monoculture and polyculture subplots, We fit a linear mixed-effects model to analyze whether mean individual biomass varied with both subplot type (monoculture or polyculture) and density in subplots; species nested within origin was specified as a random effect. Across all subplots, there was no significant effect of subplot type, density, or the interaction between them on mean individual biomass (Appendix Table 4A.8). Scatterplots of mean individual biomass in relation to total subplot density in monoculture and polyculture for the 12 focal species are shown in Appendix Fig. 4A.1.

Results

Emergence and density of focal species

Consistent with prior work in this study system, exotic species emerged significantly faster than native species ($\chi^2_{(1)} = 4.05$, $P = 0.044$; Table 4.2; Fig. 4.2). There was no difference in mean emergence time between exclusion treatments ($\chi^2_{(2)} = 4.58$, $P = 0.10$; Table 4.2) and no significant interaction between origin and exclusion treatment ($\chi^2_{(2)} = 1.61$, $P = 0.45$; Table 4.2). These results suggest that the following analysis of biomass and RPP was unlikely to be confounded by altered emergence behavior between exclusion treatments. Mean emergence time for species ranged from 9.6 days for the exotic legume *Vicia villosa*, to 26.5 days for the native legume *Acmispon americanus* (Fig. 4.2).

Overall, we observed mean densities of 13.6 individuals in monoculture and 20.2 individuals in polyculture; however, there was considerable variation amongst species (Appendix Table 4A.2). Exclusion treatment did not significantly affect the density of individuals in monoculture ($\chi^2_{(2)} = 1.565$, $P = 0.457$; Table 4.3). Native and exotic species did not differ with regards to density ($\chi^2_{(1)} = 1.306$, $P = 0.253$; Table 4.3), and there was no interaction between origin and exclusion treatment ($\chi^2_{(2)} = 2.355$, $P = 0.308$; Table 4.3).

Table 4.2: Analysis-of-deviance table derived from linear mixed-effects model of emergence time in monoculture by origin and exclusion treatment. Species nested within origin was treated as a random factor.

	Emergence Time		
	Df	χ^2	*P*
Origin	1	4.05	**0.044**
Exclusion	2	4.58	0.10
Origin*Exclusion	2	1.61	0.45

Table 4.3: Analysis-of-deviance table derived from linear mixed-effects model of density in monoculture subplots by origin and exclusion treatment. Species nested within origin was treated as a random factor.

	Density		
	Df	χ^2	*P*
Origin	1	1.31	0.25
Exclusion	2	1.57	0.46
Origin*Exclusion	2	2.36	0.31

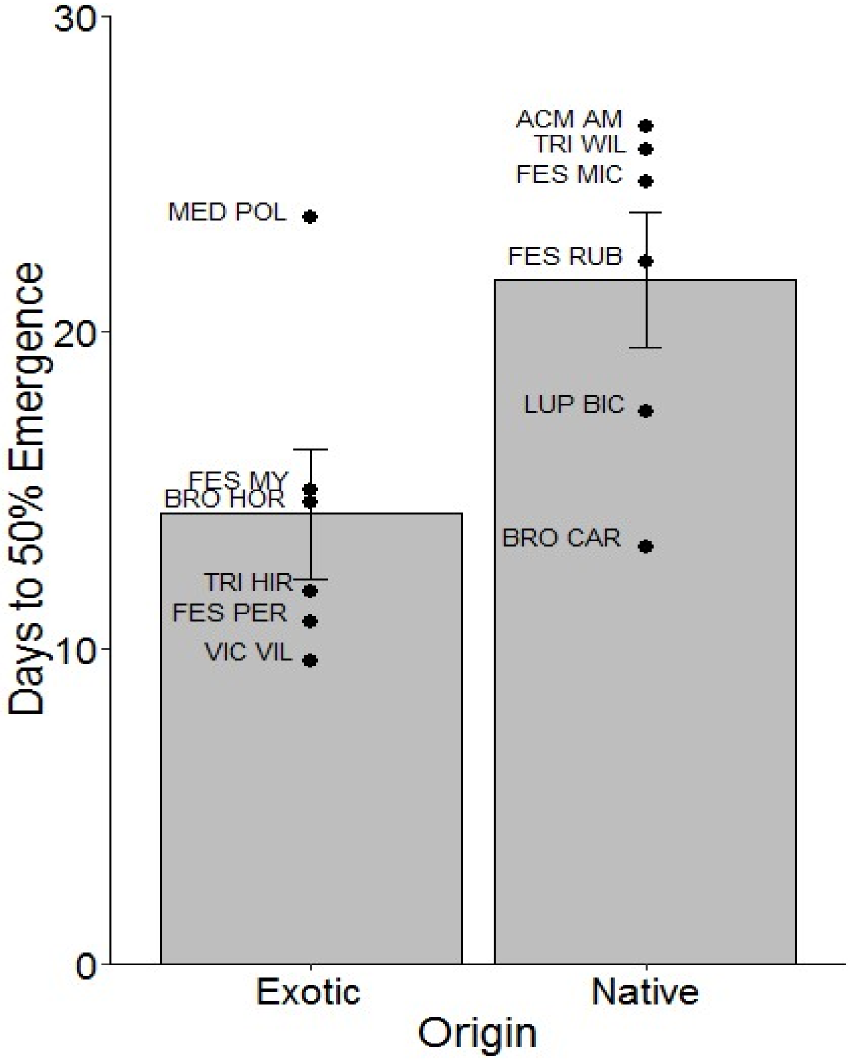

Figure 4.2: Days to reach 50% emergence for native and exotic focal species. Filled circles represent species mean values. Error bars denote one standard error of the mean where *n* = 6, the number of species within each origin.

Hypothesis 1: earlier emerging species are more susceptible to herbivores, and this susceptibility is greatest early in the growing season

Emergence time was a significant predictor of total biomass in monoculture subplots after 70 days, with earlier emergence resulting in greater biomass ($\chi^2_{(1)} = 9.014$, $P = 0.003$; Table 4.4). Density of plants was a significant predictor of subplot biomass in monoculture ($\chi^2_{(1)} = 22.298$, $P < 0.001$; Table 4.4), but not in polyculture subplots ($F_{(1)} = 1.074$, $P = 0.312$; Table 4.5). Exclusion treatment was a significant predictor of subplot biomass in both monoculture ($\chi^2_{(2)} = 27.508$, $P < 0.001$; Table 4.4; Fig. 4.3) and polyculture ($F_{(2)} = 8.094$, $P = 0.003$; Table 4.5; Fig. 4.3). Post-hoc Tukey's HSD tests showed that in monoculture, biomass in early exclusion treatments was significantly higher than the no exclusion treatment ($P = 0.001$), whilst all other pairwise comparisons were non-significant (Fig. 4.3). In polyculture, subplot biomass in the early exclusion treatment was significantly higher than both late ($P = 0.009$) and no exclusion treatments ($P = 0.004$), with these latter two groups not differing significantly from one another (Fig. 4.3). In monoculture, we observed a significant interaction between emergence time and herbivore exclusion treatment ($\chi^2_{(2)} = 28.631$, $P < 0.001$; Table 4.4), with earlier emergence associated with greater increases in biomass in early exclusion subplots compared to late and no exclusion subplots (Fig. 4.4). Biomass of native and exotic species in monoculture responded similarly to exclusion treatments, as there was no significant interaction between origin and exclusion treatment ($\chi^2_{(2)} = 2.335$, $P = 0.311$; Table 4.4).

Table 4.4: Analysis-of-deviance table derived from linear mixed-effects model of subplot biomass in monoculture by origin, exclusion treatment, emergence time and density. Species nested within origin was treated as a random factor.

	Biomass		
	Df	χ^2	*P*
Origin	1	2.62	0.11
Exclusion	2	27.51	**< 0.001**
Emergence	1	9.01	**0.003**
Density	1	22.30	**< 0.001**
Origin*Exclusion	2	2.34	0.31
Origin*Emergence	1	1.17	0.28
Exclusion*Emergence	2	28.63	**<0.001**
Origin*Exclusion*Emergence	2	0.68	0.71

Table 4.5: Analysis-of-variance table derived from linear fixed-effects model of subplot biomass in polyculture by exclusion treatment and density.

	Biomass		
	Df	F	*P*
Exclusion	2	8.09	**0.003**
Density	1	1.07	0.31
Residuals	20		

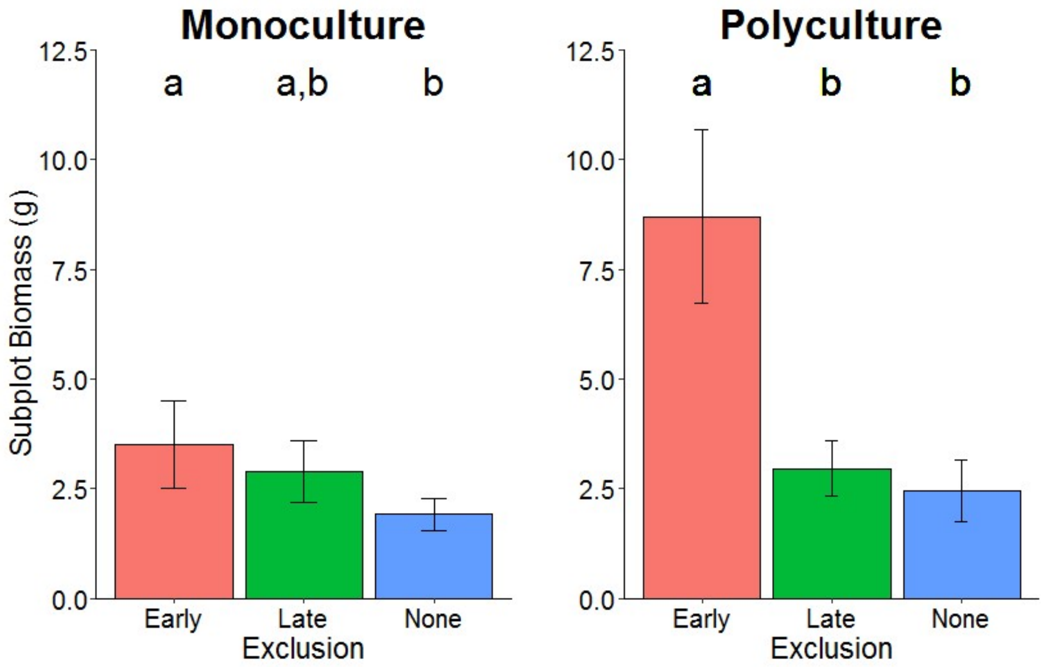

Figure 4.3: Mean biomass after 70 days in monoculture subplots (left) and polyculture subplots (right). Error bars represent one standard error of the mean: in monoculture *n* = 12, the number of species in each exclusion treatment; in polyculture *n* = 8, the number of replicates within each exclusion treatment. Groups sharing letters do not differ significantly in post hoc Tukey tests.

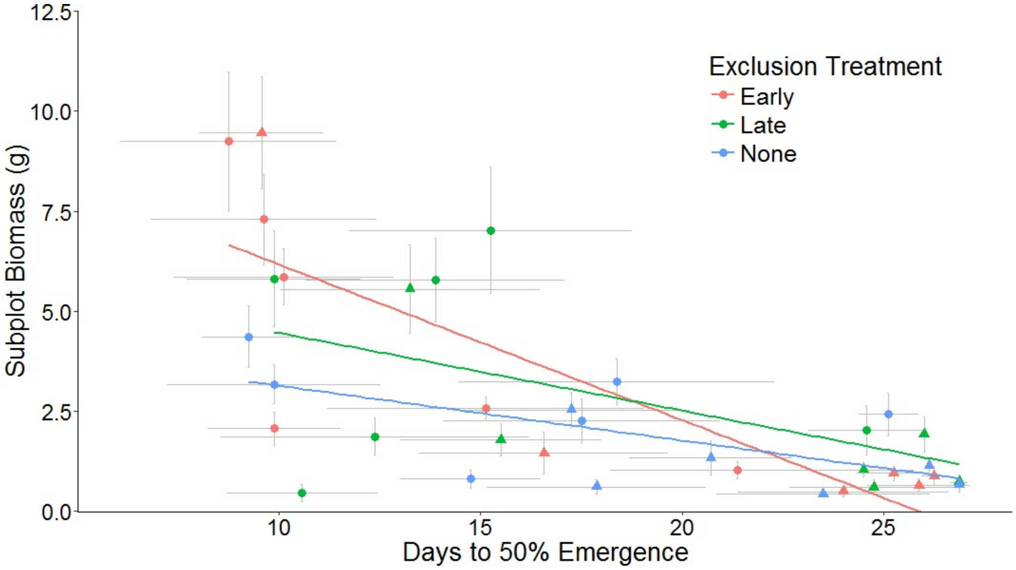

Figure 4.4: Scatterplot of monoculture subplot biomass in relation to emergence time for species in each exclusion treatment. Exotic species are denoted by circles, and native species are denoted by triangles. Horizontal and vertical grey bars denote one standard error of the mean, where *n* is the number of replicates within each exclusion treatment for each species.

Hypothesis 2: in polyculture, the competitive advantage of early-arriving exotic species is reduced by herbivory, and this effect is greatest early in the growing season

We observed lower individual biomass in polyculture (relative to monoculture) across all species and exclusion treatments with a mean RPP of -0.355 (± 0.106); likely due to the greater density at which individuals were planted in polyculture. We observed no significant effect of emergence time ($\chi^2_{(1)} = 1.755$, $P = 0.185$; Table 4.6) or exclusion treatment ($\chi^2_{(2)} = 2.110$, $P = 0.348$; Table 4.6) on RPP, and there was no significant interaction between them ($\chi^2_{(2)} = 1.870$, $P = 0.393$; Table 4.6). There was no overall difference in RPP between natives and exotics ($\chi^2_{(1)} = 0.008$, $P = 0.928$; Table 4.6). However, we observed a marginally significant interaction between origin and exclusion treatment ($\chi^2_{(2)} = 5.810$, $P = 0.055$; Table 4.6; Fig. 4.5). Post-hoc Tukey's HSD tests showed that for exotic species, RPP was significantly higher in the early exclusion treatment than in the no exclusion treatment ($P = 0.013$), whilst all other pairwise comparisons were non-significant (Fig. 4.5). For native species, RPP was significantly higher in the no exclusion treatment than early exclusion ($P = 0.037$) and late exclusion treatments ($P = 0.033$) (Fig. 4.5).

Table 4.6: Analysis-of-deviance table derived from linear mixed-effects model of RPP by origin, exclusion treatment and emergence time. Species nested within origin was treated as a random factor.

	RPP		
	Df	χ^2	*P*
Origin	1	0.008	0.93
Exclusion	2	2.11	0.35
Emergence	1	1.76	0.19
Origin*Exclusion	2	5.81	0.055
Origin*Emergence	1	0.43	0.51
Exclusion*Emergence	2	1.87	0.39
Origin*Exclusion*Emergence	2	0.20	0.91

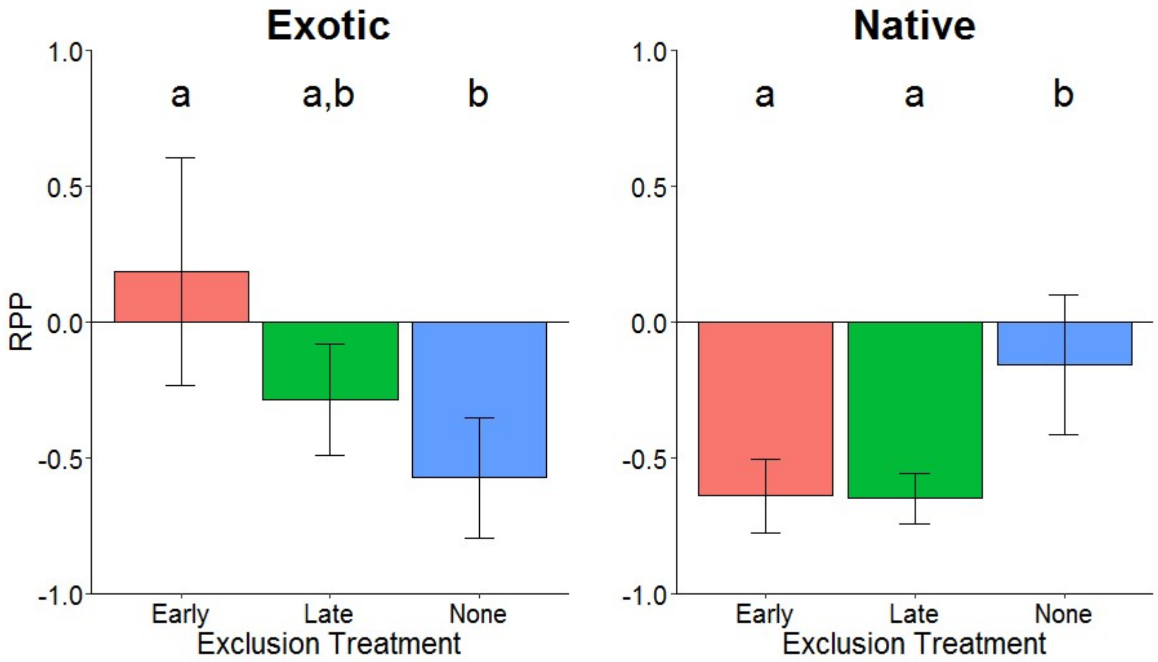

Figure 4.5: Relative performance in polyculture (RPP) in each exclusion treatment for exotic species (left) and native species (right). Error bars represent one standard error of the mean, where *n* = 6, the number of species in each exclusion treatment. Groups sharing letters do not differ significantly in post hoc Tukey tests.

Discussion

Emergence and density of focal species

Exotic species emerged faster than native species, consistent with past observations in California (Reynolds et al. 2001, Deering and Young 2006, Abraham et al. 2009, Wainwright et al. 2012, Wainwright and Cleland 2013). Although evidence is accumulating that emergence time is phylogenetically conserved (Norden et al. 2009, Xu et al. 2014), we observed significant differences between natives and exotics even when the species pool was taxonomically balanced at the family level.

The density of individuals in monoculture did not differ significantly across exclusion treatments. The differences in biomass between exclusion treatments are largely the result of differences in the size of plants harvested, rather than differences in the number of individuals remaining after 70 days.

Hypothesis 1: earlier emerging species are more susceptible to herbivores, and this susceptibility is greatest early in the growing season

As expected, earlier emergence resulted in greater biomass in monoculture subplots. This may be because such species have a longer time to accrue biomass but could also reflect higher growth rates of species that emerge earlier. The higher overall biomass observed when herbivores were excluded early in the growing season (Fig. 4.3) confirms that herbivory at this stage has disproportionately large effects on plant growth; this result is striking given that actively growing plants in the late exclusion treatment were effectively protected from herbivory for longer than plants in the early exclusion treatment (because the first 35 days of exclusion started with the very first emerging individuals, and most individuals emerged after this date). The significant interaction between emergence time and exclusion treatment, (Fig. 4.4), shows that this higher overall impact of herbivory in the early season is driven largely by the increased susceptibility of earlier emerging species which tended to have greater biomass. This result therefore lends support to our first hypothesis of a trade-off between emergence time and susceptibility to herbivory that is most prominent in the early growing season.

The greater impact of herbivory in the early growing season is likely due to greater herbivore preference for younger plants and lower ability to tolerate damage in earlier ontogenetic stages. Herbivores often prefer tissue of younger plants because they are less strongly defended and of greater nutritional quality (Quintero et al. 2014). Furthermore, younger plants have limited capacity to regrow in response to herbivory (Strauss and Agrawal 1999, Boege and Marquis 2005). Simulated herbivory in European grassland species has been shown to be most detrimental to growth when occurring earliest in ontogeny, with lower biomass persisting for longer than the duration of this experiment (Hanley and Fegan 2007). It must be noted that tolerance to herbivory also reflects the ability to reproduce following damage, and this is expected to be low if damage occurs shortly before reproduction (Strauss and Agrawal 1999). We harvested plants before they reached reproductive maturity; therefore, the fitness consequences of the timing of herbivore exposure were beyond the scope of this experiment.

As there was no significant difference in the density of individuals between exclusion treatments, the impacts of herbivory in this experiment were largely mediated by reductions in plant size rather than mortality. Although herbivory is often fatal for plants in early ontogenetic stages, this result is less surprising given that species which emerged earlier and achieved greater biomass were disproportionately impacted, and these would be expected to best tolerate herbivore damage (Strauss and Agrawal 1999, Boege and Marquis 2005).

We hypothesized that this increased susceptibility of early-emerging species could result from greater apparency to herbivores. However, the association between larger size and greater susceptibility observed in this experiment could also result from a trade-off between growth and defense. With this experimental design we are unable to conclude that this pattern was observed solely due to differences in apparency. A study in northern California by Cushman et al. (2011) found that herbivory by native black-tailed jackrabbits on the exotic grass *Ehrharta calycina* was lower when plants were growing in association with established native and exotic perennials which reduced their apparency. Therefore, apparency is likely an important mechanism underlying the trade-off between early emergence and increased susceptibility to generalist mammalian herbivores observed in this experiment. It must also be noted that herbivore feeding behavior in this experimental context (where there is a choice between treatments which are separated spatially) may not reflect herbivore in a more natural, non-choice situation such as a homogenous grassland.

We harvested all plants simultaneously after 70 days (regardless of emergence time), thus the increased susceptibility of earlier emerging species to early-season herbivory might simply be a function of the longer time that they were exposed to herbivores (approximately 17 days between fastest and slowest emerging species; Fig. 4.2). However, as discussed above, herbivore impact on biomass was not predicted by the duration of exposure to herbivores: late-emerging species showed little response to exclusion treatments that differed in herbivore exposure time by 35 days or more (Fig. 4.4). Another potential driver of the increased susceptibility associated with earlier emergence is that exotic species, which on average emerged earlier, may be less resistant to evolutionarily novel native herbivores when encountered, perhaps because of a lack of coevolved defenses (Colautti et al. 2004, Parker et al. 2006). However, we observed no significant interaction between exclusion treatment and origin, showing that origin *per se* had little effect on susceptibility in monoculture.

Hypothesis 2: in polyculture, the competitive advantage of early-arriving exotic species is reduced by herbivory, and this effect is greatest early in the growing season

We found considerable support for our second hypothesis. Exotic species, which emerged faster, had significantly higher RPP when herbivores were excluded early compared to the no exclusion treatment (Fig. 4.5). On the other hand, later-emerging natives demonstrated significantly higher RPP when herbivores were not excluded throughout the experiment (Fig. 4.5). This highlights the importance of accounting for herbivory (or lack thereof) when interpreting results from priority effects studies.

These patterns of RPP between exclusion treatments are likely observed because differences in emergence time tend to be larger between heterospecific competitors than conspecifics, which has several consequences. Firstly, individuals of early-emerging exotic species in polyculture can pre-empt resources to a greater extent because they are competing with heterospecifics that are more likely to emerge later. This could lead to improved performance of early-emerging exotic species in polyculture, consistent with priority effects mechanisms (Fukami 2015). On the other hand, later-emerging native species may display suppressed growth in polyculture because they are competing with heterospecifics that are more likely to emerge earlier. Secondly, the increased differences in emergence time between individuals in polyculture may also enhance herbivory effects. The greater size of exotic species that emerge earlier than heterospecific competitors in polyculture may mean they are relatively more apparent and accessible than when growing with conspecifics in monoculture, a form of associational susceptibility (Underwood et al. 2014). Smaller, later-emerging native species however would be relatively less apparent and accessible when growing with larger heterospecific competitors in polyculture than with similar sized conspecifics in monoculture; therefore, growth in polyculture may provide associational resistance (Underwood et al. 2014). Furthermore, native species can benefit from competitive release if early-active exotic species are preferentially consumed (Beck et al. 2015).

The greater RPP of native species observed in the no exclusion treatment suggests that in this system mammalian herbivores may play an important role in maintaining the performance of late-emerging natives in the face of competitive advantages gained by early-emerging exotics. How these changes in performance translate to population-level effects on the abundance of native and exotic species is beyond the scope of this experiment, as abundance is ultimately determined by multiple demographic processes such as recruitment and mortality (Crawley 2007). However, field experiments have shown that in Mediterranean ecosystems, herbivores can indeed reduce the abundance of early-active exotic species or functional groups and increase the abundance of later-active species. For example, in California grasslands, decreases in the abundance of early-active exotic annual grasses as a result of exposure to herbivores are linked to increased abundance of forb species that tend to emerge slowly in response to seasonal rains (Peters 2007, Skaer et al. 2013). Grazing in this system has also been shown to increase the cover and diversity of native forbs as well as decreasing year-to-year variability of these metrics (Beck et al. 2015).

Our results indicate that emergence time represents a trade-off between competitive ability and herbivore susceptibility, and that native and exotic species differ in their strategies. Fast emergence of exotics may allow them to benefit from seasonal priority effects (Wainwright et al. 2012), but at the cost of increased susceptibility to generalist herbivores. Previous studies have shown that exotic species in Mediterranean systems are better able to tolerate herbivore damage than natives, perhaps due to their longer evolutionary histories of intensive grazing (Holmgren 2002, HilleRisLambers et al. 2010). This may allow them to maintain a strategy of early emergence relative to the native community, despite their increased susceptibility to mammalian herbivores.

Shade effects of herbivore exclosures

Although species emergence times were not affected by exclusion treatment, a caveat of this experiment is that exclosures may also have altered biomass and RPP by reducing light availability (30% reduction in PAR). It has been shown in grassland systems that community biomass can increase under conditions of moderate shade (50%), with many species responding positively as result of stress reduction (Semchenko et al. 2012). Although the shading in this experiment is considerably lower, it is still possible that this may have affected biomass results over and above the effect of herbivore exclusion. If the partial shade provided by exclosures was a major factor promoting growth, then early-emerging species would likely benefit more from early exclusion than later-emerging species, as they have a relatively longer period of growth under shade. However, biomass would also be expected to be greatest in the late exclusion treatment where actively growing plants were effectively covered for longest. Similarly, if the contrasting RPP responses of native and exotics to herbivore exclusion treatments were driven by differential capacity to tolerate shade, then the biggest differences in RPP would be expected between late and no exclusion treatments where the effective duration of shade experienced by actively growing plants was longest and shortest, respectively. Although we cannot rule out the possibility that shade influenced differences in biomass and RPP between exclusion treatments, there is little evidence suggesting that these effects, rather than herbivory, were driving observed patterns.

Management implications

Herbivores can have contrasting effects on exotic plant invasions in Mediterranean systems. For example, continuous, heavy grazing over many years is thought to have contributed to the invasion of Chile, southwestern Australia, and California by exotic herbaceous species, many of which were likely pre-adapted to tolerate this chronic disturbance (Hobbs 2001, Holmgren 2002, HilleRisLambers et al. 2010). Despite having the potential to facilitate invasions, grazing is often suggested as an effective conservation and restoration tool to minimize exotic impacts in such regions (e.g. Beck et al. 2015). This is because it is becoming increasingly recognized that phenological differences between native and exotic species can be exploited to enhance the success of management and restoration efforts (Marushia et al. 2010, Wolkovich and Cleland 2011), and therefore prescription of grazing treatments during appropriate phenological windows can lead to more favorable outcomes for native species (Rinella and Hileman 2009). For example, short-duration grazing while exotic species are flowering is an effective restoration tool that acts by reducing their seed output (Menke 1992). Our results suggest that, in areas characterized by rapid exotic emergence, grazing during the earliest period of the growing season is likely to be another effective management strategy for improving native performance relative to exotic competitors. This is consistent with findings of studies in California showing that grazing in the winter/spring (early-season) leads to greater decreases in the abundance of early-active exotics (Skaer et al. 2013), whereas grazing applied in the spring/summer (late-season), at which point many exotic species are no longer active, leads to greater negative impacts on native abundance (HilleRisLambers et al. 2010). However, early-season grazing is unlikely to be effective where exotic species do not benefit from priority effects (Funk et al. 2015).

Conclusions

This study took place in the Mediterranean-type climate region of southern California where seasonal growth is initiated following the arrival of winter rains. However, the factors controlling the onset of the growing season vary within and across biomes, leading to differences in growing season length and speed of seasonal transitions (Pau et al. 2011). We expect that the trade-off between emergence time and herbivore susceptibility observed in this experiment will be present in all systems where growth occurs within distinct growing seasons, regardless of the factors determining their onset. In regions where growth is year-round, we would not expect to see a relationship between emergence time and herbivore susceptibility. What is less clear is how the length of growing seasons and the speed of seasonal transitions determine the magnitude of this trade-off. For example, slower seasonal transitions might result in larger differences in emergence time between early- and late-arriving species (Pau et al. 2011), and this may enhance both the competitive advantage and increased herbivore susceptibility associated with early-arrival.

The proposed mechanism by which early-active species are more susceptible to herbivores (increased apparency) might conceivably also result in increased susceptibility for species that have extended late-season phenology. Whilst this was beyond the scope of this short-term experiment, future work could explore whether an analogous trade-off exists between extended phenology and herbivore susceptibility. Such understanding would be particularly valuable given that extended phenology has been identified as a key trait in certain

Mediterranean invaders such as *Centaurea solstitialis* (Roché and Thill 2001), and invaders in other systems including deciduous forests (Fridley 2012).

This study demonstrates that in systems defined by seasonal periods of growth, emergence time is a key trait that may predict species' susceptibility to generalist herbivores. Because herbivore defense and phenology are both associated with many other correlated plant functional traits (Wolkovich and Cleland 2014), this trade-off is likely an important driver of the evolution of life-history variation in seasonally defined communities. In addition to aiding our fundamental understanding of how trade-offs predict variation in species abundances and species co-existence in diverse communities, an understanding of trade-offs may improve the efficacy of management efforts that exploit phenological differences between native and exotic species.

Acknowledgements

We thank Stuart Sandin for statistical advice, and thank Joshua Kohn, Justin Meyer and two anonymous reviewers for helpful comments on a prior version of this manuscript. We are also grateful to members of the Cleland Lab who helped with planting, harvesting and processing of samples. Funding was provided by a Jeanne M. Messier Memorial Fellowship to JW.

Chapter 4, in full, has been published in the journal *Ecology & Evolution*. Waterton, J., and E.E. Cleland. The dissertation author is the primary investigator and author of this paper.

Appendix 4A

Table 4A.1: Photosynthetically active radiation (PAR) inside and outside herbivore exclosures.

Measurement Date	Outside Exclosure (µmol $m^{-2}s^{-1}$)	Inside Exclosure (µmol $m^{-2}s^{-1}$)	Exclosure PAR Reduction (%)
02/18/2015	1030	710	31.07
04/03/2015	1220	828	32.13

Table 4A.2: Seeds planted, mean seedlings after 35 days and mean number of individuals harvested after 70 days for 12 focal species across all monoculture and polyculture subplots.

Scientific name	Monoculture				Polyculture	
	Seeds Planted	Total Seedlings at 35 Days	Percent Emergence at 35 days	Individuals Harvested at 70 days (Density)	Seeds Planted	Individuals Harvested at 70 Days
Acmispon americanus	41	11.5	28.1	10.3	6	1.6
Bromus carinatus	26	17.3	66.5	17.3	4	1.6
Bromus hordeaceus	16	12.0	75.3	12.3	2	2.2
Festuca microstachys	20	11.8	59.0	10.8	3	2.2
Festuca myuros	18	11.7	64.8	12.2	3	1.5
Festuca perennis	16	12.5	77.9	11.7	2	1.1
Festuca rubra	32	15.3	47.8	15.6	5	1.5
Lupinus bicolor	41	7.4	18.1	6.5	6	0.6
Medicago polymorpha	35	12.1	34.6	8.9	5	0.8
Trifolium hirtum	32	7.9	24.6	5.0	5	1.0
Trifolium willdenovi	233	40.1	17.2	37.7	33	4.4
Vicia villosa	21	13.6	64.7	13.0	3	1.9

Table 4A.3: Analysis-of-deviance table derived from linear mixed-effects model of emergence time in monoculture by origin and exclusion treatment, with block included as a fixed effect. Species nested within origin was treated as a random factor. Block was non-significant and therefore removed from the final model.

	Emergence Time		
	Df	χ^2	*P*
Origin	1	4.00	**0.046**
Exclusion	2	4.59	0.10
Block	7	8.67	0.28
Origin*Exclusion	2	1.66	0.44

Table 4A.4: Analysis-of-deviance table derived from linear mixed-effects model of density in monoculture by origin and exclusion treatment, with block included as a fixed effect. Species nested within origin was treated as a random factor. Block was non-significant and therefore removed from the final model.

	Density		
	Df	χ^2	*P*
Origin	1	1.31	0.25
Exclusion	2	1.50	0.47
Block	7	5.98	0.54
Origin*Exclusion	2	2.39	0.30

Table 4A.5: Analysis-of-deviance table derived from linear mixed-effects model of subplot biomass in monoculture by origin, exclusion treatment, emergence time and density, with block included as a fixed effect. Species nested within origin was treated as a random factor. Block was non-significant and therefore removed from the final model.

	Biomass		
	Df	χ^2	*P*
Origin	1	2.57	0.11
Exclusion	2	27.54	**<0.001**
Emergence	1	10.73	**0.001**
Density	1	23.28	**<0.001**
Block	7	10.96	0.14
Origin*Exclusion	2	2.90	0.24
Origin*Emergence	1	0.67	0.41
Exclusion*Emergence	2	30.29	**<0.001**
Origin*Exclusion*Emergence	2	0.57	0.75

Table 4A.6: Analysis-of-variance table derived from linear fixed-effects model of subplot biomass in polyculture by exclusion treatment and density, with block included as a fixed effect. Block was non-significant and therefore removed from the final model.

	Biomass		
	Df	F	*P*
Exclusion	2	12.30	**0.001**
Density	1	2.12	0.17
Block	7	2.39	0.083
Residuals	13		

Table 4A.7: Analysis-of-variance table derived from linear fixed-effects model of emergence time in monoculture by exclusion treatment and species.

	Emergence Time		
	Df	F	*P*
Exclusion	2	2.31	0.10
Species	11	20.46	**<0.001**
Exclusion*Species	22	1.10	0.35
Residuals	248		

Table 4A.8: Analysis-of-deviance table derived from linear mixed-effects model of individual biomass by subplot type (monoculture or polyculture) and density. Species nested within origin was treated as a random factor.

	Individual Biomass		
	Df	χ^2	*P*
Subplot	1	0.15	0.70
Density	1	2.13	0.14
Subplot*Density	1	0.059	0.81

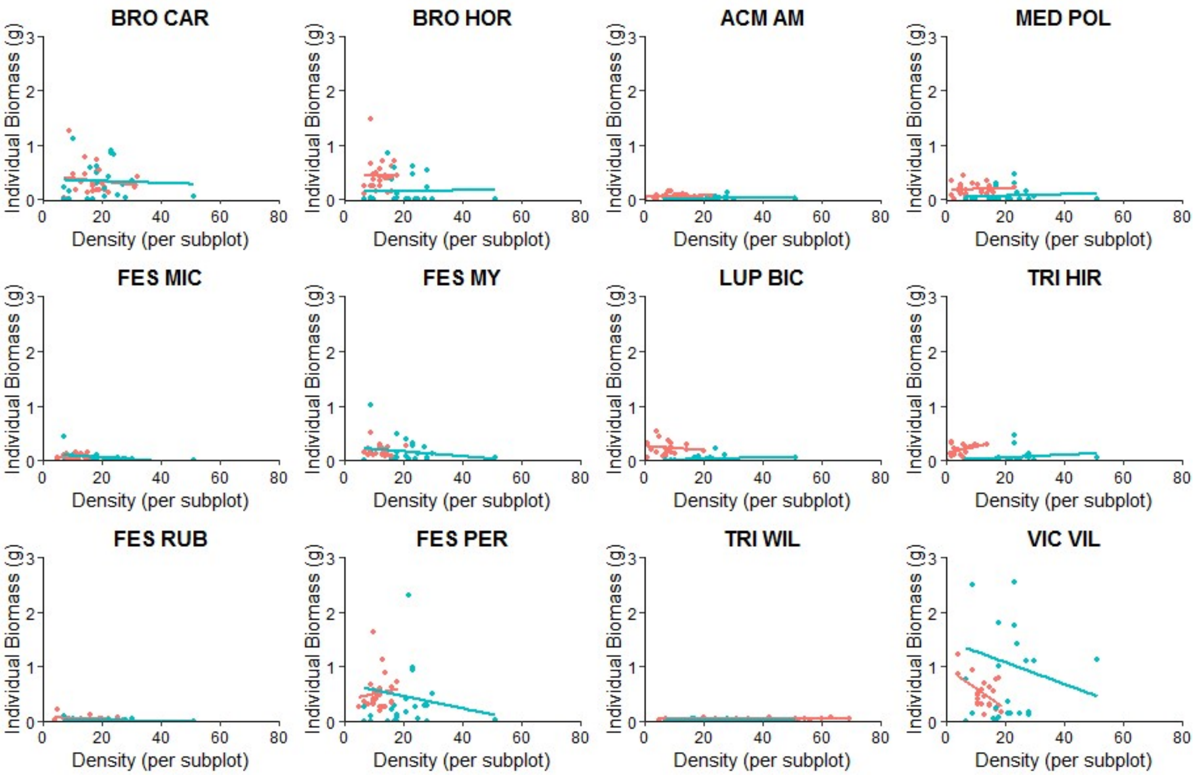

Figure 4A.1: Scatterplots of mean individual biomass in relation to total subplot density for twelve focal species in monoculture subplots (red filled circles) and polyculture subplots (cyan filled circles).

Figure 4A.2: Photograph of the experimental setup at the UC San Diego Biology Field Station.

References

Abraham, J. K., J. D. Corbin, and C. M. D'Antonio. 2009. California native and exotic perennial grasses differ in their response to soil nitrogen, exotic annual grass density, and order of emergence. Plant Ecology **201**:445-456.

Anderson, J. T., D. W. Inouye, A. M. McKinney, R. I. Colautti, and T. Mitchell-Olds. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proc Biol Sci **279**:3843-3852.

Augspurger, C. 2013. Reconstructing patterns of temperature, phenology, and frost damage over 124 years: Spring damage risk is increasing. Ecology **94**:41-50.

Bartolome, J. W. 1979. Germination and seedling establishment in California annual grassland. Journal of Ecology **67**:273-281.

Barton, K. E., and M. E. Hanley. 2013. Seedling-herbivore interactions: insights into plant defence and regeneration patterns. Ann Bot **112**:643-650.

Beck, J. J., D. L. Hernández, J. R. Pasari, and E. S. Zavaleta. 2015. Grazing maintains native plant diversity and promotes community stability in an annual grassland. Ecological Applications **25**:1259-1270.

Boege, K., and R. J. Marquis. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends Ecol Evol **20**:441-448.

Burt-Smith, G. S., J. P. Grime, and D. Tilman. 2003. Seedling resistance to herbivory as a predictor of relative abundance in a synthesised prairie community. Oikos **101**:345-353.

Cleland, E. E., E. Esch, and J. McKinney. 2015. Priority effects vary with species identity and origin in an experiment varying the timing of seed arrival. Oikos **124**:33-40.

Cleland, E. E., J. L. Funk, and E. B. Allen. 2016. Coastal Sage Scrub. Pages 429-448 *in* H. A. Mooney and E. S. Zavaleta, editors. Ecosystems of California. University of California Press, Berkeley, California, USA.

Cleland, E. E., H. A. Peters, H. A. Mooney, and C. B. Field. 2006. Gastropod herbivory in response to elevated CO2 and N addition impacts plant community composition. Ecology **87**:686-694.

Colautti, R. I., A. Ricciardi, I. A. Grigorovich, and H. J. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? Ecology Letters **7**:721-733.

Crawley, M. J. 2007. Plant population dynamics. Pages 62-83 *in* R. M. May and A. McLean, editors. Theoretical Ecology: Principles and applications. Oxford University Press, Oxford, UK.

Cushman, J. H., C. J. Lortie, and C. E. Christian. 2011. Native herbivores and plant facilitation mediate the performance and distribution of an invasive exotic grass. Journal of Ecology **99**:524-531.

Deering, R. H., and T. P. Young. 2006. Germination speeds of exotic annual and native perennial grasses in California and the potential benefits of seed priming for grassland restoration. Grasslands **16**:14-16.

Fenner, M., and K. Thompson. 2005. The ecology of seeds. Cambridge University Press, Cambridge.

Fox, J., and S. Weisberg. 2011. An {R} Companion to Applied Regression. 2nd edition. Sage, Thousand Oaks, CA.

Fridley, J. D. 2012. Extended leaf phenology and the autumn niche in deciduous forest invasions. Nature **485**:359-362.

Fukami, T. 2015. Historical contingency in community assembly: integrating niches, species pools, and priority effects. Annual Review of Ecology, Evolution, and Systematics **46**:1-23.

Funk, J. L., M. K. Hoffacker, and V. Matzek. 2015. Summer irrigation, grazing and seed addition differentially influence community composition in an invaded serpentine grassland. Restoration Ecology **23**:122-130.

Godoy, O., F. Valladares, and P. Castro-Diez. 2011. Multispecies comparison reveals that invasive and native plants differ in their traits but not in their plasticity. Functional Ecology **25**:1248-1259.

Grman, E., and K. N. Suding. 2010. Within-year soil legacies contribute to strong priority effects of exotics on native California grassland communities. Restoration Ecology **18**:664-670.

Hanley, M. 1998. Seedling herbivory, community composition and plant life history traits. Perspectives in Plant Ecology, Evolution and Systematics **1**:191-205.

Hanley, M., and E. L. Fegan. 2007. Timing of cotyledon damage affects growth and flowering in mature plants. Plant, Cell & Environment **30**:812-819.

Hanley, M. E., and R. J. Sykes. 2009. Impacts of seedling herbivory on plant competition and implications for species coexistence. Ann Bot **103**:1347-1353.

Heady, H. F. 1958. Vegetational changes in the California annual type. Ecology **39**:402–416.

HilleRisLambers, J., S. G. Yelenik, B. P. Colman, and J. M. Levine. 2010. California annual grass invaders: the drivers or passengers of change? Journal of Ecology **98**:1147-1156.

Hobbs, R. J. 2001. Synergisms among habitat fragmentation, livestock grazing, and biotic invasions in southwestern Australia. Conservation Biology **15**:1522-1528.

Holmgren, M. 2002. Exotic herbivores as drivers of plant invasion and switch to ecosystem alternative states. Biological Invasions **4**:25-33.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal **50**:346-363.

Hulme, P. E. 1994. Seedling herbivory in grassland: relative impact of vertebrate and invertebrate herbivores. Journal of Ecology **82**:873-880.

Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution **17**:164-169.

Kempel, A., M. Razanajatovo, C. Stein, S. B. Unsicker, H. Auge, W. W. Weisser, M. Fischer, and D. Prati. 2015. Herbivore preference drives plant community composition. Ecology **96**:2923-2934.

Lambrinos, J. G. 2006. Spatially variable propagule pressure and herbivory influence invasion of chaparral shrubland by an exotic grass. Oecologia **147**:327-334.

Leishman, M. R., T. Haslehurst, A. Ares, and Z. Baruch. 2007. Leaf trait relationships of native and invasive plants: community- and global-scale comparisons. New Phytologist **176**:635-643.

Loreau, M. 1998. Separating sampling and other effects in biodiversity experiments. Oikos **82**:600-602.

Marushia, R. G., M. Cadotte, and J. S. Holt. 2010. Phenology as a basis for management of exotic annual plants in desert invasions. Journal of Applied Ecology **47**:1290-1299.

Menke, J. W. 1992. Grazing and fire management for native perennial grass restoration in California grasslands. Fremontia **20**:22-25.

Norden, N., M. I. Daws, C. Antoine, M. A. Gonzalez, N. C. Garwood, and J. Chave. 2009. The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. Functional Ecology **23**:203-210.

Parker, J. D., D. E. Burkepile, and M. E. Hay. 2006. Opposing effects of native and exotic herbivores on plant invasions. Science **311**:1459-1461.

Pau, S., E. M. Wolkovich, B. I. Cook, T. J. Davies, N. J. B. Kraft, K. Bolmgren, J. L. Betancourt, and E. E. Cleland. 2011. Predicting phenology by integrating ecology, evolution and climate science. Global Change Biology **17**:3633-3643.

Pérez-Fernández, M. A., B. B. Lamont, A. L. Marwick, and W. G. Lamont. 2000. Germination of seven exotic weeds and seven native species in south-western Australia under steady and fluctuating water supply. Acta Oecologica **21**:323-336.

Peters, H. 2007. The significance of small herbivores in structuring annual grassland. Journal of Vegetation Science **18**:175-182.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2015. nlme: Linear and Nonlinear Mixed Effects Models [R package version 3.1-121]

Quintero, C., E. C. Lampert, and M. D. Bowers. 2014. Time is of the essence: direct and indirect effects of plant ontogenetic trajectories on higher trophic levels. Ecology **95**:2589-2602.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reynolds, S. A., J. D. Corbin, and C. M. D'Antonio. 2001. The effects of litter and temperature on the germination of native and exotic grasses in a coastal California grassland. Madroño **48**:230-235.

Rice, K. J. 1987. Interaction of disturbance patch size and herbivory in *Erodium* colonization. Ecology **68**:1113-1115.

Rinella, M. J., and B. J. Hileman. 2009. Efficacy of prescribed grazing depends on timing intensity and frequency. Journal of Applied Ecology **46**:796-803.

Roché, C. T., and D. C. Thill. 2001. Biology of common crupina and yellow starthistle, two Mediterranean winter annual invaders in western North America. Weed Science **49**:439-447.

Sala, O. E., F. S. Chapin III, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Semchenko, M., M. Lepik, L. Götzenberger, and K. Zobel. 2012. Positive effect of shade on plant growth: amelioration of stress or active regulation of growth rate? Journal of Ecology **100**:459-466.

Skaer, M. J., D. J. Graydon, and J. H. Cushman. 2013. Community-level consequences of cattle grazing for an invaded grassland: variable responses of native and exotic vegetation. Journal of Vegetation Science **24**:332-343.

Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends in Ecology & Evolution **14**:179-185.

Sullivan, A. T., and H. F. Howe. 2009. Prairie forb response to timing of vole herbivory. Ecology **90**:1346-1355.

Underwood, E. C., J. H. Viers, K. R. Klausmeyer, R. L. Cox, and M. R. Shaw. 2009. Threats and biodiversity in the mediterranean biome. Diversity and Distributions **15**:188-197.

Underwood, N., B. D. Inouye, and P. A. Hambäck. 2014. A conceptual framework for associational effects: when do neighbors matter and how would we know? The Quarterly Review of Biology **89**:1-19.

van Kleunen, M., E. Weber, and M. Fischer. 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. Ecology Letters **13**:235-245.

Vaughn, K. J., and T. P. Young. 2015. Short-term priority over exotic annuals increases the initial density and longer-term cover of native perennial grasses. Ecological Applications **25**:791-799.

Vilà, M., and C. M. D'Antonio. 1998. Hybrid vigor for clonal growth in *Carpobrotus* (Aizoaceae) in coastal California. Ecological Applications **8**:1196-1205.

Wainwright, C. E., and E. E. Cleland. 2013. Exotic species display greater germination plasticity and higher germination rates than native species across multiple cues. Biological Invasions **15**:2253-2264.

Wainwright, C. E., E. M. Wolkovich, and E. E. Cleland. 2012. Seasonal priority effects: implications for invasion and restoration in a semi-arid system. Journal of Applied Ecology **49**:234-241.

Wolkovich, E. M., and E. E. Cleland. 2011. The phenology of plant invasions: a community ecology perspective. Frontiers in Ecology and the Environment **9**:287-294.

Wolkovich, E. M., and E. E. Cleland. 2014. Phenological niches and the future of invaded ecosystems with climate change. AoB Plants **6**.

Xu, J., W. Li, C. Zhang, W. Liu, and G. Du. 2014. Variation in seed germination of 134 common species on the Eastern Tibetan Plateau: phylogenetic, life history and environmental correlates. PLoS One **9**:e98601.

CONCLUSIONS AND FUTURE DIRECTIONS

The timing of emergence has large impacts on plant fitness, and adaptive evolution in this trait will be a major factor contributing to the long-term persistence of plant species in the face of climate change (Walck et al. 2011, Cochrane et al. 2015). Furthermore, relative differences in emergence timing among species, and how these shift with climate change, will likely have large impacts on plant community structure (Walck et al. 2011). A major goal of my dissertation is to develop our understanding of factors that shape the evolution of emergence timing and thus influence the potential for this trait to adaptively evolve to keep pace with projected climate change. An additional goal is to develop our understanding of factors shaping the impacts of variation in emergence timing among species.

In Chapter 1, I found evidence for a genetic trade-off between within- and among-year emergence timing that is likely to constrain adaptive evolution in response to joint selection for earlier emergence within years and greater spread of emergence among years. Across an aridity gradient in the Mediterranean climate region of California, I found that populations of *S. pulchra* and *B. diandrus* with Pareto optimal combinations of earlier within-year emergence and greater potential among-year emergence experienced historically more arid climates, consistent with constrained evolution in response to such joint selection. I also found that plastic responses to variation in soil moisture in *S. pulchra*, but not *B. diandrus*, can potentially mitigate the costs associated with this constraint. The genetic trade-off demonstrated in this chapter is likely to constrain adaptive evolution in areas where future climates will exert stronger selection for both earlier within-year emergence and greater among-year emergence. Mediterranean climate regions are obvious candidates to undergo such changes as these are projected to become increasingly arid, with many areas expected to experience warmer and drier average conditions as well as increased interannual variability in precipitation (Seager et al. 2007, Alpert et al. 2008, IPCC 2013, Berg and Hall 2015, Yoon et al. 2015). The results of this chapter also highlight the need to characterize other key fitness-related traits that are genetically correlated with emerging timing, and that may therefore constrain the evolution of emergence timing. For example, it has been shown that genes influencing dormancy also influence other traits such as flowering time (Chiang et al. 2009). Testing for Pareto optimality across environmental gradients will likely be an effective approach for detecting constrained evolution in response to joint selection on correlated traits, and thus identifying scenarios where projected climate change is most likely to result in constrained evolutionary responses.

In Chapter 2, I found that variation in the surrounding plant community altered the intensity of selection for earlier emergence in *S. pulchra* and *B. diandrus*, but the selective effect of neighboring communities varied between the two focal species. I found weak support for a role of light interception and emergence timing of the surrounding community in determining the strength of selection for earlier emergence in *S. pulchra*. I also found that exotic species intensified selection for earlier emergence in *B. diandrus*. Greater diversity of neighboring species in the surrounding community did not result in stronger selection to emerge earlier. To predict how plant communities will influence the potential for adaptive evolution in emergence timing to promote long-term persistence in the face of climate change, future work will need to characterize how aspects of community context (and interactions between them), as well as the traits of focal species, determine the intensity of selection on this trait. Integrating this information with studies of how plant community composition is changing with continued plant species invasion (van Kleunen et al. 2015) and climate change (Harrison et al. 2015, Kopp and Cleland 2015, Liu et al. 2018), will allow us to better predict how emergence timing will evolve as a result of shifts in plant community composition. Low concordance between expected selection on emergence timing directly in response to climate change and in response to changes in community composition will suggest that adaptive evolution in emergence timing is less likely to promote persistence in novel climates.

In Chapter 3, I found that mammalian herbivory weakened the strength of directional selection for earlier emergence in competition, independently of reductions in mean fitness. Therefore, whether this selective effect of herbivory promotes or impedes adaptive evolution in emergence timing in response to climate change will depend on the extent to which climate change favors earlier or later emergence. For example, where climate change selects for earlier emergence, such as would be expected in response to increased drought (cf. Dickman et al. 2019), mammalian herbivory will likely impede adaptive evolutionary responses. Furthermore, studies that characterize how climate change is directly influencing the abundance or distributions of mammalian herbivores (Post and Forchhammer 2008) could provide insight into how climate change will indirectly alter selection on emergence time through changes in mammalian herbivore impacts.

In Chapter 4, I found that species that emerged earlier in the growing season achieved higher biomass than later-emerging species but were more susceptible to early-season mammalian herbivory. Furthermore, early-season mammalian herbivory resulted in the greater relative performance of later-emerging natives when growing in competition with earlier-emerging exotic species. This result shows that early-season mammalian herbivory is likely to play an important role in maintaining the performance of late-emerging species in the face of competitive advantages gained by those emerging earlier. Furthermore, this suggests that controlled grazing in the early period of growing season could be an effective management practice in areas, such as California and other Mediterranean climate regions, where dominant exotic species benefit from seasonal priority effects through earlier emergence (Reynolds et al. 2001, Deering and Young 2006, Abraham et al. 2009, Wainwright et al. 2012, Wainwright and Cleland 2013, Vaughn and Young 2015, Waterton and Cleland 2016). Furthermore, many exotic species have advanced their seasonal phenologies in response to recent climate change to a greater extent than co-occurring natives, which is associated with improved performance (Wolkovich et al. 2013, Willis et al. 2015). If similar shifts occur in emergence timing, mammalian herbivores are likely to be an important force counteracting the potential increased competitive advantages gained by exotic species. Therefore, understanding how shifts in emergence timing due to climate change will alter plant community structure will need to account for mammalian herbivory.

Together, my dissertation research shows that the timing of emergence is a trait with enormous impacts on plant fitness and is a trait that is being pulled in many directions by natural selection. Furthermore, the impacts of differences in emergence timing among species on the structure of plant communities is highly contingent on key ecological interactions, such as mammalian herbivory. The potential for adaptive evolution in emergence timing to keep pace with climate change and thus promote the long-term persistence of plants will likely depend on interactions between a multitude of ecological and evolutionary factors. These include genetic constraints, as well as selection exerted by herbivores and the surrounding plant community. In turn, to understand how adaptive evolution in emergence timing will alter the nature of interspecific plant-plant interactions and plant community structure will require that researchers consider other important interactions, such as herbivory, that can mediate the impacts of differences in emergence timing among species. Despite this apparent complexity, the timing of emergence will undoubtedly play a central role in determining the impacts of global environmental change on plants.

References

Abraham, J. K., J. D. Corbin, and C. M. D'Antonio. 2009. California native and exotic perennial grasses differ in their response to soil nitrogen, exotic annual grass density, and order of emergence. Plant Ecology **201**:445-456.

Alpert, P., S. O. Krichak, H. Shafir, D. Haim, and I. Osetinsky. 2008. Climatic trends to extremes employing regional modeling and statistical interpretation over the E. Mediterranean. Global and Planetary Change **63**:163–170.

Berg, N., and A. Hall. 2015. Increased interannual precipitation extremes over California under climate change. Journal of Climate **28**:6324–6334.

Chiang, G. C., D. Barua, E. M. Kramer, R. M. Amasino, and K. Donohue. 2009. Major flowering time gene, flowering locus C, regulates seed germination in Arabidopsis thaliana. Proceedings of the National Academy of Sciences USA **106**:11661-11666.

Cochrane, A., C. J. Yates, G. L. Hoyle, and A. B. Nicotra. 2015. Will among-population variation in seed traits improve the chance of species persistence under climate change? Global Ecology and Biogeography **24**:12-24.

Deering, R. H., and T. P. Young. 2006. Germination speeds of exotic annual and native perennial grasses in California and the potential benefits of seed priming for grassland restoration. Grasslands **16**:14-16.

Dickman, E. E., L. K. Pennington, S. J. Franks, and J. P. Sexton. 2019. Evidence for adaptive responses to historic drought across a native plant species range. Evolutionary Applications.

Harrison, S. P., E. S. Gornish, and S. Copeland. 2015. Climate-driven diversity loss in a grassland community. Proceedings of the National Academy of Sciences **112**:8672–8677.

IPCC. 2013. Annex I: Atlas of Global and Regional Climate Projections.*in* G. J. van Oldenborgh, M. Collins, J. Arblaster, J. H. Christensen, J. Marotzke, S. B. Power, M. Rummukainen, and T. Zhou, editors. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Kopp, C. W., and E. E. Cleland. 2015. A range-expanding shrub species alters plant phenological response to experimental warming. PLoS One **10**:e0139029.

Liu, H., Z. Mi, L. Lin, Y. Wang, Z. Zhang, F. Zhang, H. Wang, L. Liu, B. Zhu, G. Cao, X. Zhao, N. J. Sanders, A. T. Classen, P. B. Reich, and J. S. He. 2018. Shifting plant species composition in response to climate change stabilizes grassland primary production. Proc Natl Acad Sci U S A **115**:4051-4056.

Post, E., and M. C. Forchhammer. 2008. Climate change reduces reproductive success of an Arctic herbivore through trophic mismatch. Philos Trans R Soc Lond B Biol Sci **363**:2369-2375.

Reynolds, S. A., J. D. Corbin, and C. M. D'Antonio. 2001. The effects of litter and temperature on the germination of native and exotic grasses in a coastal California grassland. Madroño **48**:230-235.

Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H. Huang, N. Harnik, A. Leetmaa, N. Lau, C. Li, J. Velez, and N. Naik. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science **316**:1181–1184.

van Kleunen, M., W. Dawson, F. Essl, J. Pergl, M. Winter, E. Weber, H. Kreft, P. Weigelt, J. Kartesz, M. Nishino, L. A. Antonova, J. F. Barcelona, F. J. Cabezas, D. Cardenas, J. Cardenas-Toro, N. Castano, E. Chacon, C. Chatelain, A. L. Ebel, E. Figueiredo, N. Fuentes, Q. J. Groom, L. Henderson, Inderjit, A. Kupriyanov, S. Masciadri, J. Meerman, O. Morozova, D. Moser, D. L. Nickrent, A. Patzelt, P. B. Pelser, M. P. Baptiste, M. Poopath, M. Schulze, H. Seebens, W. S. Shu, J. Thomas, M. Velayos, J. J. Wieringa, and P. Pysek. 2015. Global exchange and accumulation of non-native plants. Nature **525**:100-103.

Vaughn, K. J., and T. P. Young. 2015. Short-term priority over exotic annuals increases the initial density and longer-term cover of native perennial grasses. Ecological Applications **25**:791-799.

Wainwright, C. E., and E. E. Cleland. 2013. Exotic species display greater germination plasticity and higher germination rates than native species across multiple cues. Biological Invasions **15**:2253-2264.

Wainwright, C. E., E. M. Wolkovich, and E. E. Cleland. 2012. Seasonal priority effects: implications for invasion and restoration in a semi-arid system. Journal of Applied Ecology **49**:234-241.

Walck, J. L., S. N. Hidayati, K. W. Dixon, K. Thompson, and P. Poschlod. 2011. Climate change and plant regeneration from seed. Global Change Biology **17**:2145–2161.

Waterton, J., and E. E. Cleland. 2016. Trade-off between early emergence and herbivore susceptibility mediates exotic success in an experimental California plant community. Ecology and Evolution **6**:8942-8953.

Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, J. B. Losos, and C. C. Davis. 2015. Favorable climate change response explains non-native species' success in Thoreau's Woods. PLoS One **5**:e8878.

Wolkovich, E. M., T. J. Davies, H. Schaefer, E. E. Cleland, B. I. Cook, S. E. Travers, C. G. Willis, and C. C. Davis. 2013. Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. American Journal of Botany **100**:1407-1421.

Yoon, J. H., S. Y. Wang, R. R. Gillies, B. Kravitz, L. Hipps, and P. J. Rasch. 2015. Increasing water cycle extremes in California and in relation to ENSO cycle under global warming. Nature Communications **6**:8657.