UCLA
UCLA Electronic Theses and Dissertations

Title

Evolutionary and Ecological Drivers of Invasion in the Annual Thistle, Centaurea melitensis

Permalink

https://escholarship.org/uc/item/9mx090j7

Author

Moroney, Jolene Rene

Publication Date

2012

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Evolutionary and Ecological Drivers of Invasion in the Annual Thistle,

Centaurea melitensis

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Biology

by

Jolene Rene Moroney

2012

ABSTRACT OF THE DISSERTATION

Evolutionary and Ecological Drivers of Invasion in the Annual Thistle,

Centaurea melitensis

By

Jolene Rene Moroney

Doctor of Philosophy in Biology

University of California, Los Angeles, 2012

Professor Philip W. Rundel, Chair

Invasive species have resulted in enormous economic and ecological costs and are considered a major cause of the global loss of biodiversity. The causes of invasion are complex, involving characteristics of the invading species and properties of recipient communities. Processes driving invasion are both evolutionary and ecological. Understanding the mechanisms of biological invasions is essential to reducing the threats from ongoing introductions, preventing new invasions, and controlling the spread of existing invaders. *Centaurea melitensis*, a widespread invasive plant that has established globally from its western Mediterranean center of origin, offers a unique opportunity to examine the processes that enhance invasiveness of plant species. The overall objective of this research is to examine the ecological and evolutionary factors that produce invasive populations of *C. melitensis*. The approach uses ecological and genetic data, a common garden, and species distribution modeling to compare characteristics of individuals and

environments from the native range in Spain with those from two invasive ranges of similar Mediterranean climate type: one in central Chile and the other in California. Chapter One introduces the problem and gives a background overview. Chapter Two compares population densities and dominance among regions. Chapter Three addresses variation in phenotypic plasticity and life history traits among regions by comparing plants grown in a common greenhouse. In Chapter Four, molecular genetic markers are tested for applicability to *C. melitensis*. Chapter Five examines the role of fire and other disturbances on community dynamics. Chapter Six uses species distribution modeling to project a potential invasive range onto a non-native range to identify niche evolution. Results of this project indicate that *C. melitensis* is more abundant and more dominant in the invasive range than in the native range; that invasive populations are genetically differentiated from native ones for several life history traits and their plasticities; that microsatellite markers developed for other *Centaurea* species are not applicable to *C. melitensis*; that fire promotes the recruitment of *C. melitensis*; and that the niche of invasive plants has shifted from those of native plants. The questions in this study are not only important in terms of basic research in ecological and evolutionary processes such as how species interactions and local adaptation affect species distribution and abundance, but also are relevant to management applications by informing strategies to prevent and limit the impacts of species invasions.

The dissertation of Jolene Rene Moroney is approved.

Victoria L. Sork

Peggy Fong

Thomas W. Gillespie

Philip W. Rundel, Committee Chair

University of California, Los Angeles

2012

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 2

Chapter 3

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

VITA

2003   B.S., Biology, Summa Cum Laude
California State University
Northridge, California

2003   Awarded Bennett-Bickford Prospective Teacher Award
Department of Biology
California State University, Northridge

2007   M.S., Biology
California State University
Northridge, California

2006   Awarded Outstanding Biology Graduate Teaching Award
Department of Biology
California State University, Northridge

2005 – 2006   Teaching Assistant
Department of Biology
California State University, Northridge

2005 – 2006   Teaching Associate
Department of Biology
California State University, Northridge

2007 – 2010   Teaching Associate
Department of Ecology and Evolutionary Biology
University of California, Los Angeles

2007   Awarded Quality of Graduate Education Fellowship
Department of Ecology and Evolutionary Biology
University of California, Los Angeles

2009   Awarded Stephen A. Vavra Fellowship
Department of Ecology and Evolutionary Biology
University of California, Los Angeles

2011   Awarded Dissertation Year Fellowship
Graduate Division
University of California, Los Angeles

PUBLICATIONS AND PRESENTATIONS

Moroney, J.R. and P.W. Rundel. 2012. Abundance and dispersion of the invasive Mediterranean annual, *Centaurea melitensis* in its native and non-native ranges. Biological Invasions, DOI 10.1007/s10530-012-0302-1.

Moroney, J.R. and P.W. Rundel. 2012. A comparison of life history traits of an invasive Mediterranean forb in its native range versus two invasive ranges. Poster presentation, Southern California Botanists Annual Meeting, Pasadena, CA.

Moroney, J.R. and P.W. Rundel. 2012. A comparison of life history traits of an invasive Mediterranean forb in its native range versus two invasive ranges. Poster presentation, Ecological Society of America Annual Meeting, Portland, OR.

Moroney, J.R., P.M. Schiffman, and C.A. Brigham. 2011. Invasive European annual plants impact a rare endemic sunflower. Madroño 58(2):69-77.

Moroney, J.R. 2011. Density and dispersion of an invasive thistle in its Mediterranean range compared to two invasive ranges in California and central Chile. Oral presentation, MEDECOS XII, The International Mediterranean Ecosystems Conference Los Angeles, CA.

Sharifi, R., J.R. Moroney, S. Woo, and P.W. Rundel. 2011. Physiological response of an invasive species of the coastal sage scrub, *Centaurea melitensis*, to different levels of resources. Poster presentation, MEDECOS XII, The International Mediterranean Ecosystems Conference, Los Angeles, CA

*Pucci, J.R. 2007. The effects of invasive plants on the endangered sunflower, *Pentachaeta lyonii*. Oral presentation, The California Botanical Society Annual Meeting, San Luis Obispo, CA

Kelso, S., C.A. Brigham, *J.R. Pucci, J. Tiszler. 2006. Visual cues to seed viability in two non-native invasive plant species, Poison Hemlock (*Conium maculatum*) and Harding Grass (*Phalaris aquatica*). Ecological Restoration 24(4):279-280.

*Pucci, J.R. 2006. Conservation of the rare plant, Lyon's Pentachaeta. Oral presentation, Southern California Botanists Annual Meeting, Fullerton, CA

*Pucci, J.R., C.A. Brigham and J. Tiszler. 2004. Examining multiple impacts of exotic species on a rare plant community. Poster presentation, Ecological Society of America Annual Meeting, Portland, OR.

*Pucci, J.R. 2007. Invasive European annuals impact an endangered Santa Monica Mountains endemic sunflower. Poster presentation, University of California, Los Angeles Biology Research Symposium, Los Angeles, CA.

*Pucci, J.R. 2005. Invasive species contribute to declines of *Pentachaeta lyonii*: implications for management. Oral presentation, California State University Research Symposium, Northridge, CA.

*Pucci, J.R. 2005. Effects of competition from invasive plants on the endangered plant *Pentachaeta lyonii*. Oral presentation, California State University Research Symposium, Sacramento, CA.

CHAPTER 1

Introduction and background

INTRODUCTION

Invasive species have resulted in enormous economic and ecological costs (Wilcove et al. 1998, Pimentel et al. 2005), and are considered a major cause of the global loss of biodiversity (Olden et al. 2004), second only to habitat destruction. Understanding the mechanisms of biological invasions is essential to reducing the threats from ongoing introductions of invasive species, preventing new invasions, and controlling the spread and range expansions of existing invaders.

Biological invasions have had serious and in many cases irreversible consequences on biodiversity and ecosystem functioning (D'Antonio and Vitousek 1992, Mack and D'Antonio 1998, Gaertner et al. 2009, Vilà et al. 2011), and will continue to do so with increasing human population growth and globalization (Sala et al. 2000). For decades, biologists have sought to generalize characteristics of invasive plants in order to make predictions about which species are likely to become invaders (Baker 1965). Although some progress has been made in this area (Thompson et al. 1995, Rejmánek 1996, Daehler 1998) more work is needed to decipher how plant characteristics and life history traits interact with ecological and evolutionary processes to contribute to invasions (Castro et al. 2005, Muth and Pigliucci 2006, Moles et al. 2008). Difficulties in making generalizations about the invasion process emphasize the importance of a case study approach, which can be useful in increasing system-specific knowledge (Davis 2006). To understand invasion processes, the ecology of invasive species must be studied in both native and invaded ranges to examine the causes of divergent population dynamics (Hierro et al. 2005).

In this dissertation, I examined the invasion dynamics of *Centaurea melitensis* L. (Asteraceae), an annual thistle that is native to the western Mediterranean Basin, but has been dispersed by humans and is invasive globally. To this end, I studied populations of *C. melitensis* from three regions of similar climate type: the native range (southern Spain) and two invaded

ranges (California and central Chile) to elucidate the relative importance of species characteristics, ecological and evolutionary factors, and their interactions in producing populations of *C. melitensis* with invasive potential. First, I determined the extent of invasiveness in this species by comparing population densities and dominance in six sites in each of the three study regions. Second, I compared the seed characteristics of size and germination time, and then grew the seeds from the study populations in a common greenhouse to examine the role of phenotypic plasticity, the potential post-introduction evolution of life history traits and the evolution of the plasticities of those traits in producing invasive populations. Third, I began the process of discovery of molecular markers to be used in further post-introduction evolutionary studies. Fourth, I studied the effects of fire and firebreaks, two disturbance processes common in California that might promote or inhibit invasion in this species.

Studying the invasion process not only has practical motivations, but offers a unique natural experiment setting to further our understanding of ecology and evolution, including the issues of rapid evolution, coevolution, allelopathy, community assembly, determinants of local diversity, community effects of food webs, and links in population, community, and ecosystem ecology (Callaway and Maron 2006). The results of this study will not only increase our understanding of the process of invasion in this species, but can be applied to other invasive species within mediterranean ecosystems to help manage current invasions and prevent future invasions. This work has the potential to affect conservation policy and to preserve global biodiversity.

INVASION PROCESS

Only a small percentage of introduced species actually become invasive (Richardson and Pyšek 2006). Propagules must overcome several barriers to invasion, starting with geographic

barriers that are overcome with human-mediated long-distance dispersal. Next, there must be

suitable conditions in the new environment for the species' physiological requirements. Then, the

species must overcome reproductive barriers, in that there must be appropriate pollinators or

member of the opposite sex in close proximity, if appropriate. If a population successfully

overcomes all these barriers and becomes self-replacing, it still must disperse into the landscape

to become invasive. Once out in the landscape, there must be niche availability and suitable life

history traits to thrive and dominate and display competitive superiority relative to the native

residents. (Richardson and Pyšek 2006).

The complex causes of invasion involve characteristics of the invading species

("invasiveness") and properties of recipient communities ("invasibility"). Furthermore, the

processes driving invasion are both evolutionary and ecological, and interpretation of invasion

patterns is dependent on spatial (Hamilton et al. 2005, Pauchard and Shea 2006) and temporal

(Richardson and Pyšek 2006) scales.

Invasibility might depend on biotic factors, abiotic factors, or both. Charles Elton

hypothesized that species rich communities are less invasible than those that are relatively

species poor (Elton 1958). With more species present, more niche spaces are occupied and

therefore fewer resources are available for incoming propagules to utilize. This theory has been

supported by several studies (Tilman 1997, Naeem et al. 2000, Dukes 2002); however others

have found that native species richness is positively correlated with invasive species presence at

large scales (Lonsdale 1999).

Numerous studies have shown that relatively nutrient-rich communities are more

invasible than those that are nutrient-poor (Huenneke et al. 1990, Davis et al. 2000, Leishman

and Thomson 2005). High nutrient levels can be due to intrinsic soil characteristics or can be

elevated by anthropogenic nutrient inputs such as from urban or agricultural runoff or air

pollution. The mediterranean climate regions of both California and central Chile are rich in soil

nutrients and heavily polluted by human activity.

Disturbance is a well-studied factor in increasing invasibility (Hobbs and Huenneke

1992, Schiffman 1997, D'Antonio et al. 1999, Davis et al. 2000, Hierro et al. 2006, Pausas et al.

2006). The mechanism for disturbance increasing invasibility is the removal or reduction of

native individuals, resulting in a pulse of increased available resources. Disturbance can manifest

through many different processes, either anthropogenic or natural, long-term and repetitive or

sudden and unpredictable, each with the potential to affect invasibility uniquely (D'Antonio et al.

1999). For example, grazing removes a portion of above ground biomass, which, depending on

the growth form of the grazed species may or may not include apical meristems that affect

individual response. Grazing also involves mechanical disturbance of the soil surface by

trampling, and the input of nutrients from animal waste. In a California grassland, grazing was

found to have negative effects on native species, while invasive species were unaffected

(Kimball and Schiffman 2003). Fire, depending on the intensity, removes all or a portion of

above ground biomass, inputs nutrients, and includes effects of heating on seeds. Fires can

promote invasion of sites by nonnative species (D'Antonio et al. 1999).

Disturbance due to the construction of roads or trails might involve complete or partial

removal of biomass from mechanical disturbance of the soil. Disturbance can also come from

members of the biotic community. In California, the burrowing activities of native giant

kangaroo rats (*Dipodomys ingens*) promote invasive species colonization of disturbed ground

(Schiffman 1997). In some situations, the establishment of an invader (possibly originally due to

disturbance) can facilitate continued invasion, resulting in a positive feedback loop that sustains the presence of the invader in an altered disturbance regime (D'Antonio et al. 1999).

Invasiveness can be due to characteristics inherent in a species, or traits that evolve after introduction to a new range that promote dominance. One of the leading hypotheses for why plants that are innocuous members of the community in their native range become aggressive dominants in new ranges is the Enemy Release Hypothesis (Keane and Crawley 2002). Plants arrive in a new habitat without their natural enemies, and thus can grow larger and produce more offspring due to a plastic response to increased resource availability, potentially gaining a competitive advantage over native plants. An extension of this hypothesis, the Evolution of Increased Competitive Ability Hypothesis (EICA) suggests a competitive advantage due to evolutionary changes in resource allocation. This hypothesis states that once plants are released from their specialist enemies, selection favors plants that allocate resources less toward defense and more toward growth and reproduction, resulting in superior competitors (Blossey and Nötzold 1995). Tests of the ERH have produced conflicting results (Maron and Vila 2001, Keane and Crawley 2002, Colautti et al. 2004, Liu and Stiling 2006, Liu et al. 2007). The general lack of demographic data on the effects of natural enemies on native population dynamics obscures the relative importance of enemy release to invasion success (Hierro et al. 2005). Species that are weedy in their natural ranges are most likely not significantly regulated by natural enemies. Either they don't have important natural enemies, or they have mechanisms to compensate for damage, and so release from enemy pressure will probably not explain invasiveness in these species (Maron and Vila 2001).

Species introduced into new environments are subject to rapid evolutionary change. These evolutionary processes may be adaptive in nature or stochastic. Novel conditions in the

introduced range could exert selective pressures resulting in rapid adaptive evolution. Or differences in environmental conditions could simply select for those introduced individuals already pre-adapted to the new environment. Further, traits under selection might not be simple life history traits, but a range of tolerance of conditions, or "plasticity."

Stochastic processes such as genetic bottlenecks, sampling effects, and admixture of populations subsequent to introduction have the potential to differentiate invasive from native populations and depending on local conditions, could promote invasiveness. Generally, human-mediated plant introductions to new ranges involve only a small portion of the seed pool of a source native population. By chance, this sample might not include genotypes containing deleterious alleles, producing a more robust gene pool in the founding population. If populations in the native range are differentiated, or locally adapted to environmental conditions, then the success of the founders will depend on the population of origin. Further, if founders are introduced from differentiated populations and then admix subsequent to introduction, novel genotypes unknown in the native range will result, potentially providing genetic variation for further adaptation.

STUDY SYSTEM

Five regions of the world have a mediterranean-type climate, which is characterized by warm, dry summers and mild, rainy winters (Di Castri 1991). The Mediterranean Basin, southwestern Africa, southwest Australia, central Chile, and California share this climate type and have also experienced substantial exchange of plant species, with many of these introductions resulting in invasions (Jimenez et al. 2008, Gaertner et al. 2009). Although convergent evolution due to parallel selection pressures has resulted in similar characteristics in unrelated taxa in the native floras, the regions have major differences. Consequently, they have

been subdivided into groups based on affinities in climate and biogeography, geology and soils, disturbance and invasion, and history of human impacts (Di Castri 1991). Southern Africa is grouped with southwest Australia, California with central Chile, and the Mediterranean Basin is intermediate to the others.

Although the defining characteristic of mediterranean type climates is summer drought and winter rain, southern Africa and southern Australia can experience occasional summer rains. These two regions also share a similar topography and geology. Both have a diverse topography with east-west mountain ranges, but southern Australia is extremely flat. They both lie on stable basement complexes with nutrient poor soils from ancient parent materials, and because of their Gondwanan association, these regions share many related taxa. The history of European colonization in these areas, and resultant patterns of plant invasions are similar in that both places were initially colonized by the British and Dutch, who influenced the suite of plants introduced to the regions (Di Castri 1991).

The mediterranean regions of California and Chile share the most similar terrains and climates in the world (Di Castri 1991). They both have a large central valley between two north-south mountain ranges, and are located in areas of high tectonic activity, with moderately nutrient rich soils. The climate in these regions is tempered by coastal fog and cold ocean currents. Although the vegetation in these regions is phylogenetically dissimilar, similar abiotic conditions have resulted in convergent evolution and similarities in ecosystem functioning. After thousands of years of low-intensity land use practices by native peoples, California and Chile were both settled by people from the Mediterranean Basin, who brought with them the same plant species and disturbance regimes. California was settled with the mission system starting in 1767, and the Spanish conquest began in Chile in 1536 (Di Castri 1991). Consequently, along

8

with new disturbance regimes, non-native plants that were pre-adapted to human agricultural practices were also introduced (Rundel 1998). Although human impacts did not become severe until the early to mid 1800's, when Chile won its independence and the gold rush started in California, both areas have been continually and increasingly degraded since the time of the Spanish arrival (Aronson et al. 1998, Esler et al. 1998).

The Mediterranean Basin, because of its large geographical area, possesses diverse topography and soil types. It has undergone frequent tectonic and climatic changes. This is the least invaded region of the five, partially due to its longer history of intensive human land use, which has resulted in the coevolution of plant species with disturbance. The climate is more continental than in the other regions, with occasional killing frosts, which also may limit invasibility. This region shares different characteristics with the other four regions, such as land use patterns with Chile, phylogenetic lineages with California, and a biogeographic connection with southern Africa. These varied connections with different regions place the Mediterranean Basin among the others in an intermediate subgroup (Di Castri 1991).

Mediterranean ecosystems are associated with both natural and anthropogenic disturbance regimes. In the Mediterranean Basin, plants have coevolved with anthropogenic disturbances associated with human agricultural practices for the past 10,000 years, while intensive human activity has only been ongoing in the New World systems for a few hundred years (Rundel 1998). Today, the Mediterranean Basin, central Chile, and California all are highly altered landscapes due to human activity. However, there are many natural disturbances that have been occurring in these systems over evolutionary time including fire in the Mediterranean Basin and California, earthquakes in Chile and California, and animal activity, flooding, and

9

drought in all three areas. Native plants in each region have evolved adaptations to these disturbances.

The mediterranean climate regions posses the most diverse ecosystems in the world, next to the tropics (Moreno and Oechel 1995). Although they collectively cover only 2.25% of the earth's land surface, they support 16% of the world's plant species (Cowling et al. 1996). Many of these species are rare and endemic, and are at particular risk of extinction. This high proportion of global biodiversity, coupled with extreme pressure from human-caused habitat degradation have placed the mediterranean climate regions on Conservation International's list of "biodiversity hotspots," which are regions with top conservation priorities worldwide (Myers et al. 2000). The results of this work will be applied directly to the conservation of these ecosystems.

STUDY SPECIES

The genus *Centaurea,* known from Europe in the Pliocene (Hellwig 2004), contains 500-600 species which range throughout Eurasia (Susanna et al. 1995). *Centaurea* is divided into 40 groups, and based on pollen type, *C. melitensis* is placed into the *Jacea* group (Garcia-Jacas et al. 2000). This group is further divided into three clades: a western Mediterranean clade, an eastern Mediterranean clade, and a widely distributed group. Based on biogeography, morphology, and DNA sequences, *C. melitensis* is placed within the western Mediterranean clade (Garcia-Jacas et al. 2006).

Centaurea melitensis is the only species in the genus that produces cleistogamous capitula (Hellwig 2004), along with chasmogamous ones. The heteromorphic capitula produce seeds with different characteristics. Some seeds display dormancy, and seed size and type is dependent on the breeding type and order of origin of the capitulum (i.e. initial cleistogamous,

chasmogamous, or final cleistogamous). Smaller seeds are found in chasmogamous inflorescences, while cleistogamous capitula produce larger seeds. The smaller seeds have a wider dispersal capability, and the non-dispersed larger seeds potentially produce larger individuals with faster growth rates. Placement on the plant (height) and size of pappus in seeds from both chasmogamous and final cleistogamous inflorescences determine the potential distance of wind dispersal and dispersal via ballistic projection by shaking from a breeze or passing animal. Initial cleistogamous flowers are positioned at the base of the plant, and therefore do not disperse at all (Porras and Munoz 2000a). Cleistogamous capitulum production is a plastic trait that is increased in low resource conditions (Porras and Munoz 2000b), increasing the chances of reproductive success even in years or microhabitats without enough resources to complete a regular life cycle. The mixed mating system of *C. melitensis* might also ensure that offspring that are already adapted to the immediate environment remain in that environment, while outcrossed offspring disperse into the heterogeneous surroundings. In this way, the seeds of chasmogamous flowers would be expected to colonize new patches, while cleistogamous seeds would maintain the existing patch. *Centaurea melitensis* is a ruderal species, establishing populations in disturbed, unpredictable habitats. Cleistogamy and heteromorphic achenes result in a bet-hedging strategy that makes it well adapted for unstable environments.

In its native range, *C. melitensis* produces between two and 100 inflorescences per plant (Porras and Munoz 2000b). Seedling density can be high, potentially resulting in intense intraspecific competition during the seedling stage. Porras and Muñoz (2000a) found no effect of density on seedling survival when grown in pots, although increasing density did reduce height, biomass, and number of capitula. In a close relative, *C. maculosa,* allelochemicals present in root exudates were found to inhibit intraspecific germination without killing the seed (Perry et al.

2005), thus reducing competition, but not regulating long-term population growth. The possibility of allelopathy as a mechanism to reduce intraspecific competition has not been studied in *C. melitensis*.

Facilitated by human-mediated long distance dispersal, *C. melitensis* has spread globally from its western Mediterranean center of origin and is established in a wide variety of climate and habitat types in addition to regions with similar mediterranean climate types. It is present at high latitudes in Sweden and Norway, in areas of high rainfall in the UK and Ireland, and at lower latitudes in Peru and Ecuador (Global Biodiversity Information Facility). In the United States, it is present in 18 states, including Alaska and Hawaii (USDA).

Within California, *C. melitensis* is present in every bioregion in the California Floristic Province and the Desert Province (Hickman 1993). It occurs in a variety of ecosystems from extremely disturbed to minimally disturbed localities, from nature reserves to developed areas. Specimens have been collected from annual grasslands, coastal dunes and bluffs, creek beds, undisturbed oak chaparral, oak woodlands, serpentine habitats, wetlands, and alluvial fans (Consortium of California Herbaria). *Centaurea melitensis* has been in California at least since 1797 (Hendry 1931), and today is considered an "exotic plant of greatest ecological concern" by the California Exotic Pest Plant Council (Di Tomaso and Gerlach 2000). It is not closely related to any California native species, but has 11 close relatives naturalized in California (Di Tomaso and Gerlach 2000).

Centaurea melitensis germinates shortly after the first winter rains, emerging before many native California species. Early emergence has been shown in several studies to result in higher growth rates and survival (Miller 1987), and fecundity (Kalisz 1986) than plants germinating later. After germination, *C. melitensis* grows rapidly through the winter. The

12

flowering season is from spring through the end of summer, typically lasting longer than many native California grassland species. The ability of this species to flower well into the extremely dry late summer may be attributable to its considerable allocation of resources to a large taproot, which allows exploitation of deeper water resources than natives with smaller root systems.

In Chile, *C. melitensis* is invasive in the mediterranean climate zone, and grows in espinal, sclerophyllous matorral (Montenegro et al. 1991), coastal scrub, and montane matorral. Although date of introduction is unknown, many non-native Mediterranean herbs were probably introduced to central Chile from the time of the Spanish conquest, beginning in 1536.

Centaurea melitensis is an ideal species with which to study invasion mechanisms. Its origin in and invasion of mediterranean climate regions provides the opportunity to look beyond climatic drivers of invasion processes, and into ecological factors such as colonization/propagule pressure, competition, disturbance, and edaphic conditions. On the other hand, its broad climatic tolerance and invasion into diverse regions provide the context to address questions about factors that facilitate or constrain range expansion. Its annual life cycle and amenable germination and growth requirements make it a good laboratory and greenhouse subject. Although this species has a moderate rating from the California Invasive Plant Council, its impacts vary regionally, providing opportunities to examine factors that affect the degree of impacts. One of the few studies that has been done to test invasion impacts from this species found potentially devastating competitive effects on an endangered native annual sunflower compared to other invasive mediterranean annual species in California grasslands (Moroney et al. 2011). Considering that range expansion appears to be continuing, insight into the mechanisms that promote its invasion is imperative. Further, the study of this species not only will contribute

13

insight into basic ecological theory, but also presents an opportunity to apply knowledge gained to the conservation of imperiled ecosystems.

LITERATURE CITED

Aronson, J., A. del Pozo, C. Ovalle, J. Avendaño, A. Lavin, and M. Etienne. 1998. Land use changes and conflicts in central Chile. *in* P. W. Rundel, G. Montenegro, and F. M. Jaksic, editors. Landscape Disturbance and Biodiversity in Mediterranean-Type Ecosystems. Springer-Verlag, Berlin.

Baker, H. G. 1965. Characteristics and modes of origin of weeds. Pages 147-172 *in* H. G. Baker and G. L. Stebbins, editors. The genetics of colonizing species. Academic Press, New York, New York, USA.

Blossey, B. and R. Nötzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology 83:887-889.

Callaway, R. M. and J. L. Maron. 2006. What have exotic plant invasions taught us over the past 20 years? Trends in Ecology & Evolution 21:369-374.

Castro, S. A., J. A. Figueroa, M. Munoz-Schick, and F. M. Jaksic. 2005. Minimum residence time, biogeographical origin, and life cycle as determinants of the geographical extent of naturalized plants in continental Chile. Diversity and distributions 11:183-191.

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.

Cowling, R. M., P. W. Rundel, B. B. Lamont, M. K. Arroyo, and M. Arianoutsou. 1996. Plant diversity in mediterranean-climate regions. Trends in Ecology and Evolution 11:362-366.

D'Antonio, C. M., T. L. Dudley, and M. Mack. 1999. Disturbance and biological invasions: direct effects and feedbacks. *in* L. R. Walker, editor. Ecosystems of the world 16. Ecosystems of disturbed ground. Elsevier Science B.V., Amsterdam.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass-fire cycle, and global change. Annual Review of Ecology and Systematics 23:63-87.

Daehler, C. C. 1998. The taxonomic distribution of invasive angiosperm plants: ecological insights and comparison to agricultural weeds. Biological conservation 84:167-180.

Davis, M. A. 2006. Invasion biology 1958-2005: the pursuit of science and conservation. Pages 35-64 *in* M. W. Cadotte, S. M. McMahon, and T. Fukami, editors. Conceptual ecology and invasion biology: reciprocal approaches to nature. Springer, Dordrecht.

Davis, M. A., J. P. Grime, and K. Thompson. 2000. Fluctuating resources in plant communities: A general theory of invasibility. Journal of Ecology 88:528-534.

Di Castri, F. 1991. An ecological overview of the five regions of the world with a mediterranean climate. Pages 3-16 *in* R. H. Groves and F. Di Castri, editors. Biogeography of Mediterranean Invasions. Cambridge University Press, Cambridge, United Kingdom.

Di Tomaso, J. M. and J. D. Gerlach, Jr. 2000. *Centaurea melitensis* L. Pages 98-100 Invasive plants of California's wildlands. University of California Press, Berkeley, California, USA.

Dukes, J. S. 2002. Species composition and diversity affect grassland susceptibility and response to invasion. Ecological Applications 12:602-617.

Elton, C. S. 1958. The Ecology of Invasions by Animals and Plants. The University of Chicago Press, Chicago.

Esler, K. J., P. W. Rundel, and R. M. Cowling. 1998. Biodiversity and conservation biology of

    coastal transition zones from mediterranean to desert ecosystems: an intercontinental

    comparison. Pages 205-230 *in* P. W. Rundel, G. Montenegro, and F. M. Jaksic, editors.

    Landscape Disturbance and Biodiversity in Mediterranean-Type Ecosystems. Springer-

    Verlag, Berlin.

Gaertner, M., A. Den Breeyen, C. Hui, and D. M. Richardson. 2009. Impacts of alien plant

    invasions on species richness in Mediterranean-type ecosystems: a meta-analysis.

    Progress in Physical Geography 33:319-338.

Garcia-Jacas, N., A. Susanna, V. Mozaffarian, and R. Ilarslan. 2000. The natural delimitation of

    Centaurea (Asteraceae: Cardueae): ITS sequence analysis of the *Centaurea jacea* group.

    Plant Systematics and Evolution 223:185-199.

Garcia-Jacas, N., T. Uysal, K. Romashchenko, N. Suarez-Santiago, K. Ertugrul, and A. Susanna.

    2006. *Centaurea* revisited: A molecular survey of the *Jacea* group. Annals of Botany

    98:741-753.

Hamilton, M. A., B. R. Murray, M. W. Cadotte, G. C. Hose, A. C. Baker, C. J. Harris, and D.

    Licari. 2005. Life-history correlates of plant invasiveness at regional and continental

    scales. Ecology Letters 8:1066-1074.

Hellwig, F. H. 2004. Centaureinae (Asteraceae) in the Mediterranean - history of

    ecogeographical radiation. Plant Systematics and Evolution 246:137-162.

Hendry, G. W. 1931. The adobe brick as a historical source. Agricultural History 5:110-127.

Hickman, J. C. 1993. The Jepson manual: higher plants of California. University of California

    Press, Berkeley.

Hierro, J. L., J. L. Maron, and R. M. Callaway. 2005. A biogeographical approach to plant
invasions: the importance of studying exotics in their introduced and native range.
Journal of Ecology 93:5-15.

Hierro, J. L., D. Villarreal, O. Eren, J. M. Graham, and R. M. Callaway. 2006. Disturbance
facilitates invasion: The effects are stronger abroad than at home. American Naturalist
168:144-156.

Hobbs, R. J. and L. F. Huenneke. 1992. Disturbance, diversity, and invasion: implications for
conservation. Conservation Biology 6:324-337.

Huenneke, L. F., S. P. Hamburg, R. Koide, H. A. Mooney, and P. M. Vitousek. 1990. Effects of
soil resources on plant invasion and community structure in Californian USA serpentine
grassland. Ecology 71:478-491.

Jimenez, A., A. Pauchard, L. A. Cavieres, A. Marticorena, and R. O. Bustamante. 2008. Do
climatically similar regions contain similar alien floras? A comparison between the
mediterranean areas of central Chile and California. Journal of Biogeography 35:614-
624.

Kalisz, S. 1986. Variable selection on the timing of germination in *Collinsia verna*
(Scrophulariaceae). Evolution 40:479-491.

Keane, R. M. and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis.
Trends in Ecology & Evolution 17:164-170.

Kimball, S. and P. M. Schiffman. 2003. Differing effects of cattle grazing on native and alien
plants. Conservation Biology 17:1681-1693.

Leishman, M. R. and V. P. Thomson. 2005. Experimental evidence for the effects of additional water, nutrients and physical disturbance on invasive plants in low fertility Hawkesbury Sandstone soils, Sydney, Australia. Journal of Ecology 93:38-49.

Liu, H. and P. Stiling. 2006. Testing the enemy release hypothesis: a review and meta-analysis. Biological Invasions 8:1535-1545.

Liu, H., P. Stiling, and R. W. Pemberton. 2007. Does enemy release matter for invasive plants? evidence from a comparison of insect herbivore damage among invasive, non-invasive and native congeners. Biological Invasions 9:773-781.

Lonsdale, W. M. 1999. Global patterns of plant invasions and the concept of invasibility. Ecology 80:1522-1536.

Mack, M. and C. M. D'Antonio. 1998. Impacts of biological invasions on disturbance regimes. Trends in Ecology & Evolution 13:195-198.

Maron, J. L. and M. Vila. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. Oikos 95:361-373.

Miller, T. E. 1987. Effects of emergence time on survival and growth in an early old-field plant community. Oecologia 72:272-278.

Moles, A. T., M. A. M. Gruber, and S. P. Bonser. 2008. A new framework for predicting invasive plant species. Journal of Ecology 96:13-17.

Montenegro, G., S. Teillier, P. Arce, and V. Poblete. 1991. Introduction of plants into the medierranean-type climate area of Chile. Pages 103-114 *in* R. H. Groves and F. Di Castri, editors. Biogeography of Mediterranean invasions. Cambridge University Press, Cambridge, United Kingdom.

Moreno, J. M. and W. C. Oechel. 1995. Global Change and Mediterranean-Type Ecosystems. Springer-Verlag, New York.

Moroney, J. R., P. M. Schiffman, and C. A. Brigham. 2011. Invasive European annual plants impact a rare endemic sunflower. Madroño 58:69-77.

Muth, N. Z. and M. Pigliucci. 2006. Traits of invasives reconsidered: Phenotypic comparisons of introduced invasive and introduced noninvasive plant species within two closely related clades. American Journal of Botany 93:188-196.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853-858.

Naeem, S., J. M. H. Knops, D. Tilman, K. M. Howe, T. Kennedy, and S. Gale. 2000. Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. Oikos 91:97-108.

Olden, J. D., N. L. Poff, M. R. Douglas, M. E. Douglas, and K. D. Fausch. 2004. Ecological and evolutionary consequences of biotic homogenization. Trends in Ecology & Evolution 19:18-24.

Pauchard, A. and K. Shea. 2006. Integrating the study of non-native plant invasions across spatial scales. Biological Invasions 8:399-413.

Pausas, J. G., F. Lloret, and M. Vila. 2006. Simulating the effects of different disturbance regimes on *Cortaderia selloana* invasion. Biological conservation 128:128-135.

Perry, L. G., G. C. Thelen, W. M. Ridenour, T. L. Weir, R. M. Callaway, M. W. Paschke, and J. M. Vivanco. 2005. Dual role for an allelochemical: (+-)-catechin from *Centaurea maculosa* root exudates regulates conspecific seedling establishment. Journal of Ecology 93:1126-1135.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273-288.

Porras, R. and J. M. Munoz. 2000a. Achene heteromorphism in the cleistogamous species *Centaurea melitensis*. Acta Oecologica 21:231-243.

Porras, R. and J. M. Munoz. 2000b. Cleistogamy in *Centaurea melitensis* (Asteraceae): capitulum variability and spatio-temporal development patterns. Plant Systematics and Evolution 223:251-262.

Rejmánek, M. 1996. A theory of seed plant invasiveness: the first sketch. Biological conservation 78:171-181.

Richardson, D. M. and P. Pyšek. 2006. Plant invasions: merging the concepts of species invasiveness and community invasibility. Progress in Physical Geography 30:409-431.

Rundel, P. W. 1998. Landscape disturbance in mediterreanean-type ecosystems: an overview. Springer-Verlag, Berlin Heidelberg.

Sala, O. E., F. S. Chapin, 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. Biodiversity - Global biodiversity scenarios for the year 2100. Science 287:1770-1774.

Schiffman, P. M. 1997. Animal-mediated dispersal and disturbance: driving forces behind alien plant naturalization. Pages 87-94 *in* J. O. Luken and J. W. Thieret, editors. Assessment and Management of Plant Invasions. Springer-Verlag, New York, New York, USA.

Susanna, A., N. G. Jacas, D. E. Soltis, and P. S. Soltis. 1995. Phylogenetic relationships in the tribe Cardueae (Asteraceae) based on its sequences. American Journal of Botany 82:1056-1068.

Thompson, K., J. G. Hodgson, and T. C. G. Rich. 1995. Native and alien invasive plants: More of the same? Ecography 18:390-402.

Tilman, D. 1997. Community invasibility, recruitment limitation, and grassland biodiversity. Ecology 78:81-92.

Vilà, M., J. L. Espinar, M. Hejda, P. E. Hulme, V. Jarosik, J. L. Maron, J. Pergl, U. Schaffner, Y. Sun, and P. Pysek. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters 14:702-708.

Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience 48:607-615.

CHAPTER 2

Abundance and Dispersion of the Invasive Mediterranean Annual,

Centaurea melitensis in its Native and Non-native Ranges

ORIGINAL PAPER

Abundance and dispersion of the invasive Mediterranean annual, *Centaurea melitensis* in its native and non-native ranges

Jolene R. Moroney · Philip W. Rundel

Abstract In general, invasive plants are assumed to behave more aggressively in their invasive ranges than in their native range, and studies of the mechanisms of invasion often assume these differences. However, comparisons of abundances between native and invasive ranges are rarely carried out. We compared density and dispersion of the invasive plant, *Centaurea melitensis* (Asteraceae) in its native range and two invasive ranges of similar mediterranean-climate type. The objective was to quantify the differences in its abundance among three distant regions. We surveyed six sites in the native range (Spain) and in each of two invaded ranges (California and central Chile) for population density, relative dominance and spatial distribution of *Centaurea*. *Centaurea* occurred at higher densities in invasive sites than in native ones, with a median of 100 plants per m^2 and 70 plants per m^2 in California and Chile, respectively, compared to only 4 plants per m^2 in Spain. *Centaurea* was more dominant in both invasive ranges than in the native range. *Centaurea* density and relative dominance were highly variable within regions. Plants in Spain were randomly dispersed, while those in both invasive ranges were more aggregated. Annual precipitation and mean annual temperature were the best predictors of *Centaurea* density. In California sites, density was negatively correlated with soil nutrients. The presence of at least one high-density population with near total dominance in Spain suggests that there might be ecological mechanisms for invasiveness in *Centuarea* that are not unique to invaded ranges.

Keywords *Centaurea melitensis* · Comparative biogeography · Plant demography · Invasive species · Mediterranean ecosystems · Plant invasion

Introduction

Plant invasions are a major threat to biodiversity worldwide (Mack et al. 2000), second only to land use change in driving native species extinctions. Biological invasions cause declines in native species richness (Gaertner et al. 2009), changes in ecosystem processes (Vilà et al. 2011), biotic homogenization (Olden et al. 2004), and ultimately can result in permanent changes to community and ecosystem composition and functioning (D'Antonio and Vitousek 1992).

In general, invasive plants are assumed to behave more aggressively in their invasive ranges than in their native range, and studies of the mechanisms of invasion (i.e. enemy release, novel weapons, adaptive evolution) often assume these differences. However, comparisons of abundances between native and

J. R. Moroney (✉) · P. W. Rundel
Department of Ecology and Evolutionary Biology,
University of California, 621 Charles E. Young Drive
South, Los Angeles, CA 90095, USA
e-mail: jmoroney@ucla.edu

✷ Springer

invasive ranges are rarely carried out (Hierro et al. 2005; Vilà et al. 2005). Often plants that become invasive in non-native environments are also weedy in their native ranges; their weedy characteristics contributing to their invasiveness (Baker 1965). Ruderal species have characteristics such as high fecundity and high growth rate that can be emergent in both native and non-native environments. Other characteristics such as relatively early germination or competitive advantage might only emerge in novel environments with communities of plants that have not co-evolved with the invader. Furthermore, native and novel environments might differ in factors that affect invasibility, such as disturbance regimes, soil properties, and dispersal facilitation. These differences potentially promote a competitive advantage, ultimately leading to higher abundances in the new range. However, without comparisons of community structure and dynamics between native and invasive ranges, it is impossible to determine if introduced species are more abundant in their new environment than in their native range, or if a weed is simply a weed.

The five mediterranean-type climate regions of the world are important sources of global biodiversity (Cowling et al. 1996), but they are under intense pressure from the threats associated with urbanization (Myers et al. 2000; Underwood et al. 2009). Propagules that are moved between these regions are already pre-adapted to at least the broad climatic conditions in the new range, so they can be prone to invasiveness (Gaertner et al. 2009). A well-studied example is the invasion and current domination of California grasslands by annual grasses that are native to the Mediterranean Basin (Stromberg et al. 2007). Although different mediterranean-type climate regions do not necessarily share identical invasive floras, many invasive species are common to more than one region (Jimenez et al. 2008). By comparing invasions of the same species among regions with similar climate regimes, some of the variation in climatic factors is reduced and finer-scale processes can be examined.

In this study, we compared abundance and dispersion of *Centaurea melitensis* L. in its native range and two invasive ranges. *Centaurea melitensis* (hereafter *Centaurea*) is a winter annual that is native to the Mediterranean Basin. Facilitated by human-mediated long distance dispersal, *Centaurea* has spread globally from its western Mediterranean center of origin, which includes the Balearic Islands, the Azores, Portugal,

Spain, France, Italy, Greece, Corsica, Crete, the former Yugoslavia, Sardinia, and Sicily (Tutin et al. 1976). Currently it is established in a range of climate types in addition to the four other mediterranean-climate regions. It is present at high latitudes in Sweden and Norway, in areas of high rainfall in the UK and Ireland, and at lower latitudes in Peru and Ecuador (Global Biodiversity Information Facility.). In the United States, it is present in 18 states, including Alaska and Hawaii (United States Department of Agriculture.).

To address the hypothesis that *Centaurea* is more dominant and has different patterns of dispersion in invasive communities than in native communities, population density, relative dominance and spatial distribution were quantified in both the native range (Spain) and two invaded ranges of similar climate type (California and central Chile).

Methods

Study species

Centaurea melitensis is a ruderal winter annual, commonly found in disturbed habitats. It grows up to 80 cm in height in its native range (Tutin et al. 1976), and up to 1 m in height in California (Baldwin et al. 2012). In its native range, *Centaurea* produces between two and 100 inflorescences per plant (Porras and Munoz 2000b). The mating system is mixed, with plants having both chasmogamous and cleistogamous capitula (Hellwig 2004), each producing seeds with different dormancy, size, and dispersal characteristics (Porras and Munoz 2000b). Smaller, more widely dispersed seeds are found in chasmogamous inflorescences, while cleistogamous capitula generate larger seeds, potentially producing larger individuals with faster growth rates. Initial cleistogamous flowers are positioned at the base of the plant, and therefore do not disperse at all (Porras and Munoz 2000a). Cleistogamous capitulum production is a plastic trait that is increased in low resource conditions (Porras and Munoz 2000b), increasing the chances of reproductive success in sub-optimal years or microhabitats. Cleistogamy and heteromorphic achenes result in a bet-hedging strategy that makes *Centaurea* well adapted for unstable environments.

In central Chile and California, *Centaurea* is classified as an invasive species (Montenegro et al.

1991; Di Tomaso and Gerlach 2000; Cal-IPC 2006). In both these introduced ranges as well as in the native range, it occurs in a variety of habitat types from extremely disturbed sites such as agricultural fields to minimally disturbed nature reserves. In Chile, *Centaurea* is invasive in the mediterranean climate zone (Arroyo et al. 2000), and is found in espinal, matorral, coastal scrub, and montane matorral (Montenegro et al. 1991). Although the date of introduction is unknown, many Mediterranean herbs were probably introduced to central Chile from the time of the Spanish conquest, beginning in 1536.

In California, *Centaurea* occurs in annual grasslands, coastal dunes and bluffs, creek beds, undisturbed scrub oak chaparral, oak woodlands, serpentine habitats, wetlands, and alluvial fans (Consortium of California Herbaria 2010). *Centaurea melitensis* has been present in California at least since 1797 (Hendry 1931). Today it occurs in great abundances and is undergoing high rates of increase in areas of high plant diversity in California (Cal-IPC 2006–2012). Furthermore, *Centaurea* has been shown to competitively reduce the reproductive capacity of an endangered California endemic annual sunflower, *Pentachaeta lyonii* (Moroney et al. 2011). Considering the threats from this invasion to native biodiversity, it is important to investigate the mechanisms of invasion in this species. But first it is critical to determine whether this species is more common in invasive versus native ranges.

Study areas

Population density, spatial dispersion, and vegetation cover were quantified in both the native range (Spain) and in the two invaded ranges (California and central Chile). In each region, six sites were surveyed, with sites chosen to capture a wide range of environmental conditions (Table 1). Sites were more than 20 km apart, except for two sites in California that were 15 km apart, and two in Spain that were 5 km apart. Dates of surveys were Spain: June 21, 2007 to July 19, 2007; Chile: November 2, 2007 to November 23, 2007; California: March 26, 2008 to June 28, 2008. Most of the sites were sampled from homogeneous areas at least 60 m × 24 m, with a minimum area of 25 m × 4 m. Longitude, latitude, elevation, slope, aspect, habitat type and land use were recorded for each site (Table 1). Disturbance was quantified by

scoring several variables (recent fire, bulldozing, grazing, plowing, agriculture, proximity to roads, proximity to towns) from zero to two, with zero being the least severe and two the most severe disturbance, to create an overall disturbance score for each site.

Sampling

Population density was determined using two-stage systematic sampling (Elzinga et al. 1998). A baseline was placed along the edge of the population, and transects were run perpendicular to the baseline, placed at randomly selected points. 1 m × 0.2 m quadrats were then placed at regular intervals along the transects, starting at a randomly selected point. Plants were counted within each quadrat. Quadrat totals were averaged per transect. Relative dominance was measured in the above-described quadrats by visual estimation of percent cover of *Centaurea* and of all other plants. In California, percent cover was recorded for *Centaurea*, annual grasses, other species, litter, bare ground and rock. For two sites (Guadalhorce and Puerto del Viento) percent cover of *Centaurea* was inferred from density, assuming plant size of 1 % of the quadrat (20 cm^2). For one site (Cerro Acebuchoso) these data were not collected.

Spatial dispersion was evaluated using the Clark-Evans Index of Dispersion (Clark and Evans 1954). Within six randomly placed 1 m^2 plots, nearest neighbor distances were measured and averaged, and the degree of dispersion was determined to be either aggregated ($R \approx 0$), random ($R \approx 1.0$) or regular ($R \approx 2.1$). The index of dispersion $= R =$ sample mean distance/expected mean distance. Expected mean distance $= 1/(2*\sqrt{\text{Density}})$.

Climate variables (annual mean temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, annual precipitation, and precipitation of the wettest month) were obtained from WORLDCLIM in ESRI grids of 30 arcseconds (Hijmans et al. 2005) and extracted in ArcGIS (version 10; Esri Inc., Redlands, CA, USA) for each site.

Soil properties were analyzed in the six California sites to assess population parameters in relation to soil nutrition. In each site, six 2 m^2 plots were haphazardly placed within the population. Within sampling plots, ten subsamples (5 cm^3) were collected from the surface (0–5 cm) of the mineral soil and pooled.

25

Table 1 Geographic characteristics of the study sites

Region	Site	Location		Land use	Habitat type	Elevation (m)	Slope	Aspect	Disturbance score
Spain	Puerto del Viento	N36°47′23.7″	W005°02′50.9″	Open space/goat grazing	Maquis	1,065	35	100	3
	Guadalhorce	N36°56′03.0″	W004°47′59.4″	Natural reserve/recreation	Pine woodland	390	25	340	3
	Turillas	N37°03′35.7″	W002°17′43.2″	Cultivation	Old field	539	5	300	7
	Laguna Amarga	N37°19′00.0″	W004°37′0.00″	Natural reserve/abandoned grove	Old field	300	0		5
	Cerro Acebuchoso	N37°21′10.0″	W004°35′35.3″	Abandoned grove	Old field	316	25	120	4
	Cazorla	N38°09′06.2″	W002°47′09.4″	Natural reserve/cattle grazing	Maquis	688	40	138	2
Chile	Maitencillo	S32°40′53.5″	W71°24′21.7″	Grazing	Coastal scrub	54	12	50	5
	Moscoso	S33°04′08.9″	W71°21′56.2″	Grazing	Espinal	197	30	290	5
	Farellones	S33°20′40.6″	W70°21′14.1″	Open space/grazing	Montane matorral	1,536	47	310	3
	Lagunillas	S33°38′02.0″	W70°19′11.5″	Open space/cattle grazing	Montane matorral	1,358	40	315	6
	Cuesta Zapata	S33°23′20.5″	W71°15′41.0″	Open space/roadside	Matorral	110	5	110	3
	Quebrada de la Plata	S33°29′26.4″	W70°53′15.1″	Natural reserve/recreation	Matorral	517	35	0	3
California	Temecula	N33°28′07.2	W117°00′22.5″	ORV recreation	Edge oak woodland	488	5	220	4
	Stunt Ranch	N34°05′37.2″	W118°39′16.6	Natural reserve/recreation	Chaparral	450	0	n/a	2
	Rocky Oaks	N34°05′53.2″	W118°48′54.8″	Natural reserve/recreation	Chaparral	521	0	n/a	3
	Sedgwick	N34°42′47.7″	W120°02′00.7″	Natural reserve	Chaparral	440	30	320	4
	Hastings	N36°23′03.1″	W121°33′26.0″	Natural reserve	Oak savanna	520	25	120	2
	McLaughlin	N38°50′03.7″	W122°22′41.6″	Natural reserve	Chaparral	481	25	200	0

Samples were pulverized and tested for pH in the lab. Soil fertility (nitrate-nitrogen, Bray-phosphate, exchangeable potassium, and cation exchange capacity) were analyzed using standard techniques at the University of California Agriculture and Natural Resources Analytical Laboratory in Davis, California (UC Davis Analytical Laboratory 2000–2012).

Statistical analyses

The density data did not follow a normal distribution and transformations did not improve the data, so a nested Kruskal–Wallis test was done using R statistical software (version 2.13.1, The R Foundation for Statistical Computing) with the coin package (version 1.0–19) to compare density among regions with sites nested within regions.

Relative dominance (percent cover *Centaurea*/ percent cover total vegetation) was compared among regions. The dominance data did not follow a normal distribution and transformations did not improve the data, so a nested Kruskal–Wallis was done using R with the coin package to compare relative dominance among regions, with sites nested within regions.

The index of dispersion was log transformed to approach a normal distribution. ANOVA with site nested within region was done on the log-transformed data. One-sample *t* tests were done for each region to test if the index of dispersion was significantly different from 1 (random dispersion).

As transformations for the density data failed to approximate a normal distribution, or any family of distributions, the relationship between density and several abiotic factors (annual mean temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, annual precipitation, and precipitation of the wettest month, latitude, elevation, disturbance score) was analyzed among all regions using a quasi-likelihood approach (generalized linear model) with robust (sandwich) estimates of the standard errors. Before model construction, the variables were analyzed for collinearity with a scatterplot matrix and subsequent analysis of variance inflation factors, which showed no collinearity in the variables. After the first run of the model, elevation, rainfall in the wettest month, and minimum temperature of the coldest month were the least significant variables ($P = 0.954, 0.867, 0.512$, respectively) and were removed from the model. Spearman's

rank correlations tested relationships between density and environmental variables.

Spearman's rank correlations tested relationships between density and soil properties in California. All analyses except nested Kruskal–Wallis were performed using Stata statistical software (Stata, version 10.0. Statacorp, College Station, TX).

Results

The 18 sites sampled in these three regions of mediterranean climate-type were all located between 32° and 39° latitude. Median elevations in Spain, Chile and California were 465, 357, and 485 m, respectively, with a 1,482 m elevation gradient in Chile, compared to only an 81 m range in site elevation in California and a 765 m range in Spain. Chilean sites were the most disturbed, with a median disturbance score of 4, compared to Spanish sites, which had a median disturbance score of 3.5. Californian sites were the least disturbed, with a median score of 2.5 (Table 1). Spanish sites had the highest annual precipitation (median 615 mm compared to 534 and 493 mm in California and Chile, respectively). However, the precipitation in the wettest month was highest in Chile (median 119 mm compared to 89 mm in Spain and 112 mm in California). Sites in Chile had the highest mean annual temperature (median 16 °C compared to 15 °C in Spain and 14 °C in California), but the sites in Spain were hotter in the hottest month and in the coldest month than sites in the other two regions (Table 2).

Centaurea occurred at higher densities in invasive sites than in native ones, with a median of 100 plants per m^2 and 70 plants per m^2 in California and Chile, respectively, compared to only 4 plants per m^2 in Spain (Fig. 1). These differences were significant for California (nested Kruskal–Wallis $\chi^2 = 80.3576$, df = 15, $P < 0.005$) (Levy's post hoc comparisons: $P < 0.05$ for Spain and California and Chile and California, $P > 0.05$ for Spain and Chile). Separate tests for each region showed significant differences in density between sites within regions. (Spain: Kruskal–Wallis $\chi^2 = 35.970$, df = 5, $P < 0.005$; Chile: Kruskal–Wallis $\chi^2 = 23.288$, df = 5, $P < 0.005$; California: Kruskal–Wallis $\chi^2 = 15.176$, df = 5, $P < 0.05$). In Spain, population density ranged from 260 plants per m^2 to less than 1 plant per m^2. The range was similar in

Table 2 Climate characteristics of the study sites

Region	Site	Annual precipitation (mm)	Mean annual temperature (°C)	Maximum temperature hottest month (°C)	Minimum temperature coldest month (°C)	Precipitation wettest month (mm)
Spain	Puerto del Viento	822	12.8	29.2	1.3	118
	Guadalhorce	644	14.8	31.1	5.5	101
	Turillas	326	13.1	29.9	4.5	40
	Laguna Amarga	622	16.1	32.9	4.6	90
	Cerro Acebuchoso	607	14.1	33.4	4.9	88
	Cazorla	401	17.1	33.1	2.5	51
Chile	Maitencillo	365	16.7	25.1	6.1	95
	Moscoso	484	15.4	25.2	5.7	139
	Farellones	511	14.5	25.6	−0.5	106
	Lagunillas	611	16.9	25.5	−0.9	131
	Cuesta Zapata	502	14.7	26.2	3.7	136
	Quebrada de la Plata	393	16.7	28.8	2.7	98
California	Temecula	384	15.8	33.3	3.5	73
	Stunt Ranch	525	14.5	29.1	5.3	110
	Rocky Oaks	543	13.5	28.4	4.7	114
	Sedgwick	520	10.9	28.2	3.5	106
	Hastings	636	15.3	26.9	1.7	123
	McLaughlin	822	13.9	33.1	0.8	175

Fig. 1 Boxplots of density of *Centaurea melitensis* in sites in the native range (Spain) and two invasive ranges (Chile and California). The names of the sites sampled are on the x-axis
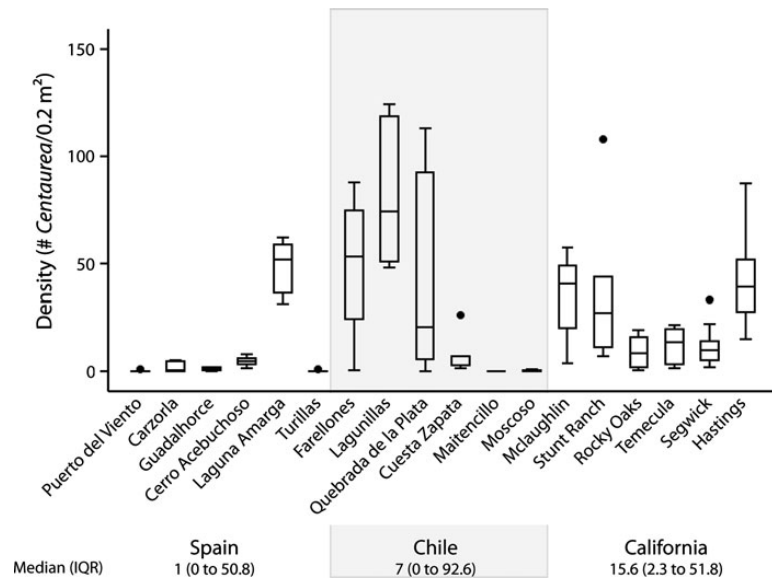
Fig. 2 Boxplots of the relative dominance (percent cover *Centaurea*/percent cover total vegetation) of *Centaurea melitensis* in sites in the native range (Spain) and two invasive ranges (Chile and California). The names of the sites sampled are on the x-axis
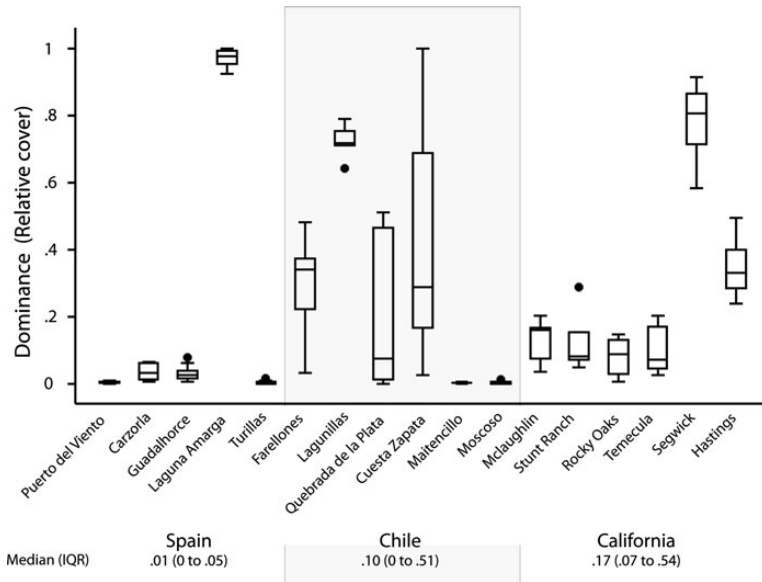
Chile, however in California, all populations were relatively dense, ranging from 44 to 203 plants per m^2.

Populations of *Centaurea* in both invasive ranges had significantly higher dominance relative to total vegetation than those in the native range, with a median 10 % of total vegetation in Chile and 17 % in California, but only 1 % in Spain (Fig. 2; nested Kruskal–Wallis $\chi^2 = 92.2561$, df = 14, $P < 0.005$). Levy's post hoc comparisons were significant for all regions ($P < 0.05$ in all cases; Fig. 4). In five of six California sites, relative dominance of annual grasses was higher than *Centaurea* (Table 3).

Plants in the native region were randomly dispersed, with an index of dispersion (R = 1.131) that was not significantly different from one (one sample *t*-test, $P < 0.05$). Populations in both invasive ranges were more aggregated than expected (Chile: R = 0.758;

California: R = 0.624), with indices of dispersion less than one (one sample *t*-tests, $P < 0.05$) and greater than zero (one sample *t*-tests, $P < 0.05$). Dispersion patterns were significantly different among regions (Fig. 3; nested ANOVA; Region: $F_{2,92} = 8.60$, $P < 0.005$; Site(Region): $F_{15,92} = 2.48$, $P < 0.005$).

The generalized linear model for all regions pooled showed that annual precipitation and mean annual temperature were the best predictors of *Centaurea* population density (Table 4). However, Spearman's correlations showed a significant relationship between minimum temperature of the coldest month and density ($r_s = -0.537$, $P = 0.022$), and weak relationships between all other environmental variables and density ($P > 0.05$ in all cases).

Soils in California sites were neutral to moderately acidic (range 5.1–7.0 pH), with variation among sites

Table 3 Means and standard deviations (in parentheses) of percent cover for sites with *Centaurea melitensis* in California

Site	*Centaurea melitensis*	Annual grass	Other species	Litter	Bare ground	Rock
Temecula	6.42 (5.3)	15.97 (5.75)	40.54 (6.04)	19.03 (4.92)	16.45 (4.84)	1.6 (1.52)
Stunt Ranch	9.15 (6.77)	17.71 (4)	48.57 (8.79)	14.93 (6.92)	9.29 (6.84)	0.42 (0.45)
Rocky Oaks	4.38 (3.3)	8.49 (3.07)	42.14 (8.97)	39.67 (6.16)	2.32 (2.03)	3.1 (1.24)
Sedgwick	37.75 (17.85)	0.25 (0.79)	9.25 (4.87)	10.25 (4.63)	39 (18.79)	4 (2.69)
Hastings	15.67 (7.95)	22.22 (4.76)	5.65 (4.45)	50.28 (8.48)	6.18 (3.49)	0 (0)
McLaughlin	6.24 (4.15)	37.86 (5.04)	10.23 (4.34)	28.18 (4.49)	13.36 (2.53)	3.77 (1.71)

Fig. 3 Clark-Evans Index of Dispersion for populations of *Centaurea melitensis* in its native range, Spain (n = 6) and two invasive ranges, Chile (n = 6) and California (n = 6). Spatial dispersion is aggregated (R ~ 0), random (R ~ 1.0) or regular (R ~ 2.1)
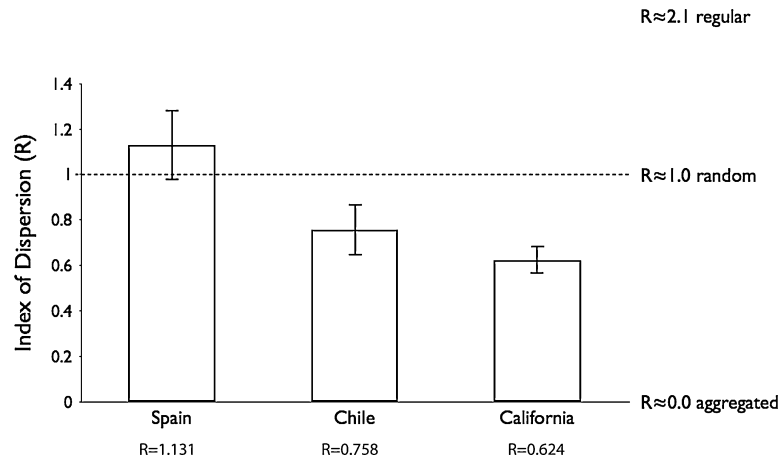
Table 4 Generalized linear model showing the best climatic predictors for *Centaurea melitensis* population density in all regions pooled

Density	Coefficient (β)	Robust SE	z	P	95 % CI
Annual precipitation	0.0074	0.0025	2.92	0.004	0.0024 to 0.0124
Mean annual temperature	0.5799	0.2637	2.20	0.028	0.0632 to 1.0967
Intercept	4.4752	6.5471	0.68	0.494	−8.3568 to 17.3072

Table 5 Mean and standard deviations (in parenthesis) of soil properties for sites with *Centaurea melitensis* in California

Site	pH	NO$_3$–N (ppm)	X-K (ppm)	Bray-P (ppm)	CEC (meq/100 g)
Temecula	6.0 (0.26)	5.43 (6.21)	297.67 (83.28)	78.75 (27.23)	24.25 (12.53)
Stunt Ranch	5.1 (0.20)	1.65 (0.44)	302.33 (32.22)	15.27 (3.24)	18.00 (3.12)
Rocky Oaks	5.6 (0.17)	8.20 (3.30)	420.50 (44.33)	62.70 (16.32)	39.55 (1.40)
Sedgwick	7.0 (012)	5.27 (1.03)	346.17 (81.14)	46.48 (12.78)	28.17 (2.31)
Hastings	5.8 (0.05)	0.52 (0.08)	190.00 (15.90)	64.28 (3.00)	15.33 (1.07)
McLaughlin	5.8 (0.26)	1.08 (0.61)	233.67 (37.44)	30.48 (18.73)	37.10 (4.21)

NO$_3$–N = soil nitrate; X-K = soil exchangeable potassium; Bray-P = extractable phosphorus, Bray method; CEC = cation exchange capacity

in fertility and cation exchange capacity (Table 5). The relationship between *Centaurea* density and soil pH was not significant ($r_s = -0.086$, $P > 0.05$), but surprisingly, density was negatively correlated with soil nutrients. This relationship was significant for nitrogen and potassium (nitrogen: $r_s = -0.386$, $P < 0.001$; Fig. 4; potassium: $r_s = -0.296$, $P < 0.005$; phosphorus: $r_s = -0.070$, $P > 0.05$). Density was also negatively correlated with cation exchange capacity ($r_s = -0.310$, $P < 0.001$), which is an indicator of nutrient availability.

Discussion

Populations of *Centaurea* in non-native ranges had higher densities, higher relative dominance in the community, and more aggregated spatial arrangements

Fig. 4 Scatterplot of the relationship between *Centaurea melitensis* density and available nitrogen in six California sites
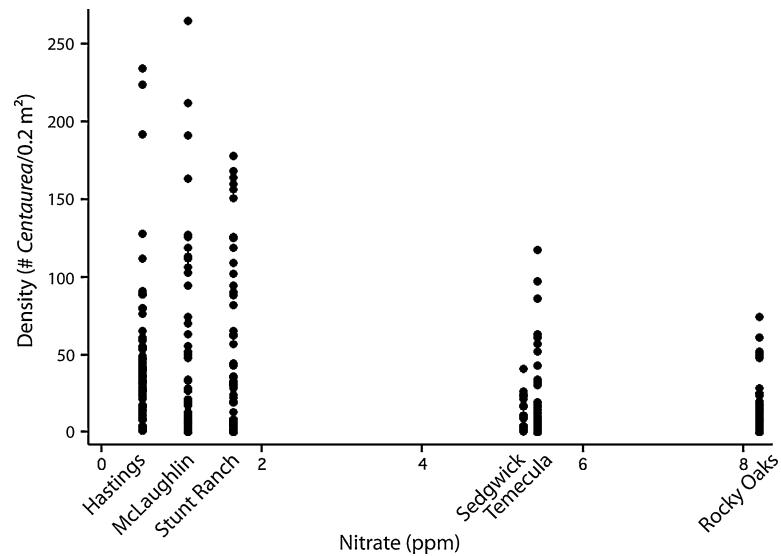
than in the native range. Sites shared overall mediterranean-type climate conditions, however, sites within regions were chosen to try to capture a range of climate, topography and land use conditions. Thus, the within-region variability in site conditions was high, contributing to significant variability of *Centaurea* density and relative dominance within regions, including within the native region. A major limitation of this study was the small number of populations sampled in each region. Ideally, sites with a wide range of environmental conditions would be replicated and matched to similar sites among regions. Furthermore, sites were chosen opportunistically, not randomly from a larger set of populations. Ideally, sampled sites would be randomly chosen from all sites where the species occurs. However, logistics dictated that sites be chosen ad hoc, with efforts made to include a range of conditions and geographic separation among sites.

Populations of *Centaurea* were at significantly higher densities in California than in the native range. Although California sites had lower median annual precipitation than sites in Spain, they did have higher median precipitation than those in Spain during the wettest month, as did sites in Chile. High winter rainfall has been implicated as an important factor in the relative domination of Mediterranean annual grasses in California grasslands, and seems to be a major reason why they have different ecological roles

in California than their much less dominant role throughout their native range (Jackson 1985). In mediterranean-climate regions, rainfall seasonality may be more important than total rainfall in determining the relative dominance of annual plants (Clary 2008).

In Chile, *Centaurea* density was positively correlated with elevation. This was unexpected because generally plant invasions are more prevalent in the lowlands. However, there has recently been growing concern that invasions into high elevation ecosystems are on the rise in many areas of the world (Pauchard et al. 2009). In Chile, extensive areas of the foothills of the Coast Range and Andes have been heavily impacted by four centuries of goat grazing and charcoal burning, providing a different land use history at high elevations than California.

Disturbance is a facilitating factor in many plant invasions (D'Antonio et al. 1999; Davis et al. 2000; Hierro et al. 2006; Hobbs and Huenneke 1992), and therefore would be expected to be associated with increased density. In central Chile, disturbances caused by grazing regime and land use are some of the most important factors in determining invasibility (Figueroa et al. 2004; Arroyo et al. 2000), and in California, disturbance has been shown to increase the invasibility of grasslands (Seabloom et al. 2003b). However, in this study, the disturbance score did not

consistently predict density. The variables used to create a disturbance score may not have encompassed all of the disturbances these particular sites have encountered over the history of the communities' assembly, and captured only current conditions. Populations in Chile and Spain showed a positive correlation between disturbance score and density, but California sites showed a negative correlation. In fact, the two populations with the highest disturbance scores in California, Sedgwick and Temecula, had the lowest densities of *Centaurea* in the region. The Temecula population also had the lowest relative dominance of *Centaurea*, and may be an example of climatic variables being more important than disturbance. It had the lowest mean annual precipitation in the California sites, with only 384 mm. Furthermore, the population with the highest disturbance score in all three regions, Turillas, Spain, had one of the lowest densities of *Centaurea*. This site had the lowest mean annual precipitation of all 18 populations. Thus, low water availability might suppress population size more than disturbance facilitates it. The Sedgwick, California site may be an illustration of how the timing and magnitude of a disturbance is important in determining community composition (Sax 2002) and population density. There was a major fire at Sedgwick in the fall prior to sampling (2007). At the time of sampling (spring 2008), *Centaurea* density was very low, as was the density of all plants. Because of the fire's effect of clearing vegetation, there was a much higher percent cover of bare ground than at the other California sites (39 %).

Despite its low density at the Sedgwick, California site, *Centaurea* at Sedgwick had the highest relative dominance of all the California sites, and the second highest overall, at 78 % of the total vegetation cover. *Centaurea* individuals were large in size, and had many seed-producing inflorescences. It is probable that the full effects of fire disturbance on increasing *Centaurea* density in that site will be realized in future years after this large input into the seed bank simultaneous to high availability of bare ground for recruitment.

The one site in the native range that was comparable in *Centaurea* density to sites in the invasive ranges was Laguna Amarga, Spain. This site had nearly 100 % cover of *Centaurea*, a much higher relative dominance than any of the other sites in all three regions. Unlike the Sedgwick site in California,

there was no evidence of any recent disturbance, and very little bare ground. In California, where overall relative dominance of *Centaurea* was highest, annual grass dominace was even higher. The Mediterranean annual grass, *Avena barbata*, has been shown to outcompete *Centaurea melitensis* in California grasslands (Callaway et al. 2003). In California sites where conditions are favorable for *Centaurea*, *Centaurea* dominance likely is held in check by competition from annual grasses. The absence of co-occurring annual grasses in high density, high dominance *Centaurea* sites in Spain (personal observation) highlights the potential of species which accompany invasive plants to a new range to alter behavior based on changes to their own ecological roles.

The aggregated distribution of plants in the invasive range, as observed in Chile and California, might indicate patchiness in resource availability. Soil nitrogen concentration can be very patchy even at small scales (Herrera 2002). In California, the soil at Hastings had the lowest nitrate concentration (0.52 ppm) and plants were closest to randomly distributed ($R = 0.928$), possibly indicating that there were not high enough concentrations of nitrogen to affect plant success within the site. On the other hand, the more aggregated distribution in invasive sites might indicate an indirect response to patchy nutrient levels via escape from competitive pressures from annual grasses, which dominate in high-nutrient sites in California. However, with the generally low nitrate levels measured in California sites overall and without nitrification rate data, it is difficult to credit nitrogen availability with the more aggregated distribution of plants in sites in the invasive range. Alternatively, differences in other processes or habitat characteristics that were not measured, such as dispersal (via cleistogamous vs. chasmogamous capitula, for example), topography, or substrate physical characteristics, might contribute to differences in spatial distribution among sites.

The density of *Centaurea* in California sites was negatively correlated with soil nitrate and potassium. These results are surprising given that numerous studies within California grassland systems have found that invasive Mediterranean annuals dominate nutrient-rich habitats, and only poorly colonize the nutrient-poor margins (Huenneke et al. 1990; Seabloom et al. 2003a; Harrison 1999). However, most studies of invasion in California grasslands have

focused on Mediterranean annual grasses, and not forbs. Nutrient availability was found to have no effect on the invasiveness of a close congener, *C. diffusa*, in Colorado prairies (LeJeune et al. 2006). If *C. melitensis* is similarly not affected by nutrient availability, then it might dominate in low-nutrient sites that exclude its nitrophilous annual grass competitors. In contrast to California, introduced grasses and forbs in Chile are more successful in low-nutrient soils (Figueroa et al. 2004; Holmgren et al. 2000). While California lacks dominant native annual grass species, a native Chilean annual grass, *Bromus berterianus*, can outcompete Mediterranean annual grasses in high-nutrient conditions (Holmgren et al. 2000). In California and Chile, there are different functional types that can be dominant in the native herbaceous flora, with potentially different effects on the ability of non-native species to colonize and persist. Differences in the dynamics of co-occurring invasive species in all regions were unknown. In many habitats in the invasive ranges, the local communities were made up of mostly non-native Mediterranean annuals. Dominant invasives can influence population dynamics of other invasives, and might have affected the results of this study.

Populations of *Centaurea* have higher densities and higher relative dominance in California and Chile than in Spain. However, the presence of at least one very high-density population with near total dominance in Spain suggests that there are ecological mechanisms not unique to invaded ranges that change population dynamics such that *Centaurea* can dominate in a particular site (i.e., "local enemy release," resource availability, or disturbance, but not evolutionary change). Nevertheless, similarities among regions in some pathways to local dominance does not preclude the possibility that there are other mechanisms of invasion ongoing in California and Chile that are unique to novel environments, as suggested by the differences in density and dominance among non-native regions. Other studies have shown divergent adaptation in non-native regions with different ecological conditions. For example, *Centaurea solstitialis* has evolved divergent germination strategies in two non-native regions of differing rainfall regimes (Hierro et al. 2009), and flowering phenology evolved along a latitudinal gradient in invasive populations of the shrub, *Hypericum canariense* throughout the non-native range (Dlugosch and Parker 2008).

The statistical similarity of population densities in Spain and Chile might suggest that *Centaurea* can be dismissed as a "weak invader" (Hierro et al. 2005; Ortega and Pearson 2005). However, its current range expansion and increase in abundance in California remind us that studies of invasion are snapshots of ongoing processes. At the present time, *Centaurea* is considered a moderate invader in California (Cal-IPC 2006), but is its status seems to be changing as it expands its range and density (Cal-IPC 2006–2012). No similar temporal data for range expansion and abundance increase exist for Chile at this time.

References

Arroyo MK, Marticorena C, Matthei O, Cavieres L (2000) Plant invasions in Chile: present patterns and future predictions. In: Mooney HA, Hobbs RJ (eds) Invasive species in a changing world. Island Press, Covelo, pp 385–421

Baker HG (1965) Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL (eds) The genetics of colonizing species. Academic Press, New York, pp 147–172

Baldwin BG, Goldman DH, Keil DJ, Patterson R, Rosatti TJ, Wilken DH (eds) (2012) The Jepson manual: vascular plants of California, 2nd edn. University of California Press, Berkeley

Cal-IPC (2006a) California invasive plant inventory. Cal-IPC Publication 2006–02. California Invasive Plant Council, Berkeley, CA

Cal-IPC (2006–2012) *Centaurea melitensis* (Malta starthistle, tocalote) statewide maps. http://www.cal-ipc.org/ip/mapping/statewide_maps/Centaurea_melitensis.php. Accessed October 6, 2011

Callaway RM, Mahall BE, Wicks C, Pankey J, Zabinski C (2003) Soil fungi and the effects of an invasive forb on grasses: neighbor identity matters. Ecology 84(1):129–135

🖄 Springer

33

Clark PJ, Evans FC (1954) Distance to nearest neighbor as a measure of spatial relationships in populations. Ecology 35:445–453

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

Consortium of California Herbaria (2010). http://ucjeps.berkeley.edu/consortium/. 2011

Cowling RM, Rundel PW, Lamont BB, Arroyo MK, Arianoutsou M (1996) Plant diversity in mediterranean-climate regions. Trends Ecol Evol 11(9):362–366

D'Antonio CM, Vitousek PM (1992) Biological invasions by exotic grasses, the grass-fire cycle, and global change. Annu Rev Ecol Syst 23:63–87

D'Antonio CM, Dudley TL, Mack M (1999) Disturbance and biological invasions: direct effects and feedbacks. In: Walker LR (ed) Ecosystems of the world 16. Ecosystems of disturbed ground. Elsevier Science B.V, Amsterdam

Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: a general theory of invasibility. J Ecol 88(3):528–534

Di Tomaso JM, Gerlach JD, Jr (2000) *Centaurea melitensis* L. In: Invasive plants of California's wildlands. University of California Press, Berkeley, pp 98–100

Dlugosch KM, Parker IM (2008) Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. Ecol Lett 11:1–9

Elzinga CL, Salzer DW, Willoughby JW (1998) Measuring and monitoring plant populations. BLM Tech Ref 1730–1731

Figueroa JA, Castro SA, Marquet PA, Jaksic FM (2004) Exotic plant invasions to the mediterranean region of Chile: causes, history and impacts. Revista Chilena de Historia Nat 77:465–483

Gaertner M, Den Breeyen A, Hui C, Richardson DM (2009) Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. Prog Phys Geogr 33(3):319–338. doi:10.1177/0309133309341607

Global Biodiversity Information Facility (2008). http://www.gbif.org/. Accessed October 2008

Harrison S (1999) Native and alien species diversity at the local and regional scales in a grazed California grassland. Oecologia 121(1):99–106

Hellwig FH (2004) Centaureinae (Asteraceae) in the Mediterranean—history of ecogeographical radiation. Plant Syst Evol 246:137–162. doi:10.1007/s00606-004-0150-2

Hendry GW (1931) The adobe brick as a historical source. Agric Hist 5:110–127

Herrera CM (2002) Topsoil properties and seedling recruitment in *Lavandula latifolia*: stage-dependence and spatial decoupling of influential parameters. Oikos 97(2):260–270

Hierro JL, Maron JL, Callaway RM (2005) A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. J Ecol 93(1):5–15

Hierro JL, Villarreal D, Eren O, Graham JM, Callaway RM (2006) Disturbance facilitates invasion: the effects are stronger abroad than at home. Am Nat 168(2):144–156

Hierro JL, Eren O, Khetsuriani L, Diaconu A, Torok K, Montesinos D, Andonian K, Kikodze D, Janoian L, Villarreal D, Estanga-Mollica ME, Callaway RM (2009) Germination responses of an invasive species in native and non-native ranges. Oikos 118(4):529–538. doi:10.1111/j.1600-0706.2009.17283.x

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25(15):1965–1978. doi:10.1002/joc.1276

Hobbs RJ, Huenneke LF (1992) Disturbance, diversity, and invasion: implications for conservation. Conserv Biol 6(3):324–337

Holmgren M, Avilés R, Sierralta L, Segura AM, Fuentes ER (2000) Why have European herbs so successfully invaded the Chilean matorral? Effects of herbivory, soil nutrients, and fire. J Arid Environ 44:197–211

Huenneke LF, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects of soil resources on plant invasion and community structure in Californian USA serpentine grassland. Ecology (Washington DC) 71(2):478–491

Jackson LE (1985) Ecological origins of California's Mediterranean grasses. J Biogeogr 12(4):349–361. doi:10.2307/2844866

Jimenez A, Pauchard A, Cavieres LA, Marticorena A, Bustamante RO (2008) Do climatically similar regions contain similar alien floras? A comparison between the mediterranean areas of central Chile and California. J Biogeogr 35(4):614–624. doi:10.1111/j.1365-2699.2007.01799.x

LeJeune KD, Suding KN, Seastedt TR (2006) Nutrient availability does not explain invasion and dominance of a mixed grass prairie by the exotic forb *Centaurea diffusa* Lam. Appl Soil Ecol 32(1):98–110

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10(3):689–710

Montenegro G, Teillier S, Arce P, Poblete V (1991) Introduction of plants into the Mediterranean-type climate area of Chile. In: Groves RH, Di Castri F (eds) Biogeography of Mediterranean invasions. Cambridge University Press, Cambridge, pp 103–114

Moroney JR, Schiffman PM, Brigham CA (2011) Invasive European annual plants impact a rare endemic sunflower. Madroño 58(2):69–77

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858

Olden JD, Poff NL, Douglas MR, Douglas ME, Fausch KD (2004) Ecological and evolutionary consequences of biotic homogenization. Trends Ecol Evol 19(1):18–24

Ortega YK, Pearson DE (2005) Weak vs. strong invaders of natural plant communities: assessing invasibility and impact. Ecol Appl 15(2):651–661. doi:10.1890/04-0119

Pauchard A, Kueffer C, Dietz H, Daehler CC, Alexander J, Edwards PJ, Arevalo JR, Cavieres LA, Guisan A, Haider S, Jakobs G, McDougall K, Millar CI, Naylor BJ, Parks CG, Rew LJ, Seipel T (2009) Ain't no mountain high enough: plant invasions reaching new elevations. Front Ecol Environ 7(9):479–486. doi:10.1890/080072

Porras R, Munoz JM (2000a) Achene heteromorphism in the cleistogamous species *Centaurea melitensis*. Acta Oecologica 21:231–243

Porras R, Munoz JM (2000b) Cleistogamy in *Centaurea melitensis* (Asteraceae): capitulum variability and spatio-temporal development patterns. Plant Syst Evol 223:251–262

Sax DF (2002) Native and naturalized plant diversity are positively correlated in scrub communities of California and Chile. Divers Distrib 8(4):193–210

Seabloom EW, Borer ET, Boucher VL, Burton RS, Cottingham KL, Goldwasser L, Gram W, Kendall BE, Micheli F (2003a) Competition, seed limitation, disturbance, and reestablishment of California native annual forbs. Ecol Appl 13(3):575–592

Seabloom EW, Harpole WS, Reichman OJ, Tilman D (2003b) Invasion, competitive dominance, and resource use by exotic and native California grassland species. Proc Nat Acad Sci 100(23):13384–13389

Stromberg MR, Corbin JD, D'Antonio CM (eds) (2007) California grasslands: ecology and management. University of California Press, Berkeley

Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds) (1976) Flora Europaea, vol 4. Plantaginaceae to Compositae (and Rubiaceae). Cambridge University Press, Cambridge

UC Davis Analytical Laboratory (2000–2012) UC Davis College of Agricultural and Environmental Sciences analytical lab. http://anlab.ucdavis.edu/analyses/soil. 2012

Underwood EC, Viers JH, Klausmeyer KR, Cox RL, Shaw MR (2009) Threats and biodiversity in the mediterranean biome. Divers Distrib 15(2):188–197. doi:10.1111/j.1472-4642.2008.00518.x

United States Department of Agriculture (2008). http://plants.usda.gov/. Accessed October 2008

Vilà M, Maron JL, Marco L (2005) Evidence for the enemy release hypothesis in *Hypericum perforatum*. Oecologia (Berlin) 142(3):474–479

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarosik V, Maron JL, Pergl J, Schaffner U, Sun Y, Pysek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol Lett 14(7):702–708. doi:10.1111/j.1461-0248.2011.01628.x

CHAPTER 3

Phenotypic plasticity and differentiation in fitness-related traits in invasive populations of the

Mediterranean forb, *Centaurea melitensis* (Asteraceae)

ABSTRACT

Biological invasions threaten global biodiversity, resulting in severe ecological and economic costs. Phenotypic plasticity and differentiation in fitness-related traits after introduction can contribute to increased performance in invasive populations of plants. The overall goal of this study was to determine the extent to which post-introduction evolution in trait means, in their plasticity, or inherent species-wide phenotypic plasticity has promoted invasiveness in a European annual forb. In a common greenhouse, we compared several fitness-related traits and the phenotypic plasticity of those traits under four levels of nutrients among native and invasive populations of *Centaurea melitensis*. We tested eighteen populations from three regions of similar mediterranean climate type: the native range (southern Spain) and two invaded ranges (California and central Chile). *C. melitensis* possesses overall phenotypic plasticity, which is a trait that promotes invasiveness. Invasive populations were differentiated from native plants for several trait means and their levels of phenotypic plasticity in directions that enhance competitive ability and success. Invasive plants flowered earlier and had greater growth rates in the early stages of growth phases, important features for invasiveness. Phenotypic plasticity, its evolution post-invasion, and the evolution of fitness-related trait means in invasive populations have potentially contributed to the invasion of *C. melitensis* in California and Chile. Along with an overall wide range of tolerance to growing conditions, *C. meltiensis* populations that have colonized habitats in California and Chile have undergone rapid evolution in several life history traits and the plasticities of those traits in directions that would promote invasiveness in mediterranean ecosystems.

INTRODUCTION

Globalization has resulted in the human-mediated introduction of thousands of species to regions where they did not evolve (Cadotte et al. 2006, Lambdon et al. 2008, Allen et al. 2009). In some instances, introduced species become invasive, establishing self-replacing populations that increase in numbers in locations far from the site of initial introduction (Richardson et al. 2000). Typically, populations of invasive species occur at higher abundances and dominance in the adventive range than they do in the native range (Vilà et al. 2005, Hierro et al. 2006, Beckmann et al. 2009, Moroney and Rundel 2012), and can have major impacts on native communities, affecting ecosystem functioning (D'Antonio and Vitousek 1992), species diversity (Gaertner et al. 2009, Vilà et al. 2011), soil microbial community composition (Batten et al. 2006), and disturbance regimes (Mack and D'Antonio 1998), ultimately permanently reducing biodiversity and ecosystem integrity. The inestimable ecological costs of invasions are compounded by enormous economic costs, which in the United States alone are estimated at $120 billion per year (Pimentel et al. 2005). Globally the damages are calculated at $1.4 trillion per year (Pimentel et al. 2001).

Many ideas have been proposed to explain why plants that are benign members of the community in their native range can become aggressive invaders in novel ranges, however, understanding and consensus remain challenging (Gurevitch et al. 2011). The multitude of overlapping and independent hypotheses (Catford et al. 2009) include mechanisms that involve genetic changes due to both stochasticity and adaptation, and others that involve phenotypic responses to different environmental conditions. Some of the leading avenues of research address: (1) a release in introduced ranges from natural enemies which decrease growth or fecundity and regulate populations in the native range (the Enemy Release Hypothesis, or ERH) (Keane and Crawley 2002, DeWalt et al. 2004b, Vilà et al. 2005); (2) selection for individuals

with increased allocation to growth and reproduction in exchange for decreased allocation to defense subsequent to release from natural enemies in the native range (the Evolution of Increased Competitive Ability, or EICA hypothesis) (Blossey and Nötzold 1995, Jakobs et al. 2004); (3) possession of a general-purpose, phenotypically plastic genotype that exhibits a wide range of tolerance to suboptimal environmental conditions or an increase in fitness in response to improved environmental conditions (Baker 1965, Parker et al. 2003, Molina-Montenegro et al. 2012); (4) a combination of plasticity and enemy release (Blumenthal et al. 2009); (5) exploitation of an empty niche (Mack 2003); (6) stochastic processes such as genetic bottlenecks (Dlugosch and Parker 2008), sampling effects (Parker et al. 2003), or admixture of introduced populations providing genetic variation for further adaptation (Lavergne and Molofsky 2007, Chun et al. 2010); or (7) adaptation to novel conditions in the invasive region (Maron et al. 2004, Dlugosch and Parker 2008, Chun et al. 2011). These mechanisms can act separately or in combination to produce populations that are higher in abundance and dominance in ranges outside their area of origin.

In invasion ecology, typically aspects of either the invaded environment or the invading species are addressed (i.e., "invasibility" or "invasiveness"). Invasibility studies are generally concerned with factors in an environment that facilitate or resist the colonization of invading species, such as species richness, disturbance, or resource availability (Lonsdale 1999, Fridley et al. 2007, Maron and Marler 2007, Milbau et al. 2009). Invasiveness studies either examine traits that make the species a superior competitor in novel environments that are inherent in the species and are present in populations across its range, both natural and adventive (Rejmánek 1996), or they show that competitive traits have evolved in populations that occupy new environments (Chun et al. 2011, Hahn et al. 2012). Associated with each trait, is its range of tolerance, or its

phenotypic plasticity, and this too can be inherent across all populations or rapidly evolve in invasive populations (Kaufman and Smouse 2001, Parker et al. 2003).

Phenotypic plasticity, the ability to tolerate low-resource conditions or to facultatively increase fitness under high levels of resources (Richards et al. 2006), could promote invasiveness in any environment. Phenotypic plasticity can facilitate an invasion in two ways. First, plasticity in some specific traits could be present in the parental populations and facilitate invasion success upon introduction (Baker 1965, Parker et al. 2003, Davidson et al. 2011). Alternatively, selection might favor the evolution of phenotypic plasticity in founder populations in the invasive range (Chun et al. 2007). Reaction norms are graphs of phenotypic responses, at the individual genotype level or at the population level, to different environments (Pigliucci and Schlichting 1995). In the first case, we would expect the reaction norms of populations from the native and invasive ranges to be parallel between ranges, with populations from both ranges showing a positive response to increased resources or a lack of response to decreased resources. In the alternative scenario, the reaction norms would show steeper increases in trait means in invasive populations as nutrient levels increase or shallower decreases in response to reduced resources compared to native populations.

Much of the research examining traits and plasticities of invasive species has been between species, comparing invasive species trait means (Godoy et al. 2009, van Kleunen et al. 2010) or plasticities (Funk 2008, Davidson et al. 2011, Godoy et al. 2011, Palacio-Lopez and Gianoli 2011) to those of related native or non-invasive species. The results have not always been consistent, especially for plasticity. This approach has been important to try to generalize what inherent traits are important for invasiveness. However, the contributions of intraspecific post-introduction evolution are not detectable with this approach. To address this issue,

investigators have compared either trait means or plasticities between native and non-native populations of invasive species (Bossdorf et al. 2005). But because a trait's associated plasticity is itself potentially part of the machinery of its evolution (West-Eberhard 1989), it is important to compare trait values *and* their plasticities within a species between native and invasive populations (DeWalt et al. 2004a, Chun et al. 2011).

The five mediterranean-climate regions of the world provide an excellent system to evaluate drivers of invasion because their abiotic similarities reduce the broad-scale variation in climatic and other factors, enabling the examination of finer-scale processes. The Mediterranean Basin, the California floristic province, central Chile, the South African western cape, and parts of western and southern Australia are widely separated geographically, but share a unique climate regime of dry summers and wet winters (Di Castri 1991). These regions are important repositories of global biodiversity, but are critically imperiled by threats related to urbanization, including the invasion of non-native species (Underwood et al. 2009). The regions have been subjected to great numbers of plant introductions, originating both from within other mediterranean-climate regions and from areas of different climate type (Jimenez et al. 2008, Gaertner et al. 2009, Arianoutsou et al. 2010). Biodiversity change due to biotic exchange is predicted to be higher in mediterranean ecosystems than in any of the other principal terrestrial biomes of the Earth by the year 2100 (Sala et al. 2000).

The mediterranean regions of California and Chile share the most similar terrains and climates in the world (Di Castri 1991). They are tied to the Mediterranean Basin not only by their climate types, but also by a shared history of colonization by people from Spain, who introduced plant species and disturbance regimes from the Mediterranean Basin. Although the introduced plants were exposed to similar climate conditions in the new ranges on a broad scale such as

41

rainfall seasonality, and similar latitudinal effects (day length, insolation, temperature ranges), they encountered novelties in soil properties, disturbance regimes, and resident communities. For example, generally, soils in the Mediterranean Basin are more nitrogen-rich than those in Chile, which in turn have more nitrogen than those in California (Rundel 1988). These novel conditions could have driven selection for genotypes that increased competitive ability in those conditions and changed the mean value of adaptive traits. If the conditions were variable enough, evolution might have occurred via changes in the plasticity of those traits with or without a change in the mean trait value. On the other hand, overall phenotypic plasticity, or a general-purpose genotype, would have promoted tolerance or a positive phenotypic reaction to novel environmental conditions without evolutionary change.

Centaurea melitensis L. (Asteraceae) is an invasive Mediterranean thistle that has been found to occur at higher densities and levels of dominance in California and Chile than in its native range (Moroney and Rundel 2012). Here we determine the extent to which genetically based quantitative trait differentiation, genetically based differentiation of the phenotypic plasticities of these traits, and overall species-wide phenotypic plasticity have contributed to the invasiveness of adventive populations of *C. melitensis*. We compared traits means and plasticities in populations from the native range in the Mediterranean Basin to those from invasive ranges in California and central Chile. The overall objective of this study is to understand the mechanisms that promote invasiveness in *C. melitensis*. The first hypothesis is that the fitness-related traits in the founder populations allowed the invasive populations to grow and reproduce better in their novel environments. The alternative hypothesis would be that rapid evolution in these traits in invasive populations was responsible for increased growth and reproduction. For the first hypothesis to be true we would expect no differences in phenotypes

between founder and invasive populations. The second key hypothesis is that selection on phenotypic plasticity after introduction enhanced the ability of this species to invade novel environments. For this hypothesis to be true, we should see greater phenotypic plasticity in introduced populations. With these findings, we will evaluate the phenotypic plasticity of fitness-related traits in this species overall.

METHODS

Study species

Centaurea melitensis is a ruderal annual that is native to the Mediterranean Basin (Tutin et al. 1976), and has been introduced to wide-ranging locations and habitat types globally, including the four other mediterranean-climate regions (Global Biodiversity Information Facility). *Centaurea melitensis* produces both chasmogamous and cleistogamous capitula (Porras and Alvarez 1999), the ratio depending on growing conditions (Porras and Munoz 2000). In California and central Chile, *C. melitensis* is an invasive pest (Montenegro et al. 1991, Arroyo et al. 2000, Cal-IPC 2006), degrading agricultural lands (Di Tomaso and Gerlach 2000) and outcompeting native species (Moroney et al. 2011). It occurs at higher densities and is more dominant in the community in California and Chile than in its native range (Moroney and Rundel 2012). Introductions into both of these regions probably occurred with the arrival of the Spanish; in Chile during the conquest from 1536, and in California with the missionaries in the late eighteenth century, with evidence from 1797 in an adobe brick (Hendry 1931).

Seed collection

Seeds (achenes) were collected in three regions: the native range (Spain) and two invaded ranges (California and central Chile) from six populations in each region. Populations were at least 20 km apart, except two populations in California, which were 12 km apart and two in

43

Spain that were 5 km apart (Fig. 3.1). In each population, seeds were collected from 30

individual mothers, which were at least 1 m apart. Seeds were collected from chasmogamous

(potentially outcrossed) capitula, so progeny from each maternal family could have been half- or

full sibs. The chasmogamous capitula of *C. melitensis* have a very high selfing rate, and

furthermore, most of the florets have been shown to display pollen tubes before the stigmas are

receptive to pollinators (Porras and Alvarez 1999), so most probably seeds were products of self-

pollination. Dates of collections were Spain: June 2009; California: Spring 2008; Chile: Dec.

2008. Seeds were placed in separate paper bags for each maternal plant and stored at room

temperature until germination.

Germination

Ten seeds from each of ten replicates (mothers) from each of the 18 populations (a total

of 1800 seeds) were weighed to the accuracy of 0.01 mg (Classic Plus microbalance; AB265-

S/FACT; Mettler Toledo, Columbus, Ohio). Each seed was placed in a separate, closed petri dish

on wet filter paper under controlled light and temperature conditions for 3.75 days (90 hours).

Seeds were checked every four hours for germination, and germination time was recorded as

number of hours until radicle emergence. Seeds that did not germinate within 90 hours were

counted as failures.

Greenhouse

In December, 2010, in the Warren Hall greenhouse at the University of California, Los

Angeles, USA, we planted five of the seedlings most similar in size from each of the ten

replicates (mothers) per population in the germination experiment into separate pots (one

individual in each pot; 6.4 x 36 cm "deepots," Stuewe & Sons, Tangent, Oregon, USA). There

were 3 regions x 6 populations per region x 10 mothers per population x 5 treatments per mother

= 900 individuals. The growing medium was a no-nutrient mix of equal parts sand, vermiculite and peat moss. All pots were watered equally and regularly. Temperature and light were ambient conditions. After an initial growing period of four weeks with daily watering, nutrient treatments (Osmocote 14-14-14 N:P:K slow-release fertilizer; Scotts Miracle-Gro Company, Marysville, Ohio, USA) were applied once. Each of four of the five seedlings was randomly assigned to one of the following nutrient treatments: (1) high nutrients (6.7 g/L), (2) medium nutrients (3.3 g/L), (3) low nutrients (1.7 g/L), or (4) zero added fertilizer. The fifth seedling was harvested and weighed (roots and shoots separately) for an initial biomass measure to calculate biomass growth rate. Pots were randomly re-positioned every two weeks for 14 weeks, after which time they remained in their random positions for the duration of the experiment. After 19 weeks, pesticide was applied to plants colonized by aphids or spider mites as needed.

The morphological and life history traits examined were germination time, rosette phase growth rate (leaf number, length of longest leaf); reproductive phase growth rate (height, number of inflorescences); days to first flower; final number of inflorescences; final height; aboveground biomass (separate shoots and inflorescences); belowground biomass (separate tap and fine roots); root:shoot ratio; tap root diameter; and specific leaf area (SLA), the area to dry mass ratio (cm^2 g^{-1}). For rosette phase growth rate, the number of leaves and the length of the longest leaf on the basal rosette were measured on January 20, February 3, February 17, and March 3. Measurements were taken at this frequency to capture fine-scale growth curves early in establishment, which is a crucial time for invasive plants to gain a competitive advantage. Growth rate was calculated for the three intervening time periods as units per day. At 12 weeks, the longest rosette leaf was collected from each plant and measured for leaf area (LI-3100 area meter, LI-COR Environmental, Lincoln, Nebraska, USA). These leaves were dried at 70°C to

45

constant mass and weighed to calculate specific leaf area. Starting at 12 weeks, reproductive

phase growth rate was measured as the difference between the height of plants between each

measurement (measured on March 3, March 18, April 12, and on the day of harvest) per number

of days between each measurement, and the difference between the number of inflorescences

produced between each measurement (measured on March 18, April 12, and on the day of

harvest) divided by the number of days between each measurement. Plants were monitored daily

for the appearance of the first flower until all individuals had flowered. At the end of the growing

period (66 days after the appearance of their first flower; range: 142 to 237days), plants were

measured for final height and final number of inflorescences, and then harvested. Shoots and

inflorescences were collected separately for aboveground biomass. Fine and tap roots were

collected separately for belowground biomass. Root:shoot ratio was calculated. Taproot diameter

was measured. All plant material was oven-dried to a constant mass. Dry mass was determined

to the nearest milligram (Mettler PC 440, Mettler Instrument Corporation, Hightstown, New

Jersey, USA).

Statistical analyses

To explain the variation in trait means, growth rates, and their plasticities among regions,

we used multilevel mixed-effects linear models (xtmixed, Stata 12.1, StataCorp, College Station,

Texas, USA) with seed mass and elevation as covariates and site and mother as random effects.

The models had three levels: (1) observations, (2) mother, and (3) site, testing the effects of

region, nutrient treatment and their interaction. If interactions were significant, the regression

was followed by *post hoc* testing using simple effects to test for the differences in means at each

treatment level, and partial interactions to test for the differences in the slopes of the lines for

each region between treatment levels. The simple effects tests addressed our first question,

testing for statistical differences in trait means among regions at each nutrient treatment level. The partial interactions tests addressed our second question. The slopes in the three sections of the line between treatments (zero to low nutrients, low to medium nutrients, and medium to high nutrients), represent the reaction of plants in each region to differing environments (nutrient levels). Thus, the differences in the slopes of the lines among regions represent the differences in phenotypic plasticity. Differences in plasticities were quantified by comparing the slopes using partial interactions. We used a multilevel approach because of its flexibility in the analysis of relationships at multiple levels and across levels. The approach can handle more complex sampling structures than traditional linear models, recognizing the variability among individuals within each unit, i.e. the clustering or non-independence of samples within groups. Multilevel procedures also display the variability in regression coefficients across groups. The results are expressed as predicted values.

To minimize maternal effects, we selected seedlings of similar size, and included seed size and elevation of the mother's site as covariates and mother and site as random effects in the statistical models.

RESULTS

The mean values of several traits in invasive plants were significantly greater than those found in the plants from the native range (Table 3.1). Invasive plants, at all nutrient levels above zero, had significantly heavier inflorescences (Fig. 3.2A), more belowground biomass (Fig. 3.2B), less aboveground biomass (Fig. 3.2C), less shoot mass (Fig. 3.2D), higher root:shoot ratio (Fig. 3.2E), and higher specific leaf area (Fig. 3.2F) than native plants. We did not find overall significant differences in final height (Fig. 3.2G), but at low nutrient levels native plants were the tallest. For final number of inflorescences, we found that at low nutrient levels, Chilean plants

had the most inflorescences (Fig. 3.2H). None of the variables significantly covaried with elevation, and only specific leaf area significantly covaried with seed mass (Table 3.1).

Native plants flowered significantly later than non-native plants (Fig. 3.2I). The predicted time to flowering for plants (treatments pooled) from Spain was 114 days after planting, which was 22 days later than plants from Chile flowered and 13 days later than those from California. Elevation of the mother's site and seed mass had no effect on flowering day (Table 3.1). However, the effects of nutrient treatment, region and their interaction on flowering day were all significant (Fig. 3.2I).

Invasive populations were differentiated from natives in levels of phenotypic plasticity in all life history traits except for number of inflorescences (Table 3.1), where California plants were less plastic than plants from Chile and Spain (Fig. 3.2H). Populations of invasive plants were significantly more plastic than native plants in total mass of inflorescences (Fig. 3.2A) and belowground biomass (Fig. 3.2B) between zero and low nutrient levels, and Chile more than Spain between low and medium levels, and medium and high levels. Although native plants' SLA (Fig. 3.2F) and root:shoot ratio (Fig. 3.2E) were lower than invasive plants in all nutrient levels except zero, they were higher than or similar to invasive plants at zero nutrients. Native plants' SLA increased more than California plants between medium and high nutrient levels (Fig. 3.2F). For height (Fig. 3.2G) and aboveground biomass (Fig. 3.2C), native plants had higher plasticity than non-native plants when nutrient levels were increased from zero to low levels, and also from medium to high levels for aboveground biomass.

The growth rates of native plants were variably different from those of non-native plants depending on the trait measured and the growth period (Table 3.2, Fig. 3.3). In the rosette phase, native rosette leaves grew slower in length than non-native leaves in the first month of

measurements, and then grew faster in the last two weeks (Fig. 3.3A). Native plants added

rosette leaves faster than non-native plants in the first month, but equally in the last two weeks,

with a stronger response to nutrients from zero to low levels in the last month (Fig. 3.3B). In the

reproductive phase, native plants grew in height more slowly than non-native plants in the first

six weeks of measurements, with a weaker response to nutrient addition from zero to low levels,

but in the final period before harvest, they grew faster in height, with a stronger response to

nutrient addition from zero to low levels (Fig. 3.3C).

Native plants added inflorescences more slowly than non-native plants and had a weaker

response to nutrient addition from zero to low levels but a stronger response from medium to

high levels in the first month of measurements. In the final period before harvest, native plants

added inflorescences at an equal rate to non-native plants, except for at high nutrient levels,

where they added them more slowly. They had a weaker response to nutrient addition from

medium to high levels (Fig. 3.3D). Native plants added biomass more slowly than non-native

plants at all nutrient levels except zero nutrients, and had a weaker response to nutrient addition

between zero and low nutrients and between medium and high nutrients compared to Chile only

(Fig. 3.3E).

Plants from all three regions demonstrated considerable phenotypic plasticity, mostly

displayed as a positive response to increasing resources. The most dramatic increases in

performance occurred between zero and low nutrients for both life history traits (Fig. 3.2) and

growth rates (Fig. 3.3).

Seeds from California and Chile were larger than those from Spain (Wald $X^2_{(2)} = 24.55$,

$P < 0.0001$; Fig. 3.4). Accounting for non-independence in sites and mothers, and adjusting for

elevation of the site of origin, seeds from California (B = 0.633, $P < 0.0001$) and Chile (B =

0.527, $P < 0.0001$) were significantly larger than those from Spain. Californian seeds were the largest, with a predicted value of 1.93 mg. Chilean seeds were not significantly different from those from California at 1.83 mg ($P = 0.436$). Spanish seeds were predicted to weigh only 1.30 mg. The mean seed mass was 1.69 mg (95% CI, 1.67 to 1.71) for all regions pooled. Elevation of the site of origin did not affect seed size (B = 0.00005, $P = 0.754$).

Germination time averaged 42 hours (95% CI, 41.7 to 42.8) for all regions pooled, with an overall 81% germination rate (1,462/1,800) within 90 hours. For Spain, 80% of seeds germinated and 82% of seeds from both California and Chile germinated. Region had a significant effect on germination time ($X^2 = 12.42$, df = 2, $P = 0.002$), but seed mass did not (B = 91.307, $P = 0.184$), and elevation did not (B = -0.200, $P = 0.092$) once site and maternal effects were controlled for. Every 1 mg increase in seed mass increased germination time by 1.5 hours and every 1 m increase in elevation decreased germination time by 0.2 minutes. Seeds from Chile were predicted to germinate 4.7 hours later than those from Spain and 6.3 hours later than those from California. Germination time for seeds from California did not differ from those from Spain (B = -96.87, $P = 0.414$; Fig. 3.5).

DISCUSSION

The findings of this study indicate that the invasive populations of *C. melitensis* studied here have undergone rapid evolution since arrival in their non-native sites. Invasive populations of *C. melitensis* were genetically differentiated from native populations in both trait means and the plasticity of those traits, in directions that promote invasiveness. It is compelling that many of the phenotypic differences among the native versus non-native sites seem to reflect adaptive response to the new environments because they enhance fitness in our experimental conditions,

but it is possible that stochastic events such as founder effects, genetic bottleneck, or genetic drift have also contributed to genetic differentiation among populations.

Centaurea melitensis' general-purpose genotype, evident by the plasticity in both native and invasive populations, is an adaptation to the variable conditions within the native range. This characteristic has not only promoted invasiveness independently in this species, but also potentially provided the raw material for selection for further change. This flexibility would be advantageous in the colonization of a new range, allowing expansion into multiple habitat conditions. Plants from all three regions exhibited plasticity in several traits, but they varied in the direction and intensity, whether they tolerated low resources or responded to higher resources.

The growth rates of invasive plants were faster and more plastic than those of native plants in the early part of each growing phase. The timing of allocation to growth was the important factor in increasing competitive advantage and promoting invasiveness. Our frequent measures of growth allowed us to capture changing dynamics in growth rate differences that were not apparent in comparisons of overall growth rates. If we had only looked at trait means of final measurements and not their incremental growth rates and plasticity we would not have identified the more subtle mechanisms of invasion in this case.

The length of invasive rosette leaves grew faster in the early part of the rosette phase, when it would be important to establish ground space to inhibit potential competitors. This rate increased with added nutrients more steeply than the rate for native plants. Adding to their competitive advantage, invasives increased in height faster than natives in the early part of the reproductive phase, increasing the ability to intercept light from competitors. Furthermore, invasive plants added inflorescences earlier, potentially bet hedging against an unpredictable end

of the rainy season and the onset of drought. The rate of increase of inflorescences was much more pronounced under higher nutrients for non-native plants than for native plants. Native plants added leaves faster than non-native plants, but this trait does not seem to have as clear a fitness advantage for invasion. In the native range, however, adding leaves might be an adaptation to heavy herbivory.

Plants adapted to lower-resource environments typically have slower growth rates, which are associated with higher physical and chemical herbivore defenses in the leaves (Coley 1983, 1988). Leaf toughness, indicated by low specific leaf area (SLA), is a significant factor in herbivore defense (Coley 1983), so leaves with lower SLA would be expected to be better defended from herbivores. Low SLA is an adaptation to nutrient limitation because it slows growth rates, reducing nutrient turnover. It can also be adaptive to drought stress because of the reduction in the area available for water loss. Native plants maintained lower SLA than invasive plants at all nutrients levels except for zero where the natives' SLA surpassed those of invasive plants. This response was the opposite of an adaptive plastic downturn in SLA in response to reduced nutrient levels, and may be an indication that defense from native herbivores is more important in the native range than plasticity in growth rate or SLA in response to limited nutrients or drought stress. The higher SLA of invasive plants may indicate a release from those herbivores has allowed a competitively adaptive faster growth rate, the effects of which are stronger than the effects of soils potentially lower in nutrients than Mediterranean soils on slowing growth rates.

High root:shoot ratio is an adaptation to low nutrient availability because nutrient uptake is maximized (Dent and Burslem 2009). Invasive plants had higher mean root:shoot ratio than native plants under all nutrient levels except for zero nutrients, where Californian plants did not

differ from native ones. Plants from both Spain and Chile showed plasticity for root:shoot ratio by increasing allocation to roots at low nutrient levels. Although the nutrient concentrations of the treatments were much higher than field levels at collection sites in California (e.g. nitrogen: range 0.52 to 8.20 ppm), this nitrophilous ruderal species continued to respond positively for many traits through the highest level of nutrients (6700 ppm). This ability of this species to increase fitness-related traits such as inflorescence number and mass, and aboveground biomass, in response to very high nitrogen inputs could have implications for increasing nitrogen deposition that is expected with global change (Padgett et al. 1999).

Native plants had a larger response to nutrient addition for aboveground biomass, but this did not correlate with an increase in flower and seed production, as there was no difference in the final number of inflorescences, and native inflorescences weighed less than invasive ones. But, again, more biomass would be advantageous not necessarily in the invasive ranges where coevolved herbivores have been excluded, but in the native range where grazing pressures might be heavier.

Flowering phenology is an adaptive trait, in that in different environments a particular timing of the production of flowers and seeds can increase fitness, and the most advantageous timing depends on environmental conditions. For an annual plant in a mediterranean-climate facing uncertain rainfall resources and the onset of drought, the earlier the plant begins to produce flowers the better the chance that it will obtain enough resources to fill a large number of seeds. Flowering time is expected to correlate with day length (latitude), or elevation, but these effects were controlled for in this study by including elevation in the model and restricting seed source populations to the mediterranean-climate zone (between 32 and 39 degrees latitude). When species of mediterranean-climate origins invade regions of similar climate type, flowering

phenology is generally conserved (Godoy et al. 2009). Invasive populations of *C. melitensis* flowered 13 (California) and 22 (Chile) days earlier than native populations. If release from native herbivores eliminates the need for more protected leaves that require a slower growth rate, then invasive populations that have been released from natural enemies and can grow faster and flower sooner would be favored even if their leaves are less protected.

The larger seeds of invasive populations would be expected to produce seedlings with a higher probability of survival than the smaller seeds from the native range. Larger seed size is associated with higher seedling survival, first because developing seedlings are supplied with more reserves, and second because larger seedlings perform better under hazardous conditions such as competition from established vegetation and other seedlings, shading, defoliation, nutrient shortage, burial under litter, or drought (Westoby et al. 2002). Larger invasive seedlings would be expected to be better competitors than their smaller-seeded native counterparts (Stanton 1984, Eriksson 1999), but more vulnerable to seed predation (Reader 1993). If larger seeds confer an overall advantage in the absence of predators, an increase in seed size would be expected in invasive populations that have been released from natural predators from the native range (Blossey and Nötzold 1995). Larger seed mass is generally correlated with slower growth rates in interspecific comparisons (Swanborough and Westoby 1996). However, seed size within species has been positively correlated with growth rate at the seedling stage, but associations between seed mass and germination time and germination probability are variable depending on species (Stanton 1984, Mogie et al. 1990, Eriksson 1999).

Seeds from Chile germinated more slowly than those from California and Spain. These differences were statistically significant, but the scale of the difference was not great: the Chilean seeds germinated only 4.5 hours after Spanish seeds, on average, and 6 hours after Californian

ones. Early emergence is a competitive trait that is important for invasiveness, but a difference of a few hours might not be ecologically significant.

We used methodological and statistical approaches that minimize maternal effects, however, it is possible that variation in maternal environments nevertheless affected the results of this study. Nutrient supply, water supply, temperature, shading, or grazing in the maternal environment can affect seed size. Similarly, germination time can be affected by light quality, elevation, temperature, day length, nutrient availability, or drought stress. These effects can be positive, negative, or neutral depending on species. However, most maternal effects are on the seed and germination and seedling stages, not later growth stages (Roach and Wulff 1987).

The plants all grew for the same period of time after flowering. Because of the later onset of flowering in native populations, Spanish plants grew for more time overall, and thus ended with higher final aboveground biomass and the same number of inflorescences as invasive plants. The growth rates of native plants for rosette leaf length exceeded those for invasive plants in the last two weeks of the rosette phase and for height in the last segment of the reproductive phase. However, growing in isolation in separate pots and under optimal conditions, the experimental plants were not exposed to the numerous and widespread hazards and interactions in the natural environment. Outcomes in field conditions would surely favor invasives if early season growth rate is important for competitive advantage, if earlier onset of flowering increases the range of time that resources are available to produce viable seeds, and if unpredictable onset of drought sometimes cuts short reproductive opportunities.

In conclusion, this study of plant performance in a common environment setting provides strong evidence of rapid evolution flowing introduction. The changes in both phenotypic traits and phenotypic plasticity are consistent with what we would expect for early colonizing species

and suggest that natural selection has played an important role. These findings illustrate the importance of rapid evolution in the ability of introduced species to invade novel environments, despite any environmental perturbations that might also exist.

Table 3.1. Twelve multi-level mixed effects linear regression models for twelve key life history traits in *Centaurea melitensis*: days to flower, height, inflorescence number, inflorescences mass, shoots mass, aboveground biomass, fine root mass, tap root mass, belowground biomass, root:shoot ratio, taproot diameter, and specific leaf area. The main effects were followed by contrasts of marginal linear predictions of final measurement variables. B is the slope (coefficient), and refers to the amount of change in the response variable per unit increase in the fixed effect at the reference region and treatment. Spain is the reference region and zero-added-nutrients is the reference treatment. Elevation and seed mass are covariates.

	Source	df	Days to flower			Height			Inflorescence number		
			B	X^2	P	B	X^2	P	B	X^2	P
Fixed effects	Overall test of treatment	3		231.25	***		4377.4	***		2045.3	***
	Low nutrients		2.39	7.13	**	658.81	1390.5	***	43.61	130	***
	Medium nutrients		3.85	18.84	***	602.27	1165.5	***	70.23	337.46	***
	High nutrients		3.84	18.15	***	472.7	711.29	***	100.09	697.64	***
	Overall test of region	2		43.15	***		3.58	0.167		2.88	0.237
	California		-12.76	6.15	*	16.9	0.13	0.716	3.43	0.303	0.584
	Chile		-21.97	18.4	***	-21.22	0.212	0.643	3.39	0.314	0.578
	Treatment x region	6		279.33	***		31.25	***		11.93	0.064
	Elevation	1	0.008	2.103	0.147	0.01	0.048	0.825	-0.007	1.8	0.181
	Seed mass	1	-1.37	1.44	0.229	2.26	0.017	0.898	-5.265	2.62	0.106
	Constant		113.91	546.16	***	262.63	32.6	***	14.83	5.2	*
				Variance			Variance			Variance	
Random effects	Site			72.08			4995.6			59.61	
	Mother			28.45			1126.32			4.21	
	Residual			22.5			8986.37			423.24	

57

Table 3.1, continued

	Source	df	Inflorescence mass			Shoots mass			Aboveground biomass		
			B	X^2	P	B	X^2	P	B	X^2	P
Fixed effects	Overall test of treatment	3		2702.5	***		8974.9	***		8440.9	***
	Low nutrients		2.43	232.26	***	6.08	1408.5	***	8.49	1085.7	***
	Medium nutrients		3.02	363.28	***	8.68	2874.03	***	11.68	2075.7	***
	High nutrients		3.46	477.86	***	10.8	4456.9	***	14.24	3089.1	***
	Overall test of region	2		22.26	***		137.47	***		40.25	***
	California		0.117	0.194	0.657	0.119	0.23	0.631	0.161	0.26	0.608
	Chile		0.117	0.212	0.649	0.05	0.044	0.833	0.109	0.123	0.718
	Treatment x region	6		59.77	***		417.88	***		66.79	***
	Elevation	1	-.001	0.689	0.407	0.0002	0.922	0.338	-.001	0.0004	0.983
	Seed mass	1	-0.199	2.04	0.152	-0.104	0.504	0.475	-0.215	1.25	0.263
	Constant		0.397	2.07	0.15	0.163	0.372	0.541	0.464	1.93	0.164
				Variance			Variance			Variance	
Random effects	Site			0.106			0.061			0.042	
	Mother			0.03			0.115			0.111	
	Residual			0.726			0.749			1.88	

Table 3.1, continued

			Fine root mass			Taproot mass			Belowground biomass		
Source		df	B	X^2	P	B	X^2	P	B	X^2	P
Fixed effects	Overall test of treatment	3		270.07	***		1493.9	***		560.93	***
	Low nutrients		0.763	17.56	***	0.564	154	***	1.33	44.09	***
	Medium nutrients		0.927	25.91	***	0.672	219.34	***	1.6	64.16	***
	High nutrients		0.777	17.98	***	0.699	235.32	***	1.48	54.17	***
	Overall test of region	2		11.48	**		19364	***		15.44	***
	California		-0.025	0.01	0.923	0.004	0.004	0.955	-.028	0.01	0.924
	Chile		-0.014	0.004	0.955	0.006	0.01	0.922	-.013	0.003	0.963
	Treatment x region	6		35.74	***		36.08	***		42.17	***
	Elevation	1	0.0002	0.884	0.348	0.0001	0.014	0.901	0.0002	0.706	0.399
	Seed mass	1	0.022	0.02	0.886	0.018	0.366	0.616	0.05	0.09	0.766
	Constant		-.076	0.073	0.786	0.008	0.014	0.907	-.080	0.068	0.797
				Variance			Variance			Variance	
Random effects	Site			0.069			0.005			0.094	
	Mother			0.062			0.0003			0.07	
	Residual			0.96			0.06			1.15	

Table 3.1, continued

			Root:shoot ratio			Taproot diameter			Specific leaf area		
Source		df	B	X^2	P	B	X^2	P	B	X^2	P
Fixed effects	Overall test of treatment	3		306.66	***		9300.5	***		67.77	***
	Low nutrients		-.284	135.96	***	3.21	1374.9	***	-81.32	65.45	***
	Medium nutrients		-.304	157.25	***	3.97	2111.4	***	-93.10	85.93	***
	High nutrients		-.340	194.88	***	4.76	3008.5	***	-90.56	81.36	***
	Overall test of region	2		19.66	***		10.58	**		41.65	***
	California		-.081	6.05	*	-.048	0.102	0.751	-3.01	0.053	0.815
	Chile		0.001	.002	0.969	-.011	0.006	0.939	-11.61	0.884	0.349
	Treatment x region	6		69.18	***		32.05	***		72.42	***
	Elevation	1	-0.001	0.102	0.747	0.001	0.176	0.674	-.01	1.19	0.276
	Seed mass	1	0.004	0.036	0.846	0.073	0.865	0.354	-35.23	21.62	***
	Constant		0.438	157.25	***	1.23	61.15	***	411.81	918.09	***
				Variance			Variance			Variance	
Random effects	Site			0.001			0.036			119.68	
	Mother			0.001			0.016			21.64	
	Residual			0.169			0.216			2897.28	

*$P<0.05$, **$P<0.01$, ***$P<0.001$

Table 3.2. Twelve multi-level mixed effects linear regression models for the growth rates of five different plant parts in *Centaurea melitensis*: length of the longest leaf, number of leaves, plant height, number of inflorescences, and biomass. Each growth rate is determined for separate increments of growth, except for biomass growth rate, which was determined over one overall time period. The main effects were followed by contrasts of marginal linear predictions of final measurement variables. B is the slope (coefficient), and refers to the amount of change in the response variable per unit increase in the fixed effect at the reference region and treatment. Spain is the reference region and zero-added-nutrients is the reference treatment. Elevation and seed mass are covariates.

	Source	df	Length of longest leaf Jan. 20 - Feb. 3			Length of longest leaf Feb. 3 - Feb. 17			Length of longest leaf Feb. 17 - March 3		
			B	X^2	P	B	X^2	P	B	X^2	P
Fixed effects	Overall test of treatment	3		484.07	***		2372.72	***		514.63	***
	Low nutrients		0.905	42.51	***	3.708	390.85	***	3.152	96.24	***
	Medium nutrients		1.074	58.22	***	3.931	427.25	***	4.066	155.50	***
	High nutrients		0.940	46.24	***	3.788	408.04	***	4.445	175.03	***
	Overall test of region	2		20.69	***		7.300	*		46.03	***
	California		0.189	0.846	0.356	0.064	0.058	0.811	-0.891	5.808	*
	Chile		0.044	0.048	0.826	-0.08	0.096	0.757	-0.684	3.686	0.054
	Treatment x region	6		29.61	***		30.92	***		8.800	0.185
	Elevation	1	0.001	2.890	0.089	-.001	0.036	0.846	0.001	0.026	0.872
	Seed mass	1	0.104	2.074	0.360	-0.13	0.706	0.401	0.083	0.152	0.697
	Constant		1.333	37.95	***	1.018	12.89	***	1.334	12.32	***
				Variance			Variance			Variance	
Random effects	Site			0.053			0.084			0.031	
	Mother			0.444			0.796			2.172	
	Residual			0.065			0.116			0.299	

61

Table 3.2, continued

			Number of leaves Jan. 20 - Feb. 3			Number of leaves Feb. 3 - Feb. 17			Number of leaves Feb. 17 - March 3		
Source	**df**	**B**	X^2	**P**	**B**	X^2	**P**	**B**	X^2	**P**
Fixed effects Overall test of treatment	3		321.64	***		1151.92	***		423.25	***
Low nutrients		0.081	71.57	***	0.326	323.64	***	0.439	108.16	***
Medium nutrients		0.104	116.64	***	0.360	384.94	***	0.469	118.81	***
High nutrients		0.082	75.00	***	0.369	414.94	***	0.503	130.87	***
Overall test of region	2		25.21	***		45.05	***		3.260	0.196
California		-0.04	10.63	**	-0.035	2.310	0.128	0.027	0.160	0.692
Chile		-0.21	2.822	0.094	-0.01	0.281	0.597	0.085	1.613	0.203
Treatment x region	6		12.65	0.049		33.83	***		12.14	0.059
Elevation	1	0.001	0.001	0.974	-0.01	0.260	0.610	-0.001	0.260	0.613
Seed mass	1	0.044	35.17	***	0.047	11.97	**	0.075	4.666	*
Constant		0.105	60.22	***	0.176	50.41	***	0.111	2.434	0.118
			Variance			Variance			Variance	
Random effects Site			0.001			0.001			0.006	
Mother			0.002			0.007			0.034	
Residual			0.001			0.001			0.005	

62

Table 3.2, continued

Fixed effects	Source	df	Height March 3 - March 18			Height March 18 - April 12			Height April 12 - Harvest		
			B	x^2	P	B	x^2	P	B	x^2	P
Fixed effects	Overall test of treatment	3		414.01	***		688.91	***		130.72	***
	Low nutrients		1.269	3.920	*	8.118	71.91	***	5.971	164.35	***
	Medium nutrients		1.006	2.465	0.117	7.781	64.32	***	5.190	122.10	***
	High nutrients		1.264	3.960	*	6.988	52.85	***	3.813	66.26	***
	Overall test of region	2		21.74	***		11.44	0.003		77.22	***
	California		1.825	0.810	0.366	2.620	1.061	0.305	-1.295	3.312	0.069
	Chile		2.640	1.742	0.188	1.762	0.490	0.487	-1.865	7.129	**
	Treatment x region	6		142.40	***		47.82	***		84.68	***
	Elevation	1	-0.01	0.036	0.852	0.001	0.001	0.983	-0.001	0.240	0.624
	Seed mass	1	0.460	0.640	0.426	-0.74	0.740	0.390	0.767	3.960	*
	Constant		1.367	0.504	0.481	4.899	3.842	*	1.199	2.560	0.110
				Variance			Variance			Variance	
Random effects	Site			10.56			15.89			0.705	
	Mother			9.012			20.31			4.944	
	Residual			1.577			3.400			0.730	

Table 3.2, continued

		Number of inflorescences March 18 - April 12			Number of inflorescences April 12 - Harvest			Biomass Jan. 20 – Harvest		
Source	df	B	X^2	P	B	X^2	P	B	X^2	P
Fixed effects Overall test of treatment	3		666.12	***		1003.08	***		449.37	***
Low nutrients		0.066	2.657	0.103	0.622	37.58	***	0.009	29.05	***
Medium nutrients		0.072	3.063	0.080	1.040	103.23	***	0.011	44.36	***
High nutrients		0.068	2.756	0.097	1.613	253.13	***	0.010	34.22	***
Overall test of region	2		59.51	***		3.740	0.154		14.55	***
California		0.029	0.116	0.736	0.045	0.053	0.820	-0.001	0.001	0.996
Chile		0.077	0.810	0.368	0.077	0.160	0.688	-0.001	0.001	0.973
Treatment x region	6		261.56	***		14.64	*		46.00	***
Elevation	1	-.001	1.153	0.220	-0.01	1.020	0.313	0.000	0.073	0.784
Seed mass	1	0.036	1.020	0.312	-0.05	0.348	0.556	0.001	0.314	0.573
Constant		0.021	0.063	0.804	0.207	1.082	0.298	-0.001	0.137	0.709
			Variance			Variance			Variance	
Random effects Site			0.016			0.075			0.000	
Mother			0.037			0.233			0.000	
Residual			0.006			0.036			0.000	

$*P<0.05$, $**P<0.01$, $***P<0.001$

64
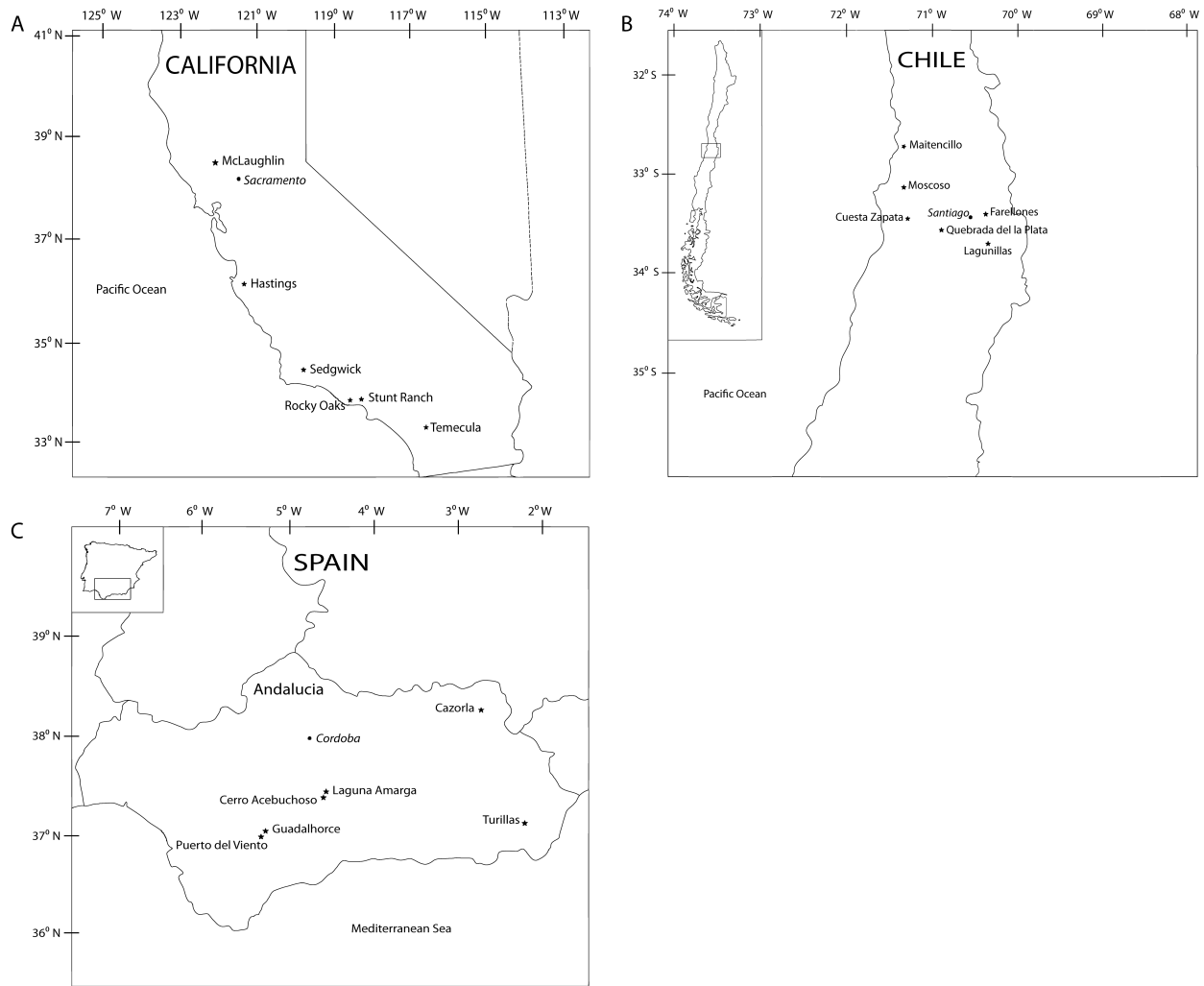
Figure 3.1. Geographic locations of the 18 *Centaurea melitensis* seed collection sites. The six sites in each region are indicated with stars in: (A) the invasive range, California; (B) the invasive range, Chile; and (C) the native range Spain.
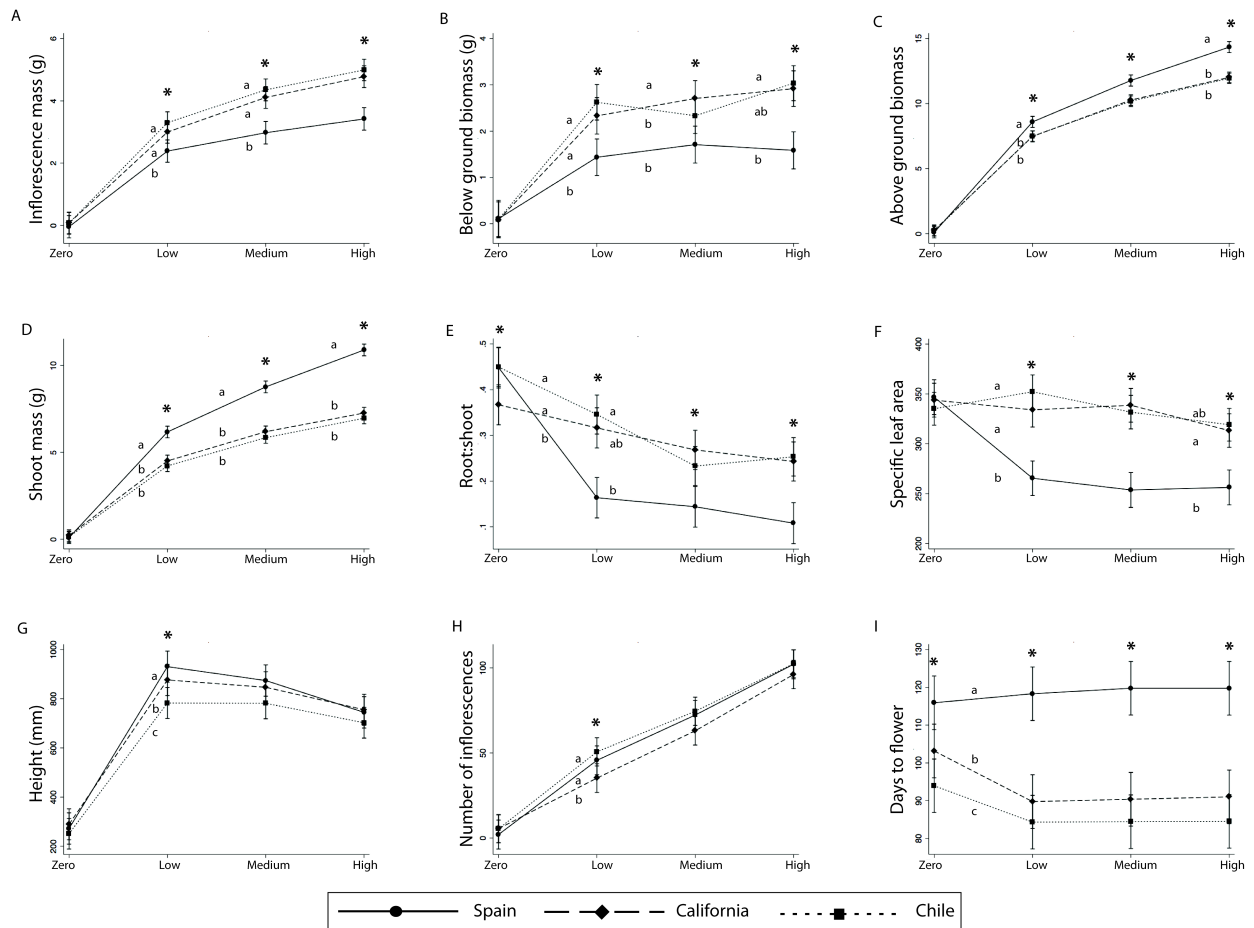
Figure 3.2. Predicted values of the final measurement variables for *Centaurea melitensis* from three regions, grown under four nutrient levels. (A) inflorescence mass; (B) belowground biomass; (C) aboveground biomass; (D) shoot mass; (E) root:shoot ratio; (F) specific leaf area; (G) height; (H) number of inflorescences; and (I) days to flower. The x-axis is four levels of nutrients treatments. The y-axis is the predictive margins with 95% confidence intervals for each trait in each region, accounting for non-independence in sites and mothers. Asterisks indicate significant differences between regions of trait values at a nutrient level. Letters next to line segments are the results of partial interaction tests and indicate statistically significant differences in the slopes of the lines for each region, or differing response to a change in nutrient level/environment (plasticity).

66
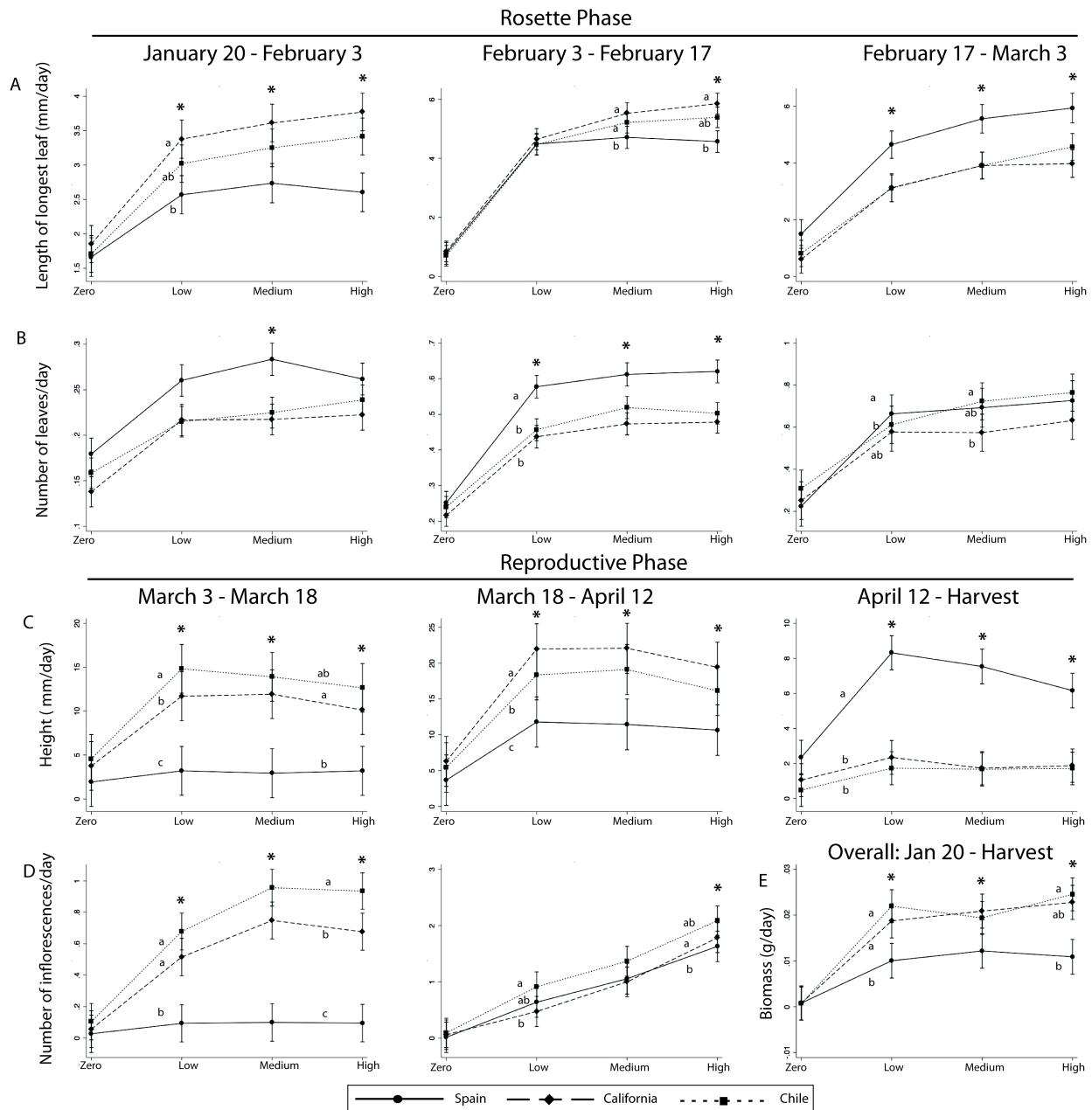
Figure 3.3. Predicted values of growth rates for *Centaurea melitensis* from three regions, grown under four nutrient levels for four variables during three growth phases: the rosette phase: (A) length of the longest leaf and (B) number of leaves; the reproductive phase: (C) height and (D) number of inflorescences; and the overall growth rate: (E) total biomass. Columns within growth phase are increments over the entire measurement period. The x-axis is the nutrient treatments. The y-axis is the predictive margins with 95% confidence intervals for each trait in each region, accounting for non-independence in sites and mothers. Asterisks indicate significant differences between regions of trait values at a nutrient level. Letters next to line segments indicate statistically significant differences in the slopes of the lines for each region, or differing response to a change in nutrient level/environment (plasticity).
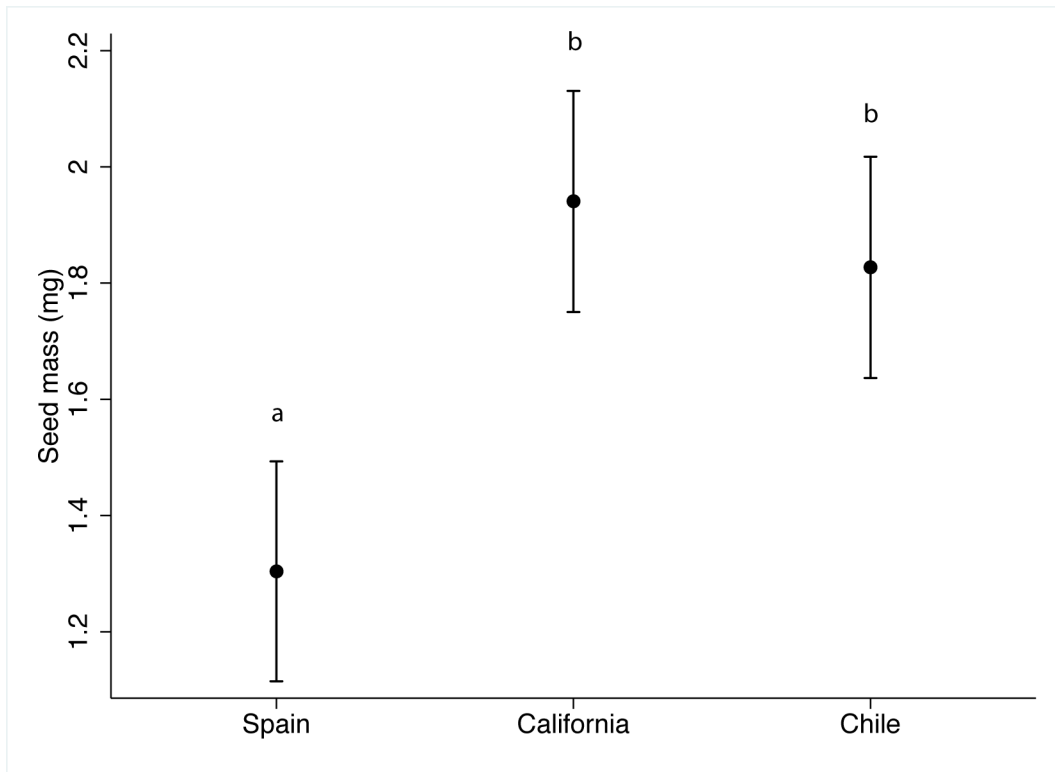
Figure 3.4. Predictive margins with 95% confidence intervals for *Centaurea melitensis* seed mass in each region, for a seed originating from a site of average elevation, and accounting for non-independence in sites and mothers. Letters that are different from each other indicate significant differences among regions.
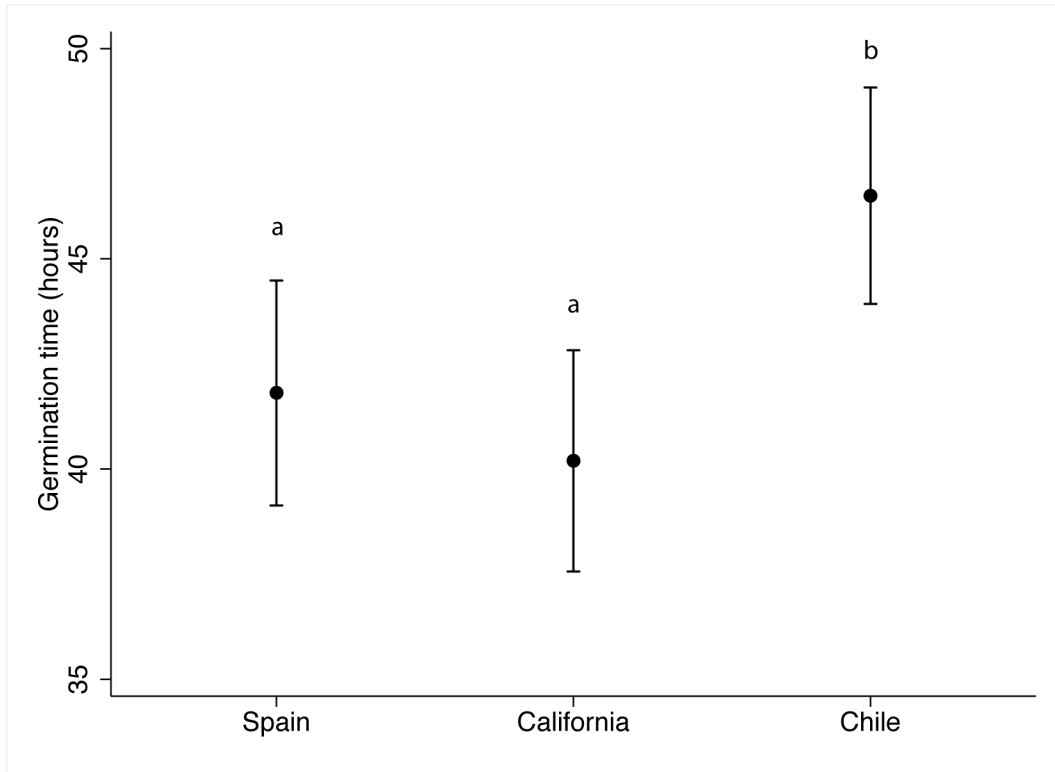
Figure 3.5. Predictive margins with 95% confidence intervals for *Centaurea melitensis* germination time in each region for a seed of average mass and originating from a site of average elevation, adjusted for the non-independence in sites and mothers. Letters that are different from each other indicate significant differences among regions.

LITERATURE CITED

Allen, J. A., C. S. Brown, and T. J. Stohlgren. 2009. Non-native plant invasions of United States National Parks. Biological Invasions 11:2195-2207.

Arianoutsou, M., P. Delipetrou, L. Celesti-Grapow, C. Basnou, I. Bazos, Y. Kokkoris, C. Blasi, and M. Vila. 2010. Comparing naturalized alien plants and recipient habitats across an east-west gradient in the Mediterranean Basin. Journal of Biogeography 37:1811-1823.

Arroyo, M. K., C. Marticorena, O. Matthei, and L. Cavieres. 2000. Plant invasions in Chile: present patterns and future predictions. Pages 385-421 *in* H. A. Mooney and R. J. Hobbs, editors. Invasive species in a changing world. Island Press, Covelo, California, USA.

Baker, H. G. 1965. Characteristics and modes of origin of weeds. Pages 147-172 *in* H. G. Baker and G. L. Stebbins, editors. The genetics of colonizing species. Academic Press, New York, New York, USA.

Batten, K. M., K. M. Scow, K. F. Davies, and S. P. Harrison. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions 8:217-230.

Beckmann, M., A. Erfmeier, and H. Bruelheide. 2009. A comparison of native and invasive populations of three clonal plant species in Germany and New Zealand. Journal of Biogeography 36:865-878.

Blossey, B. and R. Nötzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology 83:887-889.

Blumenthal, D. M., C. E. Mitchell, P. Pyšek, and V. Jarosik. 2009. Synergy between pathogen release and resource availability in plant invasion. Proceedings of the National Academy of Sciences 106:7899-7904.

Bossdorf, O., H. Auge, L. Lafuma, W. E. Rogers, E. Siemann, and D. Prati. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1-11.

Cadotte, M. W., B. R. Murray, and J. Lovett-Doust. 2006. Ecological patterns and biological invasions: Using regional species inventories in macroecology. Biological Invasions 8:809-821.

Cal-IPC. 2006. California invasive plant inventory. Cal-IPC Publication 2006-02. California Invasive Plant Council, Berkeley, CA.

Catford, J. A., R. Jansson, and C. Nilsson. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Diversity and distributions 15:22-40.

Chun, Y. J., M. L. Collyer, K. A. Moloney, and J. D. Nason. 2007. Phenotypic plasticity of native vs. invasive purple loosestrife: A two-state multivariate approach. Ecology 88:1499-1512.

Chun, Y. J., B. Fumanal, B. Laitung, and F. Bretagnolle. 2010. Gene flow and population admixture as the primary post-invasion processes in common ragweed (*Ambrosia artemisiifolia*) populations in France. New Phytologist 185:1100-1107.

Chun, Y. J., V. Le Corre, and F. Bretagnolle. 2011. Adaptive divergence for a fitness-related trait among invasive *Ambrosia artemisiifolia* populations in France. Molecular Ecology 20:1378-1388.

Coley, P. D. 1983. Herbivory and defensive characteristics of tree species in a lowland

    tropical forest. Ecological Monographs 53:209-233.

Coley, P. D. 1988. Effects of plant growth rate and leaf lifetime on the amount and type

    of anti-herbivore defense. Oecologia 74:531-536.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the

    grass-fire cycle, and global change. Annual Review of Ecology and Systematics

    23:63-87.

Davidson, A. M., M. Jennions, and A. B. Nicotra. 2011. Do invasive species show higher

    phenotypic plasticity than native species and, if so, is it adaptive? A meta-

    analysis. Ecology Letters 14:419-431.

Dent, D. H. and D. Burslem. 2009. Performance trade-offs driven by morphological

    plasticity contribute to habitat specialization of Bornean tree species. Biotropica

    41:424-434.

DeWalt, S. J., J. S. Denslow, and J. L. Hamrick. 2004a. Biomass allocation, growth, and

    photosynthesis of genotypes from native and introduced ranges of the tropical

    shrub *Clidemia hirta*. Oecologia 138:521-531.

DeWalt, S. J., J. S. Denslow, and K. Ickes. 2004b. Natural-enemy release facilitates

    habitat expansion of the invasive tropical shrub *Clidemia hirta*. Ecology 85:471-

    483.

Di Castri, F. 1991. An ecological overview of the five regions of the world with a

    mediterranean climate. Pages 3-16 *in* R. H. Groves and F. Di Castri, editors.

    Biogeography of Mediterranean Invasions. Cambridge University Press,

    Cambridge, United Kingdom.

72

Di Tomaso, J. M. and J. D. Gerlach, Jr. 2000. Invasive plants of California's wildlands. University of California Press, Berkeley, California, USA.

Dlugosch, K. M. and I. M. Parker. 2008. Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. Ecology Letters 11:1-9.

Eriksson, O. 1999. Seed size variation and its effect on germination and seedling performance in the clonal herb *Convallaria majalis*. Acta Oecologica-International Journal of Ecology 20:61-66.

Fridley, J. D., J. J. Stachowicz, S. Naeem, D. F. Sax, E. W. Seabloom, M. D. Smith, T. J. Stohlgren, D. Tilman, and B. Von Holle. 2007. The invasion paradox: Reconciling pattern and process in species invasions. Ecology 88:3-17.

Funk, J. L. 2008. Differences in plasticity between invasive and native plants from a low resource environment. Journal of Ecology 96:1162-1173.

Gaertner, M., A. Den Breeyen, C. Hui, and D. M. Richardson. 2009. Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. Progress in Physical Geography 33:319-338.

Global Biodiversity Information Facility. http://www.gbif.org/ October 2008

Godoy, O., D. M. Richardson, F. Valladares, and P. Castro-Diez. 2009. Flowering phenology of invasive alien plant species compared with native species in three Mediterranean-type ecosystems. Annals of Botany 103:485-494.

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.

Gurevitch, J., G. A. Fox, G. M. Wardle, Inderjit, and D. Taub. 2011. Emergent insights from the synthesis of conceptual frameworks for biological invasions. Ecology Letters 14:407-418.

Hahn, M. A., Y. M. Buckley, and H. Muller-Scharer. 2012. Increased population growth rate in invasive polyploid Centaurea stoebe in a common garden. Ecology Letters 15:947-954.

Hendry, G. W. 1931. The adobe brick as a historical source. Agricultural History 5:110-127.

Hierro, J. L., D. Villarreal, O. Eren, J. M. Graham, and R. M. Callaway. 2006. Disturbance facilitates invasion: The effects are stronger abroad than at home. American Naturalist 168:144-156.

Jakobs, G., E. Weber, and P. J. Edwards. 2004. Introduced plants of the invasive *Solidago gigantea* (Asteraceae) are larger and grow denser than conspecifics in the native range. Diversity and distributions 10:11-19.

Jimenez, A., A. Pauchard, L. A. Cavieres, A. Marticorena, and R. O. Bustamante. 2008. Do climatically similar regions contain similar alien floras? A comparison between the mediterranean areas of central Chile and California. Journal of Biogeography 35:614-624.

Kaufman, S. R. and P. E. Smouse. 2001. Comparing indigenous and introduced populations of Melaleuca quinquenervia (Cav.) Blake: Response of seedlings to water and pH levels. Oecologia 127:487-494.

Keane, R. M. and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17:164-170.

Lambdon, P. W., P. Pysek, C. Basnou, M. Hejda, M. Arianoutsou, F. Essl, V. Jarosik, J. Pergl, M. Winter, P. Anastasiu, P. Andriopoulos, I. Bazos, G. Brundu, L. Celesti-Grapow, P. Chassot, P. Delipetrou, M. Josefsson, S. Kark, S. Klotz, Y. Kokkoris, I. Kuhn, H. Marchante, I. Perglova, J. Pino, M. Vila, A. Zikos, D. Roy, and P. E. Hulme. 2008. Alien flora of Europe: species diversity, temporal trends, geographical patterns and research needs. Preslia 80:101-149.

Lavergne, S. and J. Molofsky. 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proceedings of the National Academy of Sciences of the United States of America 104:3883-3888.

Lonsdale, W. M. 1999. Global patterns of plant invasions and the concept of invasibility. Ecology 80:1522-1536.

Mack, M. and C. M. D'Antonio. 1998. Impacts of biological invasions on disturbance regimes. Trends in Ecology & Evolution 13:195-198.

Mack, R. N. 2003. Phylogenetic constraint, absent life forms, and preadapted alien plants: A prescription for biological invasions. International Journal of Plant Sciences 164:S185-S196.

Maron, J. and M. Marler. 2007. Native plant diversity resists invasion at both low and high resource levels. Ecology 88:2651-2661.

Maron, J. L., M. Vila, R. Bommarco, S. Elmendorf, and P. Beardsley. 2004. Rapid evolution of an invasive plant. Ecological Monographs 74:261-280.

Milbau, A., J. C. Stout, B. J. Graae, and I. Nijs. 2009. A hierarchical framework for integrating invasibility experiments incorporating different factors and spatial scales. Biological Invasions 11:941-950.

75

Mogie, M., J. R. Latham, and E. A. Warman. 1990. Genotype-indpepndent aspects of seed ecology in *Taraxacum*. Oikos 59:175-182.

Molina-Montenegro, M. A., J. Penuelas, S. Munne-Bosch, and J. Sardans. 2012. Higher plasticity in ecophysiological traits enhances the performance and invasion success of *Taraxacum officinale* (dandelion) in alpine environments. Biological Invasions 14:21-33.

Montenegro, G., S. Teillier, P. Arce, and V. Poblete. 1991. Introduction of plants into the medierranean-type climate area of Chile. Pages 103-114 *in* R. H. Groves and F. Di Castri, editors. Biogeography of Mediterranean invasions. Cambridge University Press, Cambridge, United Kingdom.

Moroney, J. R. and P. W. Rundel. 2012. Abundance and dispersion of the invasive Mediterranean annual, *Centaurea melitensis* in its native and non-native ranges. Biological Invasions:10.1007/s10530-10012-10302-10531.

Moroney, J. R., P. M. Schiffman, and C. A. Brigham. 2011. Invasive European annual plants impact a rare endemic sunflower. Madroño 58:69-77.

Padgett, P. E., E. B. Allen, A. Bytnerowicz, and R. A. Minich. 1999. Changes in soil inorganic nitrogen as related to atmospheric nitrogenous pollutants in southern California. Atmospheric Environment 33:769-781.

Palacio-Lopez, K. and E. Gianoli. 2011. Invasive plants do not display greater phenotypic plasticity than their native or non-invasive counterparts: a meta-analysis. Oikos 120:1393-1401.

Parker, I. M., J. Rodriguez, and M. E. Loik. 2003. An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. Conservation Biology 17:59-72.

Pigliucci, M. and C. D. Schlichting. 1995. Reaction norms of *Arabidopsis* (Brassicaceae) 3. Response to nutrients in 26 populations from a worldwide collection. American Journal of Botany 82:1117-1125.

Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O'Connell, E. Wong, L. Russel, J. Zern, T. Aquino, and T. Tsomondo. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. Agriculture Ecosystems & Environment 84:1-20.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273-288.

Porras, R. and J. M. M. Alvarez. 1999. Breeding system in the cleistogamous species *Centaurea melitensis* (Asteraceae). Canadian Journal of Botany-Revue Canadienne De Botanique 77:1632-1640.

Porras, R. and J. M. Munoz. 2000. Cleistogamy in *Centaurea melitensis* (Asteraceae): capitulum variability and spatio-temporal development patterns. Plant Systematics and Evolution 223:251-262.

Reader, R. J. 1993. Control of seedling emergence by ground cover and seed predation in relation to seed size for some old-field species. Journal of Ecology 81:169-175.

Rejmánek, M. 1996. A theory of seed plant invasiveness: the first sketch. Biological conservation 78:171-181.

Richards, C. L., O. Bossdorf, N. Z. Muth, J. Gurevitch, and M. Pigliucci. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9:981-993.

Richardson, D. M., P. Pyšek, M. Rejmánek, M. G. Barbour, F. D. Panetta, and C. J. West. 2000. Naturalization and invasion of alien plants: concepts and definitions. Diversity and distributions 6:93-107.

Roach, D. A. and R. D. Wulff. 1987. Maternal Effects in Plants. Annual Review of Ecology and Systematics 18:209-235.

Rundel, P. W. 1988. Vegetation, nutrition and climate - data-tables (2) Foliar analyses. Pages 63-80 *in* R. L. Specht, editor. Mediterranean-Type Ecosystems: A Data Source Book. Kluwer Academic, Dordrecht, Netherlands.

Sala, O. E., F. S. Chapin, 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. Biodiversity - Global biodiversity scenarios for the year 2100. Science 287:1770-1774.

Stanton, M. L. 1984. Seed variation in wild radish - effect of seed size on components of seedling and adult fitness Ecology 65:1105-1112.

Swanborough, P. and M. Westoby. 1996. Seedling relative growth rate and its components in relation to seed size: Phylogenetically independent contrasts. Functional Ecology 10:176-184.

Tutin, T. G., V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb, editors. 1976. Flora Europaea. Cambridge University Press, Cambridge, United Kingdom.

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.

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.

Vilà, M., J. L. Espinar, M. Hejda, P. E. Hulme, V. Jarosik, J. L. Maron, J. Pergl, U. Schaffner, Y. Sun, and P. Pysek. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters 14:702-708.

Vilà, M., J. L. Maron, and L. Marco. 2005. Evidence for the enemy release hypothesis in *Hypericum perforatum*. Oecologia 142:474-479.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics 20:249-278.

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: Some leading dimensions of variation between species. Annual Review of Ecology and Systematics 33:125-159.

CHAPTER 4

Neutral differentiation of populations of an invasive plant: Testing primers for microsatellite

markers developed for closely related species

ABSTRACT

Common garden comparisons of native and nonnative populations of invasive plants can reveal genetic differences among plants from different regions. The causes of changes in gene frequencies between native and invasive populations can include genetic bottlenecks, genetic drift, post-introduction admixture, sampling effects, and adaptive evolution. To determine the mechanism of evolution in *Centaurea melitensis* plant populations, which were sampled from different countries and found through a common garden experiment to be differentiated, we attempted to develop microsatellite markers for the species. We tested 22 primers for microsatellite markers that had previously been developed for four species that are closely related to *C. melitensis*. Our ultimate objective was to determine the relative contributions of stochastic demographic vs. adaptive evolutionary processes to the invasiveness of *C. melitensis*. Six primer pairs were successfully amplified by PCR, producing a product large enough for genotyping. Genotyping showed that none of the microsatellite markers amplified adequately for *C. melitensis*. All of the eight potential fragments were too small for genotyping, and they were all monomorphic. Therefore, none of the 22 previously developed *Centaurea* species microsatellite markers were adaptable to *C. melitensis*. This experiment represents a first attempt at obtaining genetic data in this invasive species that can be used to inform demographic investigations, and ultimately management plans to control *C. melitensis* invasions.

INTRODUCTION

Common garden experiments suggest that invasive plants can evolve rapidly in their adventive range, but without molecular data the mechanism of differentiation is unknown. Aside from adaptive evolution, the causes of changes in gene frequencies between native and invasive populations can include genetic bottlenecks, genetic drift, post-introduction admixture, or sampling effects. Any of these mechanisms could work in conjunction with adaptive evolution.

Given that invasive species colonizing new environments are exposed to novel selection regimes, it is not surprising that rapid adaptive evolution in invasive plant populations has been demonstrated in a number of studies (Maron et al. 2004, Lavergne and Molofsky 2007, Dlugosch and Parker 2008, Chun et al. 2011). However, other evolutionary processes can have profound impacts on the genetic differentiation of invasive populations relative to native ones. Long-distance human-mediated dispersal of propagules only transports a portion of the genetic diversity from the native range, potentially resulting in a loss of genetic diversity due to founder effects in the invasive range. Additionally, sampling effects and the evolutionary history of the source region can affect the genetics of introduced populations. If source populations are differentiated, repeated introductions to the new range could result in an increase of genetic diversity in invasive populations due to admixture of genotypes, despite potential initial reductions in diversity due to founder effects (Keller and Taylor 2008). However, if there is widespread gene flow in the native range and native populations are not differentiated, then even multiple introductions would not have an effect on the genetic diversity of invasive populations. If the selection regimes in the invasive ranges are different from the native range, or if there has been a release from native enemies in the new range, then adaptive divergent evolution can occur in invasive populations. Alternatively, if similar conditions between ranges drive invasion, then trait values would stabilize despite potential neutral differentiation.

Common gardens have been used to suggest rapid adaptive evolution in invasive plant populations by comparing quantitative traits (Leger and Rice 2003, Stastny et al. 2005). However, without also examining neutral variation, it is impossible to decipher whether the mechanism of genetic change is founder effects, sampling effects, or adaptive evolution. In a previous study (Chapter 2), a common garden was established for native and invasive populations of the Mediterranean thistle, *Centaurea melitensis* L. Greenhouse-grown plants were compared for differences in quantitative traits between populations from three regions (the native range: Spain; and two invasive ranges: California and central Chile), and differentiation was found in several traits and their plasticities. Here, we undertake the preliminary step for a genetic analysis of those same greenhouse plants by testing microsatellite markers in *C. melitensis*, which have been developed for several closely related species. With microsatellite data, we can determine introduction history, group individuals into distinct populations, estimate allele frequencies within these populations, identify admixed individuals, compare population differentiation, determine the source populations of differentiated invasive populations, and compare genetic diversity among native and invasive populations by looking for a reduction (genetic bottleneck) or an increase (admixture) in the diversity of invasive populations. By comparing the percentage of total neutral genetic variation (F_{ST}) between native and invasive ranges, among regions, and among populations within each region with the divergence in quantitative traits between these populations (Q_{ST}; (Spitze 1993), we can determine if there has been (1) divergent selection due to adaptation to different environments (if $Q_{ST} > F_{ST}$), (2) stabilizing selection, where the trait is important in all environments ($Q_{ST} < F_{ST}$), or (3) if differentiation has been due to stochastic processes alone ($Q_{ST} = F_{ST}$). In this manner, F_{ST} is the null expectation, where differentiation only appears in neutral markers and any divergence from

F_{ST} in the differentiation in quantitative traits (Q_{ST}) must be due to selection (Merila and Crnokrak 2001, Keller and Taylor 2008).

Nuclear microsatellite markers provide the basis for the investigation of neutral genetic variation. To address the hypothesis that processes such as genetic bottlenecks, sampling effect, intraspecific hybridization, or adaptive evolution have produced populations of invasive genotypes that are differentiated from native populations of *C. melitensis,* we first need to develop appropriate microsatellite markers. In this study, we tested 22 primers for microsatellite markers that had previously been developed for four species that are closely related to *C. melitensis*. Our ultimate objective was to determine the relative contributions of stochastic demographic vs. adaptive evolutionary processes to the invasiveness of *C. melitensis.*

METHODS

We tested 22 primers on eight *C. melitensis* individuals to determine which, if any, of them amplify and are polymorphic for *C. melitensis*. The microsatellite markers that were tested had been previously developed for four closely related species: six markers had been developed for *Centaurea solstitialis* L. (Anderson and Luster 2005), the species most closely related to *C. melitensis* (Garcia-Jacas et al. 2006); nine microsatellite markers had been developed for *Centaurea diffusa* Lam. and *Centaurea stoebe* L. (Marrs et al. 2006); and seven microsatellite markers had been developed for *Centaurea corymbosa* Pourr. (Freville et al. 2000), which have also amplified successfully in *C. stoebe* (Table 4.1).

DNA was extracted from fresh leaf tissue in greenhouse-grown *C. melitensis* seedlings. The plants originated from three regions: the native range, Spain, and two invasive ranges, California and Chile, with two populations representing each region. There were eight *C. melitensis* individuals tested in total. Three individuals from both Spain and Chile were tested:

two from one population, and one from another. Two individuals from California were tested, each from a separate population.

Laboratory methods

DNA was extracted from the eight samples, and the 22 primers were prepared. Polymerase chain reactions (PCR) were performed in the lab to determine if any of the primers amplified a product. Primers that produced a product were prepared for genotyping. PCR products were genotyped at the UCLA Genotyping and Sequencing Core.

RESULTS

Six primer pairs were successfully amplified by PCR, producing a product large enough for genotyping (Table 4.1). These were 17E3, 42CM27, 28A7, CM15, 21CM36, and Csol04. Very small fragments were detected in Csol02 and 16G1, and they were also genotyped. Genotyping showed that none of the microsatellite markers amplified adequately for *C. melitensis*. The small fragments that amplified were approximately 100 base pairs in length, much smaller than the expected length of 250+. Furthermore, these loci were all monomorphic.

DISCUSSION

In order for microsatellites to be informative, they must be polymorphic fragments of a length of at least 250 base pairs. All of the eight potential fragments were too small for genotyping, and they were all monomorphic. Therefore, none of the 22 previously developed *Centaurea* species microsatellite markers were adaptable to *C. melitensis*. An alternative option is to study allozymes. However, allozymes can be even less polymorphic than microsatellites, and can underestimate heterozygosity (Freville et al. 2000). The fact that the *C. melitensis* microsatellites were all monomorphic implies that allozymes may be problematic. A more

efficient option with a better chance of detecting polymorphisms is next-generation sequencing (Chabot and Nigenda 2011).

Although we already have genetic differentiation data for *C. melitensis* as seen for quantitative traits in a common garden, neutral molecular markers will allow us to do two things: (1) to determine if the differences observed are due to adaptive evolution or to stochastic processes by comparison of neutral differentiation to quantitative trait differentiation, and (2) to examine demographic processes such as introduction history, genetic bottlenecks or admixture, assignment of invasive populations to their sources in the native range, differences in genetic variation between ranges, and population structuring due to adaptive evolution or genetic drift. Knowledge of these processes can expand our understanding of the invasion process and also inform management decisions such as the selection of appropriate biocontrol agents.

This experiment represents a first attempt at obtaining genetic data in this invasive species. This will be the first step in a discovery process of unearthing exploitable genetic markers that will inform demographic investigations, and ultimately management plans to control *C. melitensis* invasions.

Table 4.1. Microsatellite marker information for primers tested on *Centaurea melitensis*. All of the forward markers had the sequence GTAAAACGACGGCCAG pasted to the front to attach a color dye. Species indicates the species in which the marker was originally developed.

Species	Locus	Primer Sequence (5' - 3')	Repeat Motif
C. solstitialis	Csol01-F-M29	GACAAGTCACTGATCATCAC	TG
	Csol01-R	TGGGGTTTCCTTTCTTTTTG	
	*Csol02-F-M30	CGCCCCTTGTTCATAGGTATT	$(TA)_x(TG)_y$
	Csol02-R	ATGCTACCCGCTTGAACATC	
	Csol03-F-M31	GGCAATTCGTAGCATCCTCT	GA
	Csol03-R	GGAACCCGCTGCTCAACTA	
	**Csol04-F-M32	TCCTTGGCTCAAACACATGA	TTC
	Csol04-R	CTGAAATCCATTCCCATGCT	
	Csol05-F-M33	ACGCCCATCATATCAGTTCA	TAA
	Csol05-R	GCTTTATTGGAATGGCATCTTC	
	Csol06-F-M34	CCCTACAGTCCCTCAACCAA	$(ATG)_x \ldots (ATG)_y$
	Csol06-R	CATATCGTCCTCATTAGCAAGG	
C. stoebe	CM26-F-M13	GAAGGGCTACGAGGGTGTTC	$(TG)_9T(TA)_3$
	CM26-R	GAAGTGTTGTGCATTTCAATCTATT	
	**21CM36-F-M15	GCTATTAACAACTCCAAAATGAACAG	$(CA)_6(TA)_5(TG)_{16}$
	21CM36-R	CCTGCTCCAACAAGTTTCCTC	
	38CM22-F-M16	GGCTACATTAAGCTTATCCATTC	$(GA)_{12}(AA(GA)_8$
	38CM22-R	CTCGCATGTTATCCTCCCTC	
	**42CM27-F-M18	TGGGATATTCGTTGGTTTAGTTTT	$(TG)_{14}$
	42CM27-R	CCTCCCACTCCCGTTTGAC	
	**CM15-F-M19	GGAGGGCATGGGATTAAAGAGAT	$(GT)_9$
	CM15-R	TGGATGCATCGGTCTGGAAATA	
	25CM6-F-M20	ATGGGACATAAGATCCACAACAG	$(CA)_9$
	25CM6-R	TAATTCAGCATTCAAAAATTTAGAAGAC	
	CM17-F-M21	TACTTGGGCTTTTCGCTAATGAT	$(AC)_9$
	CM17-R	ACAAACGTGTTCCAGCAGCAG	
C. diffusa	CD9-F-M17	GGTCCCCATACTTTCAAGCTAATAAC	$(CA)_{17}$
	CD9-R	ATGCTTCCCTTCTCAATGTTTTCTCT	
	CD37-F-M14	AGGTGCACTTTCCTGTTCAAC	$(CA)_9$
	CD37-R	CAACCCAATAAGATTACTTCCACTTC	
C. corymbosa	12B1-F-M22	CACACTCACGCTCAGCATTC	$(TA)_{27}(GA)_{22}$
	12B1-R	CATCGTTTCCAAACTTCCTC	
	13B7-F-M23	TTTTCAAATATCTTGGTCAAT	$(AC)_{12}(AT)_5$

Table 4.1, continued

13B7-R	TGCTGCCATTAATTTTGTCA	
13D10-F-M24	GGAGGCATGCGAACTAAAAG	$(AC)_7ATAC(AT)_{10}$
13D10-R	CCGGTCTCATGAAAACAACT	
*16G1-F-M25	GTGCTCCGTCAGCAATCTTT	$(TA)_7(TG)_8$
16G1-R	GGATGGAGGTGGTGAGGTTA	
**17E3-F-M26	TGTTAGAAACACAAAAGCATGC	$(CA)_{11}$
17E3-R	TTTCCAAAATGAAGTTGAAGGC	
21D9-F-M27	CATATACACCCACGCACAGC	$(CA)_{20}$
21D9-R	GGTGCAGCAAGGAGAGGAC	
**28A7-F-M28	TTTCTATGCTGTTTGTTTTTGG	$(CA)_{16}$
28A7-R	CCCATACGTCGTCTTCCC	

**Indicates that the primer pair was successfully amplified by PCR and genotyped.
*Indicates that the primer pair was amplified, but very small in length. These were also genotyped.

LITERATURE CITED

Anderson, S. and D. Luster. 2005. Molecular Variation in *Centaurea Solstitialis* L. The ASA-CSSA-SSSA International Annual Meetings. Salt Lake City, UT.

Chabot, C. L. and S. Nigenda. 2011. Characterization of 13 microsatellite loci for the tope shark, *Galeorhinus galeus*, discovered with next-generation sequencing and their utility for eastern Pacific smooth-hound sharks (*Mustelus*). Conservation Genetics Resources 3:553-555.

Chun, Y. J., V. Le Corre, and F. Bretagnolle. 2011. Adaptive divergence for a fitness-related trait among invasive *Ambrosia artemisiifolia* populations in France. Molecular Ecology 20:1378-1388.

Dlugosch, K. M. and I. M. Parker. 2008. Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. Ecology Letters 11:1-9.

Freville, H., E. Imbert, F. Justy, R. Vitalis, and I. Olivieri. 2000. Isolation and characterization of microsatellites in the endemic species *Centaurea corymbosa* Pourret (Asteraceae) and other related species. Molecular Ecology 9:1671-1672.

Garcia-Jacas, N., T. Uysal, K. Romashchenko, N. Suarez-Santiago, K. Ertugrul, and A. Susanna. 2006. *Centaurea* revisited: A molecular survey of the *Jacea* group. Annals of Botany 98:741-753.

Keller, S. R. and D. R. Taylor. 2008. History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. Ecology Letters 11:852-866.

Lavergne, S. and J. Molofsky. 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proceedings of the National Academy of Sciences of the United States of America 104:3883-3888.

Leger, E. A. and K. J. Rice. 2003. Invasive California poppies (*Eschscholzia californica* Cham.) grow larger than native individuals under reduced competition. Ecology Letters 6:257-264.

Maron, J. L., M. Vila, R. Bommarco, S. Elmendorf, and P. Beardsley. 2004. Rapid evolution of an invasive plant. Ecological Monographs 74:261-280.

Marrs, R. A., R. A. Hufbauer, S. M. Bogdanowicz, and R. Sforza. 2006. Nine polymorphic microsatellite markers in *Centaurea stoebe* L. [subspecies *C.s. stoebe* and *C.s. micranthos* (S. G. Gmelin ex Gugler) Hayek] and *C. diffusa* Lam. (Asteraceae). Molecular Ecology Notes 6:897-899.

Merila, J. and P. Crnokrak. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology 14:892-903.

Spitze, K. 1993. Population structure in *Daphnia obtusa*: Quantitative genetic and allozymic variation. Genetics 135:367-374.

Stastny, M., U. Schaffner, and E. Elle. 2005. Do vigour of introduced populations and escape from specialist herbivores contribute to invasiveness? Journal of Ecology 93:27-37.

CHAPTER 5

Persistence of invasive *Centaurea melitensis* (Asteraceae) in a southern California chaparral

firebreak four years after fire

ABSTRACT

Native plants in most California ecosystems are adapted to fire, but within a particular fire regime. Changes in the fire regime can facilitate nonnative invaders and exclude native species. *Centaurea melitensis* is a common invader after fire in California chaparral. In the spring following a fire in Santa Barbara County, California, October 2007, we sampled the plant community in a burned firebreak. Four years later, we resampled this site, plus two adjacent sites with similar elevation, slope and aspect. The second site was burned but with no firebreak, and the third site had no firebreak and was unburned. The overall goal of this study was to test the hypothesis that fire and other disturbances promote the colonization of nonnative species, and these nonnatives persist after fire in disturbed areas. We quantified population density and relative cover of *C. melitensis,* and characteristics of the associated community. After four years within the firebreak, the mean relative cover of *C. melitensis* decreased from 72% to 28%, and the cover of other nonnative annual forbs decreased, but the cover of annual grasses increased. Both of the burned sites had more than 25% cover of *C. melitensis,* and the unburned site had only 5% cover. *Avena* species occurred most commonly in the two sites with no firebreak, both burned and unburned. The burned site with no firebreak had the greatest native species richness, while the unburned site with no firebreak had the lowest. The results of our study suggest that the recruitment of *C. melitensis,* along with some native species, is promoted by fire. However, in the absence of additional disturbance by firebreaks, its persistence is limited by competition from introduced Mediterranean annual grasses.

INTRODUCTION

Fire is a natural process in California ecosystems (Keeley et al. 2012). In California forests, woodlands, shrublands, and grasslands, fire has been a presence for thousands of years and an evolutionary force on the resident plant species (Pausas et al. 2006, Keeley et al. 2012). For California grasslands in particular, fire has been so important that it is one of the most significant factors shaping their current distribution and composition (Keeley 2002). From prehistoric infrequent lightning-ignited fires, to controlled burning by Native Americans, to the increasing frequency of human-influenced fires since the arrival of the Europeans to the present, fire has in some sites permanently converted woodlands and shrublands to grasslands and influenced the composition of the resident species in both converted and natural grasslands (Keeley 2002). Most significantly, since the arrival of the Europeans, California grasslands have become dominated by nonnative, invasive plants, and fire has played a critical role in this invasion (Keeley et al. 2011).

Native plants in most California ecosystems are adapted to fire, but they are so within a particular regime, which includes the range of frequency, seasonality, and intensity. Changes in the fire regime can facilitate the recruitment of nonnative invaders (Hobbs and Huenneke 1992), support their persistence (Haidinger and Keeley 1993), and engender the exclusion of native species (Keeley and Brennan 2012). With high fire frequency, chaparral can be type converted to an annual grassland dominated by nonnative herbaceous species (Haidinger and Keeley 1993, Syphard et al. 2006). Furthering the exclusion of natives and persistence of nonnatives, the domination of Mediterranean annual grasses is itself a driver of change in the fire regime (D'Antonio and Vitousek 1992, Brooks et al. 2004). Annual grasses, with their high surface area and dry fall vegetation produce a highly flammable ignition source, and sometimes increase the fire intensity potential, resulting in a feedback loop known as the grass/fire cycle, which results

93

in permanent type conversion to Mediterranean annual grassland (D'Antonio and Vitousek 1992, Dukes and Mooney 2004).

Centaurea melitensis L. (Asteraceae) is a weedy annual forb that is native to the Mediterranean Basin and an aggressive invader in California shrublands and grasslands (Moroney and Rundel 2012). It is commonly observed after fire in California chaparral (Keeley et al. 2005a). In October 2007, the Sedgwick Fire burned 710 acres through a chaparral/grassland mosaic on the Sedgwick Reserve in the Santa Ynez Valley of Santa Barbara County, California, providing an opportunity to quantify fire and disturbance impacts on invasive and native species in chaparral under differing disturbance treatment conditions. The objectives of this study were first, to quantify the progression of *C. melitensis* and the associated plant community in a burned firebreak directly following the fire and four years later. Our second objective was to compare the presence of *C. melitensis* and the associated community composition four years after fire within a burned firebreak to burned sites outside the firebreak, and to sites with no disturbance. The fire gave us an opportunity to address these objectives and look at the relationship of the disturbance regime to patterns of native and invasive dominance by testing the hypothesis that fire and other disturbances promote the colonization of nonnative species, and these nonnatives persist after fire in disturbed areas. Of particular interest in these questions was the comparative behavior of nonnative annual grasses with the forb, *C. melitensis*.

METHODS

Study species

Centaurea melitensis is one of the most common nonnative plants in the first five years after fire and other disturbance in chaparral and coastal sage scrub of southern California, with an average density of >16,000 individuals ha^{-1} in chaparral sites and >285,000 individuals ha^{-1} in

sage scrub sites (Keeley et al. 2005a). It is a problem pest in California because it threatens the health of livestock and the persistence of native plants and animals (Di Tomaso and Gerlach 2000, Moroney et al. 2011). It has been in California since at least 1797 (Hendry 1931).

<u>Study sites</u>

We sampled three sites located in a grassland savanna/chaparral mosaic at the Sedgwick Reserve, University of California Natural Reserve System (N34°42'47.7" W120°02'00.7"). The area is on the Paso Robles Formation, and all three of the sites were on a Shedd silty clay loam soil classification. The two years surveyed, 2008 and 2012, had 739 mm and 577 mm of precipitation, respectively, during the rainy season (Lisque weather station, Sedgwick Reserve, 34.72449 N, 120.0635 W).

The first of the three sites (burned firebreak) was located along a ridge that was both burned and bulldozed during the 2007 Sedgwick Fire. A bulldozer cleared a one-blade width (12 feet) firebreak over a ridgeline after the fire burned through the area to create access to the rest of the fire. This firebreak had existed before the Sedgwick fire, but the original date of construction is unknown (Scot Alderete, personal communication). In the spring following the fire, we sampled the vegetation on the firebreak in association with a previous study that compared the density and dominance of *Centaurea melitensis* L. in its native and invasive ranges (Moroney and Rundel 2012).

The second site (burned no firebreak) was located along the same ridge and adjacent to the burned firebreak. This site burned in the Sedgwick fire but was not disturbed by a firebreak. The third site (unburned no firebreak) was on the ridge parallel and adjacent to the burned firebreak, with a similar elevation, slope and aspect. This site was not burned nor disturbed in the Sedgwick Fire.

Sampling

In 2008, sampling was conducted in the burned firebreak. In June 2012, the same firebreak was resampled, and the two additional sites were also sampled as follows. Population density of *C. melitensis* was determined using two-stage systematic sampling (Elzinga et al. 1998). Ten transects were placed in each site, perpendicular to the ridgeline. The transects were placed at randomly selected points along a baseline that followed the ridgeline. A series of 1 m x 0.2 m plots were then placed at regular intervals along each transect, starting at a randomly selected point. *Centaurea melitensis* individuals were counted within each plot, and plot totals were averaged per transect. We focused on the density and relative cover of *C. melitensis*, and we characterized the associated community. Along with *C. melitensis*, the percent cover of each of the following groups was measured in the plots: annual grasses, litter, nonnative species other than *C. melitensis* and annual grasses, native species, bare ground, and rock. All taxa encountered in the plots were recorded.

Statistical analyses

We used negative binomial regressions to compare the effect of site on the density of *C. melitensis* and the relative cover of the following variables: *C. melitensis*, annual grasses, nonnative plants, native plants, and native richness. The negative binomial was used because the data were overdispersed; the variance was larger than the mean. Relative cover was calculated as the percentage of the target group divided by the total percentage of the plot covered by vegetation.

We compared the proportions of the plots covered with litter, rock and bare ground using generalized linear models with a logit link and a binomial distribution. All statistical analyses were done using Stata statistical software (Stata, version 12.1. Statacorp, College Station, TX).

RESULTS

A total of 24 species occurred in the plots sampled, representing 16 native species, and 8 nonnative species. All of the nonnative plants were annuals, with five annual forb species and three annual grass species.

Centaurea melitensis was present in 74% of the plots, and was the species that occurred most commonly overall. *Centaurea melitensis* occurred most commonly in the 2008 firebreak, *Bromus* species occurred most commonly in the 2012 firebreak, and *Avena* species occurred most commonly in the two sites with no firebreak, both burned and unburned. The burned site with no firebreak had the greatest native species richness, while the unburned site with no firebreak had the lowest. Although the burned no firebreak site had the highest native species richness, these species were mostly annual forbs, with one perennial grass occurrence, one geophyte, and no shrubs. The firebreak in both years had mostly native perennial species, with only one annual forb appearance in 2008. The unburned site with no firebreak had only one occurrence each of native forbs, shrubs, and perennial grasses (Table 5.1).

Firebreak Between Years

After four years in the firebreak, the density of *C. melitensis* remained the same (Table 5.2, Fig. 5.1). However, relative cover of *C. melitensis* decreased, but not significantly so (Table 5.2, Fig. 5.2). Meanwhile, relative cover of annual grasses increased significantly (Table 5.2, Fig. 5.2). The decrease in relative cover of all nonnatives was not significant (Table 5.2, Fig. 5.2), nor was the decrease in relative native richness (Table 5.2, Fig. 5.2). Relative cover of natives increased, but not significantly so (Table 5.2). The percent cover of litter increased significantly (Table 5.3, Fig. 5.3), while the percentage of rock and bare ground decreased, but not significantly so (Table 5.3, Fig. 5.3). The number of plots with native shrubs, geophytes, and

97

annual forbs decreased in four years, but the number of plots with native perennial grasses increased (Table 5.1).

<u>Sites Within Year</u>

In 2012, the density of *C. melitensis* was the greatest in the burned no firebreak site, and the lowest in the unburned site (Fig 5.1). The only significant difference in the density of *C. melitensis* was that the unburned site had lower density than both of the other sites (Table 5.2, Fig. 5.1). Relative cover of *C. melitensis* was greater in burned areas, both with and without a firebreak, than it was in the unburned area, and significantly greater in the burned firebreak (Table 5.2, Fig. 5.2). Relative cover of annual grass was greater in the two no firebreak sites than in the firebreak, but not significantly so (Table 5.2, Fig. 5.2). There were no significant differences in the relative cover of nonnative species or in native richness among the sites (Table 5.2, Fig. 5.2). Litter was significantly reduced in the firebreak compared to in the burned no firebreak site (Table 5.3, Fig. 5.3). There was significantly more rock and bare ground on the surface of the firebreak than in both sites without a firebreak (Table 5.3, Fig. 5.3). The unburned site had fewer native species than both of the other two sites (Table 5.1). The three sites all had the same number (7) of nonnative species (Table 5.1). Every plot in the sites with no firebreak contained *Avena* species, while only three plots in the firebreak contained *Avena*. However, only 30% of the plots in the unburned site contained *C. melitensis*, while in both burned sites (with and without a firebreak) 85% had *C. melitensis* (Table 5.1). All of the native species found in the firebreak four years after fire were perennials, with four shrub species and one perennial grass (Table 5.1). However, in the burned site without a firebreak, all of the native species were annual forbs except for a perennial grass. In the unburned site, there was one annual forb, one shrub, and one perennial grass (Table 5.1).

98

The ratio of native to nonnative relative cover increased slightly within the firebreak, despite the slight decline in native species richness. The most common natives were shrub seedlings in 2008, but the most common group in 2012 was perennial grasses. None of the shrub species that appeared in 2008 were lost by 2012. The number of shrub species found within the firebreak increased from three to four. The reduction in native species richness was due to the loss of the geophytes and an annual forb, which might have been a temporary retreat due to the reduced water availability in 2012. Therefore, the overall native species recovery was positive, with only transitory losses in species and an increase in cover.

DISCUSSION

The differences in the three treatment sites in 2012 were most dramatic in the relative cover and frequency of *C. melitensis*. While both of the burned sites had more than 25% cover of *C. melitensis*, the 2012 unburned site had only 5% cover. This suggests that the disturbance caused by fire, regardless of the additional clearing by bulldozer, opens colonization sites sufficiently for *C. melitensis* to establish. The reduced cover of *C. melitensis* in the unburned site might be linked to the higher cover of annual grasses and its associated litter, as their relative covers are inversely related to that of *C. melitensis*. Annual grasses germinate and grow tall earlier in the season than *C. melitensis*, possibly blocking out light and preempting germination potential. Litter accumulation may also suppress germination by limiting light and changing the temperature and moisture availability on the soil surface.

Of the three 2012 sites, the firebreak had the lowest relative cover of annual grasses, while the relative cover of *C. melitensis* was the highest. Furthermore, this site had the lowest total nonnative relative cover and the highest native cover. This may be related to the depth of the seed banks of annual grasses and *C. melitensis*. Smaller seeds are generally shallower in the

soil than heavier seeded species, and thus more vulnerable to mortality from fire (Bond et al. 1999). If the heavier, more compact *C. melitensis* seeds are buried deeper in the soil, while the lighter grass seeds stay nearer the soil surface, then both the fire intensity and the depth of the bulldozer blade might have been factors in the reduction of annual grasses and the persistence of *C. melitensis*. High intensity, warm-season fires can kill annual grass seeds on the surface of the soil and increase the cover of native species (Meyer and Schiffman 1999). Furthermore, the bulldozer might have cleared surface seeds, exposing the deeper *C. melitensis* seeds to the surface. Alternatively, clearing the litter may have been the more important effect of the bulldozer and the fire. With germination interference removed, *C. melitensis* and native seeds in the seed bank would have had an opportunity to recruit. The disturbances of fire and bulldozing might reduce annual grasses and recover forbs and shrubs in the short term as long as the seed bank is deep enough and remains intact.

Within the firebreak, four years after the disturbances of fire and bulldozing, the mean relative cover of *Centaurea melitensis* decreased from 72% to 28%. Although the decrease in relative cover was not statistically significant because of the high variance in the sample plots, the pattern seems ecologically significant. Furthermore, the cover of other nonnative annual forbs also decreased. In contrast, the cover of annual grasses and their associated litter increased in the firebreak in four years from almost zero to a level not statistically different from the other two sites. This lack of difference suggests that even after severe disturbances such as bulldozing and fire reduce the cover of annual grasses, these grasses quickly regain dominance by competitively displacing annual forbs. Despite the decrease in relative cover of *C. melitensis*, the density remained the same. The same number of individuals germinated and survived, but they were smaller in size. This could be due to the reduction in water availability in 2012 compared to

2008, or to competition with annual grasses that were not present in 2008. Most likely, both factors had an effect on the reduced size of *C. melitensis* individuals.

Woody plant canopy closure (i.e., native shrubs) has been shown to be the most important direct factor in explaining alien plant dominance in southern California chaparral and sage scrub sites within five years after fire (Keeley et al. 2005a). After four years in the firebreak, none of the shrubs were large enough to close the canopy sufficiently to shade out annual plants. The slow recovery of woody plants in this site may have been due to fire history or other disturbance history, including bulldozing, or aridity, or a combination of these factors (Keeley et al. 2005a). Additionally, the only site with substantial woody plant recruits four years after fire was the bulldozed firebreak, with no shrubs in the burned no firebreak site, and the unburned site supporting only a few small individuals of one shrub species (*Hazardia squarrosa*).

The use of firebreaks has been controversial for several reasons (Agee et al. 2000). The effects of firebreaks, both on changing fire behavior and on impacts to the native community, are only recently being studied. Many of the firebreaks constructed by the federal government, which have cost several billion dollars over the last decade, may have limited value in protecting the wildland-urban interface (Schoennagel et al. 2009). Many firebreaks rarely intersect with fire, and firebreaks often do little to impede fire spread unless firefighters are present (Syphard et al. 2011). The disturbance effects of the construction and maintenance of firebreaks can promote nonnative plant colonization (D'Antonio et al. 1999, Merriam et al. 2006). In addition, fire is a well-known agent of invasion (Hobbs and Huenneke 1992). The interaction of effects of the disturbance from firebreaks with the effects of the disturbance from fire would be expected to promote the colonization of nonnative annual species (Merriam et al. 2006). However, our

results suggest that relative to undisturbed sites, sites that have been disturbed by fire and firebreaks might promote the recruitment of more native species, at least in the first several years after fire, and in sites where Mediterranean annual grasses are present.

For other *Centaurea* species, fire frequency can have important impacts on density. Prescribed burns three years in a row in a northern California site infested with *C. solstitialis* resulted in a more than 99% reduction in seed bank and seedling density of *C. solstitialis*, and increased native species richness (DiTomaso et al. 1999). However, despite the reduction during fire years, *C. solstitialis* returned to dominance within four years after the burning was stopped (Kyser and DiTomaso 2002).

Fire frequency is an important determinant of the relative success of native versus nonnative species in chaparral. Species diversity is typically highest in the first few years following fire (Keeley and Fotheringham 2003) at sites that burn at intermediate fire frequencies. Native annuals dominate after fire in sites not burned for several decades, but nonnative annuals dominate after fire in sites that have burned repeatedly (Haidinger and Keeley 1993), and in postfire stands of chaparral that had previously not burned within the outside range of historical fire regimes (>100 years) (Keeley et al. 2005b). However, in our study, native richness was lowest in the undisturbed (unburned no firebreak) site, suggesting the persistent dominance of nonnative annual grasses after some past disturbance other than fire. The recorded fire history for the Sedgwick Reserve begins in 1912, and there have been no fires recorded on the reserve other than the 2007 Sedgwick Fire (Scot Alderete, personal communication). Prior to the establishment of the Natural Reserve on the site, there was a history of grazing. In the absence of fires at least in the last 100 years, perhaps the disturbance from historical grazing has been a factor in determining the present composition of the community, and the domination of annual grasses.

The availability of different areas with different management regimes presented an opportunity to examine factors affecting the invasion of *C. melitensis*. However, a major limitation of this study was that we were able to study only one site with each condition. It is possible that the prior histories of these sites created differences that are confounded with fire history. This lack of replication may have emphasized any underlying heterogeneity in the sites that in a replicated study would be minimized.

In conclusion, this study process has explored how fire affects species composition and in particular invasion by *C. melitensis*. Post-fire recovery is a successional process, but with the introduction of altered disturbance regimes, both by fire and firebreak construction and maintenance, that process becomes unpredictable. Multi-year and multi-site monitoring of the community is important to assess the fate of early colonizers. The results of our study suggest that the recruitment of *C. melitensis*, along with some native species, is promoted by fire. But in the absence of additional disturbance by firebreaks, its persistence is limited by competition, not from woody native annuals, but from another group of nonnative invaders, Mediterranean annual grasses.

Table 5.1. Taxa present in the sample plots. Percentage of plots each taxon was found in per site and percentage of transects per site in parentheses.

	Species	Life-form	2008 Firebreak	2012 Firebreak	2012 No FB Burned	2012 No FB Unburned
Native	*Adenostoma fasciculatum*	S	20(30)	5(10)	0	0
	Amsinckia sp.	AF	0	0	5(10)	15(20)
	Artemisia californica	S	10(20)	20(30)	0	0
	Calochortus sp.	G	25(40)	0	0	0
	Ceanothus sp.	S	35(50)	5(10)	0	0
	Daucus pusillus	AF	0	0	10(10)	0
	Dichelostemma capitatum	G	10(20)	0	0	0
	Galium sp.	AF	5(10)	0	0	0
	Hazardia squarrosa	S	0	0	0	20(30)
	Deinandra fasciculata	AF	0	0	25(40)	0
	Lupinus sp.	AF	0	0	30(50)	0
	Navarretia sp.	AF	0	0	30(50)	0
	Plantago erecta	AF	0	0	10(20)	0
	Salvia mellifera	S	0	5(10)	0	0
	Sisyrinchium bellum	G	0	0	5(10)	0
	Stipa sp.	PG	15(30)	40(50)	5(10)	15(20)
	Native species richness		**6**	**5**	**8**	**3**
Nonnative	*Anagallis arvensis*	AF	70(100)	35(50)	25(40)	0
	Avena sp.	AG	0	15(30)	100(100)	100(100)
	Bromus sp.	AG	5(10)	95(100)	85(100)	90(100)
	Carduus pycnocephalus	AF	0	5(10)	0	10(20)
	Centaurea melitensis	AF	95(100)	85(90)	85(100)	30(40)
	Erodium sp.	AF	35(50)	45(60)	60(80)	30(40)
	Hordeum murinum	AG	0	25(30)	25(40)	30(50)
	Lactuca serriola	AF	0	0	5(10)	25(50)
	Nonnative species richness		**4**	**7**	**7**	**7**
	Total species richness		**10**	**12**	**15**	**10**

AF=Annual forb
S=Shrub
G=Geophyte
PG=Perennial grass
AG=Annual grass

Table 5.2. Six negative binomial regression models for the density of *C. melitensis*, and the relative cover of *C. meltiensis*, annual grasses, nonnative plants, native plants, and relative native richness. The reference site is Firebreak 2008.

Source	Coefficient	X^2	P
C. melitensis density			
Firebreak 2012	0.677	2.528	0.112
Burned no firebreak	1.023	5.808	*
No burn no firebreak	-2.059	17.556	***
Constant	2.497	67.24	***
Relative cover of C. melitensis			
Firebreak 2012	-0.974	2.25	0.134
Burned no firebreak	-1.041	2.56	0.109
No burn no firebreak	-2.646	16.565	***
Constant	-0.287	0.372	0.543
Relative cover of annual grasses			
Firebreak 2012	4.323	68.228	***
Burned no firebreak	4.862	86.676	***
No burn no firebreak	5.296	102.82	***
Constant	-5.678	221.117	***
Relative cover of nonnative plants			
Firebreak 2012	-0.324	6.554	**
Burned no firebreak	-0.086	0.462	0.495
No burn no firebreak	-0.114	0.81	0.366
Constant	-0.089	0.941	0.331
Relative cover of native plants			
Firebreak 2012	1.383	3.803	0.052
Burned no firebreak	0.636	0.81	0.371
No burn no firebreak	0.771	1.188	0.278
Constant	-2.469	22.944	***
Relative native richness			
Firebreak 2012	-0.042	0.006	0.933
Burned no firebreak	0.125	0.068	0.798
No burn no firebreak	-0.853	3.028	0.082
Constant	0.354	104.448	***

*$P<0.05$
**$P<0.01$
***$P<0.001$

Table 5.3. Three generalized linear models with logit links and binomial distributions for percent cover of non-vegetation (litter, rock, and bare ground) in the quadrats. The reference site is Firebreak 2008.

Source	Coefficient	$X2$	P
Percent cover of litter			
Firebreak 2012	1.274	19.448	***
Burned no firebreak	2.023	70.224	***
No burn no firebreak	1.995	56.55	***
Constant	-2.17	201.072	***
Percent cover of rock			
Firebreak 2012	-0.766	3.1	0.078
Burned no firebreak	-17.068	1971.36	***
No burn no firebreak	-17.068	1971.36	***
Constant	-3.178	223.503	***
Percent cover of bare ground			
Firebreak 2012	-0.15	0.176	0.677
Burned no firebreak	-1.885	15.288	***
No burn no firebreak	-2.705	28.837	***
Constant	-0.447	3.46	0.062

*$P<0.05$
**$P<0.01$
***$P<0.001$
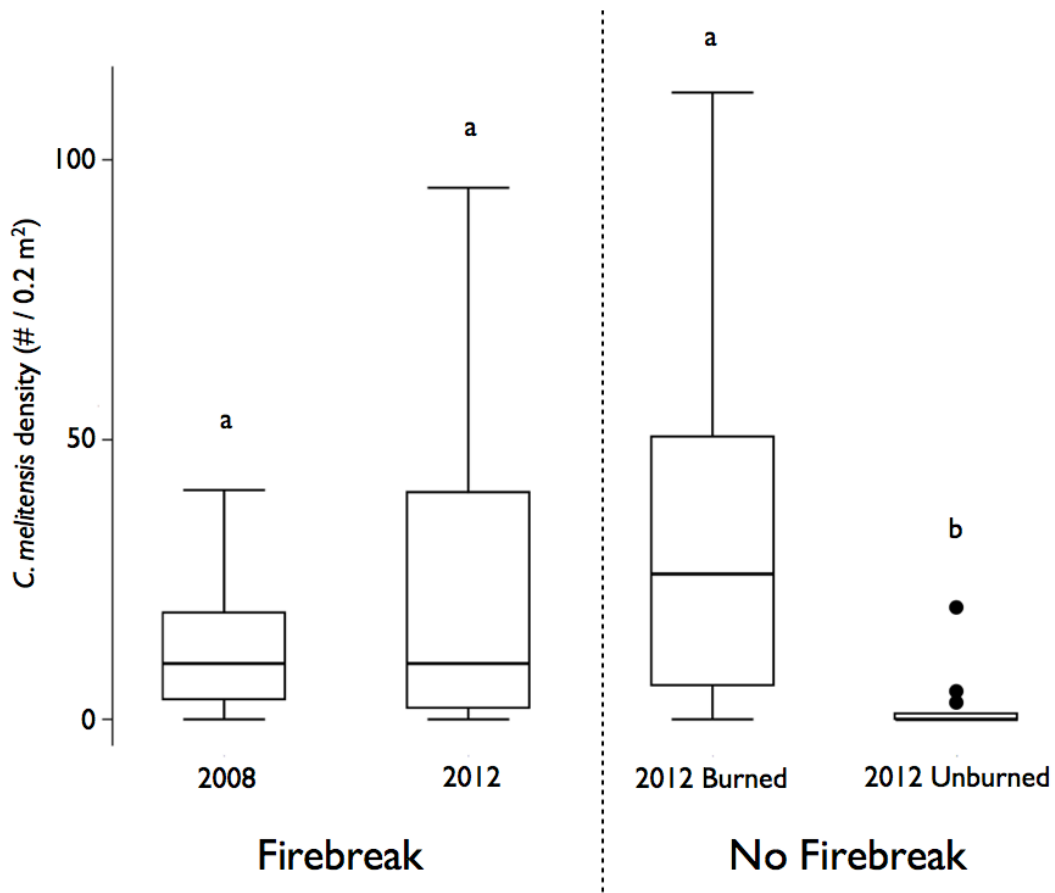
Figure 5.1. Density box plots of *C. melitensis* measured in four sites at the Sedgwick Reserve. The two boxes on the left represent sites within a bulldozed firebreak, with data for density measured in 2008 and 2012. The two boxes on the right represent two sites adjacent to the firebreak, but not bulldozed, with data for density measured in 2012 from one burned site and one unburned site.
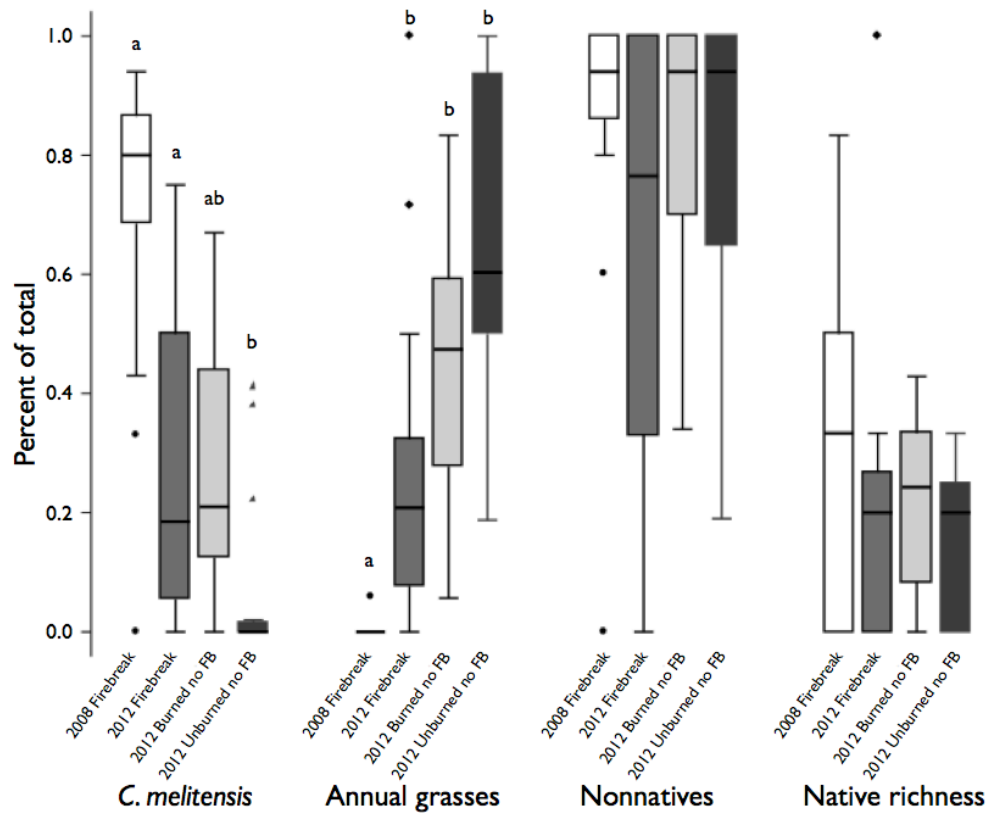
Figure 5.2. Box plots of the relative cover of *C. melitensis*, annual grasses, all nonnative plants, (percent cover/percentage of total vegetation cover) and native richness (native richness/total richness) compared in four sites within the Sedgwick Reserve.

108
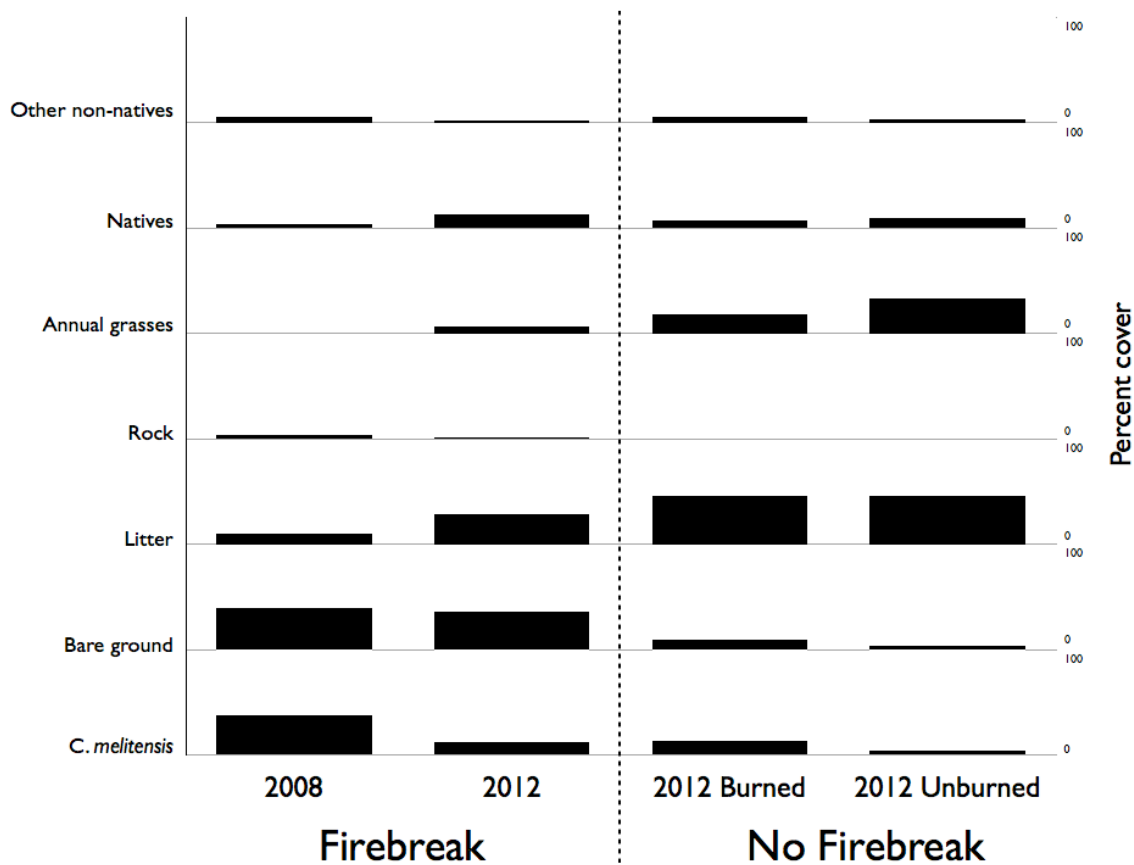
Figure 5.3. The percent cover of seven elements in plots in four sites. The two boxes on the left represent sites within a bulldozed firebreak, with data measured in 2008 and 2012. The two boxes on the right represent two sites adjacent to the firebreak, but not bulldozed, with data measured in 2012 from one burned site and one unburned site. Each bar represents the mean proportion of the plots that were covered by *C. melitensis*, bare ground, litter, rock, annual grasses, native plants, and nonnative plants other than *C. melitensis* and annual grasses.

LITERATURE CITED

Agee, J. K., B. Bahro, M. A. Finney, P. N. Omi, D. B. Sapsis, C. N. Skinner, J. W. van Wagtendonk, and C. P. Weatherspoon. 2000. The use of shaded fuelbreaks in landscape fire management. Forest Ecology and Management 127:55-66.

Bond, W. J., M. Honig, and K. E. Maze. 1999. Seed size and seedling emergence: an allometric relationship and some ecological implications. Oecologia 120:132-136.

Brooks, M. L., C. M. D'Antonio, D. M. Richardson, J. B. Grace, J. E. Keeley, J. M. DiTomaso, R. J. Hobbs, M. Pellant, and D. Pyke. 2004. Effects of invasive alien plants on fire regimes. BioScience 54:677-688.

D'Antonio, C. M., T. L. Dudley, and M. Mack. 1999. Disturbance and biological invasions: direct effects and feedbacks. *in* L. R. Walker, editor. Ecosystems of the world 16. Ecosystems of disturbed ground. Elsevier Science B.V., Amsterdam.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass-fire cycle, and global change. Annual Review of Ecology and Systematics 23:63-87.

Di Tomaso, J. M. and J. D. Gerlach, Jr. 2000. Invasive plants of California's wildlands. University of California Press, Berkeley, California, USA.

DiTomaso, J. M., G. B. Kyser, and M. S. Hastings. 1999. Prescribed burning for control of yellow starthistle (*Centaurea solstitialis*) and enhanced native plant diversity. Weed Science 47:233-242.

Dukes, J. S. and H. A. Mooney. 2004. Disruption of ecosystem processes in western North America by invasive species. Revista Chilena de Historia Natural 77:411-437.

Haidinger, T. L. and J. E. Keeley. 1993. Role of high fire frequency in destruction of mixed chaparral. Madroño 40:141-147.

Hendry, G. W. 1931. The adobe brick as a historical source. Agricultural History 5:110-127.

Hobbs, R. J. and L. F. Huenneke. 1992. Disturbance, diversity, and invasion: implications for conservation. Conservation Biology 6:324-337.

Keeley, J. E. 2002. Native American impacts on fire regimes of the California coastal ranges. Journal of Biogeography 29:303-320.

Keeley, J. E., M. Baer-Keeley, and C. J. Fotheringham. 2005a. Alien plant dynamics following fire in Mediterranean-climate California shrublands. Ecological Applications 15:2109-2125.

Keeley, J. E., W. J. Bond, R. A. Bradstock, J. G. Pausas, and P. W. Rundel. 2012. Fire in Mediterranean Ecosystems: Ecology, Evolution, and Management. Cambridge University Press, New York, New York.

Keeley, J. E. and T. J. Brennan. 2012. Fire-driven alien invasion in a fire-adapted ecosystem. Oecologia 169:1043-1052.

Keeley, J. E. and C. J. Fotheringham. 2003. Species-area relationships in Mediterranean-climate plant communities. Journal of Biogeography 30:1629-1657.

Keeley, J. E., J. Franklin, and C. M. D'Antonio. 2011. Fire and invasive plants on California landscapes. Pages 193-221 *in* D. McKenzie, C. Miller, and D. A. Falk, editors. The Landscape Ecology of Fire. Springer, New York, New York.

Keeley, J. E., A. H. Pfaff, and H. D. Safford. 2005b. Fire suppression impacts on postfire recovery of Sierra Nevada chaparral shrublands. International Journal of Wildland Fire 14:255-265.

Kyser, G. B. and J. M. DiTomaso. 2002. Instability in a grassland community after the control of yellow starthistle (*Centaurea solstitialis*) with prescribed burning. Weed Science 50:648-657.

Merriam, K. E., J. E. Keeley, and J. L. Beyers. 2006. Fuel breaks affect nonnative species abundance in Californian plant communities. Ecological Applications 16:515-527.

Meyer, M. D. and P. M. Schiffman. 1999. Fire season and mulch reduction in a California grassland: a comparison of restoration strategies. Madroño 46:25-37.

Moroney, J. R. and P. W. Rundel. 2012. Abundance and dispersion of the invasive Mediterranean annual, *Centaurea melitensis* in its native and non-native ranges. Biological Invasions:10.1007/s10530-10012-10302-10531.

Moroney, J. R., P. M. Schiffman, and C. A. Brigham. 2011. Invasive European annual plants impact a rare endemic sunflower. Madroño 58:69-77.

Pausas, J. G., J. E. Keeley, and M. Verdu. 2006. Inferring differential evolutionary processes of plant persistence traits in Northern Hemisphere Mediterranean fire-prone ecosystems. Journal of Ecology 94:31-39.

Schoennagel, T., C. R. Nelson, D. M. Theobald, G. C. Carnwath, and T. B. Chapman. 2009. Implementation of National Fire Plan treatments near the wildland-urban interface in the western United States. Proceedings of the National Academy of Sciences of the United States of America 106:10706-10711.

Syphard, A. D., J. Franklin, and J. E. Keeley. 2006. Simulating the effects of frequent fire on southern California coastal shrublands. Ecological Applications 16:1744-1756.

Syphard, A. D., J. E. Keeley, and T. J. Brennan. 2011. Comparing the role of fuel breaks across southern California national forests. Forest Ecology and Management 261:2038-2048.

CHAPTER 6

Climatic niche modeling to predict *Centaurea melitensis* invasion

ABSTRACT

Species distribution models are valuable tools that can be used to identify geographical areas with environmental conditions that are suitable for the colonization and invasion of nonnative species, and for the analysis of changes in the niche of invading populations. We used Maxent to construct bioclimatic species distribution models to predict areas of suitable habitat and potential invasion for the Mediterranean Basin annual forb, *Centaurea melitensis* L. (Asteraceae) in California, and we tested the hypothesis that *C. melitensis* has undergone a niche shift in the invasive range. We constructed two models, each at two thresholds, the first based on occurrences in Spain, representing the native range, and projected onto California, and the second based on occurrences in California, and projected onto Spain. The models performed well, with AUC's of more than 0.70, but at the most conservative threshold, the model trained in Spain successfully predicted only 57% of occurrence points in California, and the model trained in California only predicted 2% of points in Spain. The results indicate that the niche for invasive populations has shifted by expanding into more coastal habitats than in the native range. These results can focus directed study of the mechanisms of the niche shift by closer examination of the plants and environments that occur in coastal areas. For example, testing for the effects of enemy release to potentially identify candidate populations for a biocontrol program. There are substantial areas of suitable habitat with no occurrence records in the Mojave Desert, and on the eastern side of the Peninsular, Transverse, Sierra Nevada Ranges, and Cascade Ranges. To prevent further spread of invasion of *C. melitensis*, managers of unoccupied areas of high suitability must enact risk screening, early detection, and eradication plans.

INTRODUCTION

Invasive species pose one of the most pressing problems threatening biodiversity globally (Wilcove et al. 1998, Sala et al. 2000, Olden et al. 2004). They can have competitive impacts on native species (Brooks 2000) affect disturbance regimes (Mack and D'Antonio 1998), and cause changes in ecosystem processes (Vilà et al. 2011). Ultimately they result in global economic damages valued at more than $1 trillion per year (Pimentel et al. 2001). Only a small portion of introduced species establish and become invasive (Richardson and Pyšek 2006), but once they do, eradication is very difficult and in many cases impossible (Lodge et al. 2006, Bhagwat et al. 2012). Given the extremely high costs of control once invasive species are established, the best strategy to reduce the costs and impacts of harmful biological invasions is to prevent them from becoming established (Leung et al. 2002, Lodge et al. 2006, Finnoff et al. 2007).

The identification of geographical areas with environmental conditions that are suitable for the colonization and invasion of nonnative species is a valuable tool to prevent species invasions (Lodge et al. 2006). Species distribution models can characterize the climatic niche of a species in its native range and project that niche onto potential invasive ranges to predict areas climatically suitable for habitation (Peterson 2003, Thuiller et al. 2005, Jeschke and Strayer 2008). In the case of species distribution models, the niche is defined in terms of only the bioclimatic niche, not the overall niche, which would also include substrate properties and biotic interactions. The bioclimatic niche, although not encompassing all factors comprising a niche, provides a reasonable approximation of the distribution of a species (Araujo and Peterson 2012).

The niche is defined as an *n*-dimensional hypervolume, a multivariate niche space that includes all of the environmental requirements of a species (Hutchinson 1957). The fundamental niche includes the physiological requirements of the species and the range of environmental conditions, including climatic and substrate requirements, in which the species can survive, but

does not include the effects of interactions with other species. Biotic interactions and dispersal limitations constrain the fundamental niche to the actual distribution of the species, or the realized niche. Species with a broad fundamental niche could colonize a wide range of environments, possibly well outside their realized niche in their native range. For invasive species, this would be advantageous because in new ranges there might be novel climate conditions that the species is preadapted to (Jackson and Overpeck 2000).

Species distribution models for predicting invasions are especially useful between regions of analogous climate types (Petitpierre et al. 2012). The mediterranean type climate regions of the world provide a system that lends itself to the application of species distribution models. Biotic exchanges among these regions are common (Jimenez et al. 2008), and inter-mediterranean invasions can have bigger impacts on native species richness than invasions among areas of dissimilar climate type (Gaertner et al. 2009).

The objectives of this study were first to predict areas of suitable habitat and potential invasion for the Mediterranean Basin annual forb, *Centaurea melitensis* L. (Asteraceae) in California; and second, to test the hypothesis that *C. melitensis* has undergone a niche shift in the invasive range. There are a number of reasons to suggest that *C. melitensis* might be expected to have undergone a niche shift from its range in Spain to its current expansion in California based on prior evidence that invasive populations have undergone rapid evolution (Chapter 2). Not only has this species undergone rapid evolution in several quantitative traits and their plasticities, but individuals from both the native and invasive ranges seem to possess a phenotypically plastic general purpose genotype that could allow the occupancy of a wide range of environments. Furthermore, *C. melitensis* occurs at higher abundances in California than in the native range (Moroney and Rundel 2012). If the niche has shifted in the invasive range, occurrences might be

found outside modeled suitable climate areas on a model trained in the native range and projected onto California. Alternatively, propagule limitation might have constrained the occupancy of *C. melitensis* in the full range of suitable habitats, which is possible for an invasion that has not yet reached equilibrium. Identification of either of these scenarios would be applicable to invasion prevention management strategies for *C. melitensis*, which might include biocontrol efforts on populations found outside of predicted habitats, assuming that niche expansion is due to enemy release, and dispersal regulation in areas of unoccupied suitable habitat.

METHODS

Data collection

Centaurea melitensis occurrence records for the native range were obtained from the Global Biodiversity Information Facility (http://www.gbif.org; accessed April 2009). The native range includes much of the Mediterranean Basin (Tutin et al. 1976), however I used only the georeferenced points from Spain because founding colonizers in California would be expected to have originated in Spain. Occurrence records for California were obtained from the Consortium of California Herbaria (http://ucjeps.berkeley.edu/ consortium; accessed April 2009).

Climate variables were obtained from WorldClim in ESRI grids of 30 arc-seconds (Hijmans et al. 2005) and extracted in ArcGIS (version 10; Esri Inc., Redlands, CA, USA). All 19 of the available climate variables were used in the model (Table 6.1).

Model parameterization

Maxent (version 3.3.2) is an algorithm that uses presence-only data to model species distributions, based on environmental variables that are associated with geographic occurrence points, to calculate an index of habitat suitability with values ranging from 0 to 1 (least to most

suitable habitat) (Phillips et al. 2006). I used the logistic output format because it estimates the probability of presence with presence-only data. The default regularization parameters were: linear, quadratic, and product: 0.100, categorical: 0.250, threshold: 1.070, hinge: 0.500. The sampling technique or replicate type was subsample, using a random seed.

The Maxent model, trained using data from the native range, Spain, was run three times: First, under the default threshold. Second, holding all parameters constant except the threshold, which was changed to the minimum training presence threshold of 0.096 to include the maximum area of suitable habitats vulnerable to invasion. Third, we used the equal training sensitivity and specificity threshold of 0.452 because it is the most conservative threshold. The model trained in Spain under the default threshold used 10,085 points to determine the Maxent distribution (background points and presence points), with a maximum 5,000 iterations. A random test point percentage of 30% resulted in 93 presence records being used for training and 39 for testing.

The Maxent model, trained using California data, was also run three times. Under the default threshold 10,196 points were used to determine the Maxent distribution (background points and presence points), with a maximum 5,000 iterations. A random test point percentage of 30% resulted in 202 presence records being used for training and 86 for testing. The remaining parameters were identical to the model trained in Spain. The minimum training presence threshold was 0.005, and the equal training sensitivity and specificity threshold was 0.225.

Model performance

The receiver operating characteristic (ROC) curve plots sensitivity vs. 1 − specificity. Sensitivity describes the rate of successful predictions of presence, and specificity represents how well absences are predicted. ROC curves for both the training presence records and the test

records was evaluated for the Area Under the Curve (AUC), which describes the probability that a presence point will be ranked higher than a random background pseudo-absence point. This measure will lie between 0.50 and 1.0, where 0.50 represents a random outcome, and 1.0 represents a perfect prediction. The AUC was calculated for both training data and test points.

RESULTS

The association of *C. melitensis* occurrence points with climatic data in Spain resulted in a map of areas of predicted climatic suitability within Spain. Areas in the southwestern and northeastern regions of Spain were predicted to have highly suitable habitat. Projecting this model onto California resulted in a predictive map showing areas of predicted climatic suitability for *C. melitensis* in California (Fig. 6.1). The Coastal regions were predicted to be unsuitable habitat, as were the Outer North Coast Ranges. Highly suitable areas were predicted in the Central Valley, the South Coast Ranges, the Peninsular Ranges, and portions of the Transverse Ranges, and eastern side of the Cascade Ranges and northern Sierra Nevada. Moderately suitable areas were predicted in the Mojave Desert (Fig. 6.1a). Training the model in California and projecting it onto Spain resulted in a predictive map with divergent suitable areas for *C. melitensis* both in California and in Spain. The central and south coastal regions of California were predicted to be highly suitable habitat. Portions of the North Coast and Outer North Coast Ranges became moderately suitable. The Outer South Coast Ranges remained as highly suitable, but the Inner South Coast Ranges diminished in suitability, as did the Central Valley and the eastern side of the Cascade Ranges and northern Sierra Nevada, and the Mojave Desert. The projection of this California-trained model onto Spain resulted in a reduction of total area predicted as suitable, and a shift in highly suitable areas to the northwest, with a small area

119

retained in the southwest, shifted slightly southward. The previously predicted highly suitable area in the northeast became unsuitable (Fig. 6.1b).

Both models performed well, with AUC's of greater than 0.7 and predictive power significantly greater than random (referring to the range in which the model was trained). The model that was trained in California performed better than the model trained in Spain (AUC: 0.907 on the training data, and 0.896 on the test data versus AUC: 0.858 on the training data, and 0.731 on the test data; Table 6.2). The Spain algorithm converged after 1160 iterations and the California algorithm converged after 1220 iterations. However, for the California-trained model projected onto Spain, the range of successfully predicted occurrence points in Spain (depending on threshold) was larger than the range for the Spain-trained model projected onto California for successfully predicted occurrence points in California (2% to 92% versus 57% to 88%; Table 6.2).

For Spain, the variable that had the most influence on the distribution was Bio 1, the annual mean temperature (Table 6.1). This variable had the most influence, both when used in isolation and when omitted from the model. For California, the variables that had the most influence on the distribution were Bio 3, isothermality (mean diurnal range/temperature annual range (maximum temperature of the warmest month – minimum temperature of the coldest month)(*100)), and Bio 1, the annual mean temperature (Table 6.1). Bio 3 had the most influence when used in isolation, and Bio 1 had the most influence when omitted from the model.

DISCUSSION

These findings showing divergence in the two predictive models and their associated projections provide clear evidence that invasive populations of *C. melitensis* have undergone

120

shifts in their climatic niche. The overlap of the predicted invasive range with a high proportion of occurrence points in California, however, suggest a potential expansion of the realized niche, as opposed to a shift in the fundamental niche.

The use of bioclimatic niche models for the prediction of invasive species ranges has been controversial because of concerns over the apparent violation of assumptions in the models (Jeschke and Strayer 2008, Araujo and Peterson 2012, Petitpierre et al. 2012). Traditionally, niche conservatism and equilibrium with the environment is assumed in species distribution models (Peterson and Vieglais 2001). However, in the case of a biological invasion, it is possible that the species has not yet dispersed to all available habitats, and therefore is not at equilibrium. Furthermore, invasive species commonly occupy niche space outside their niche as realized in the native range (Broennimann et al. 2007, Mukherjee et al. 2012). This observation can be an indication that the fundamental niche is much broader than realized in the native range, and a different portion of the fundamental niche is being exploited in the new range due to a release from constraining biotic interactions or a plastic response to novel conditions. Alternatively, the species may have rapidly evolved to adapt to the new environment, thus expanding or changing the fundamental niche (Maron et al. 2004, Dlugosch and Parker 2008, Xu et al. 2010, Chun et al. 2011).

In predictive species distribution models, occurrences that appear outside the predicted area of suitability can indicate either changes in the fundamental niche, or changes in the realized niche (Broennimann et al. 2007). Because introductions involve only a small portion of the gene pool, founder effects are common. If genetic drift and selection shape genotypes that are adapted to novel climatic conditions, the fundamental niche may change in the new range. If the fundamental niche is constrained in the native region by biotic interactions that are not present in

the invaded range, the realized niche may expand in the new range without changes in the underlying fundamental niche. If a predicted area of suitability in the model does not have any occurrences, then either (1) the species has not yet dispersed to that area (it is not yet at equilibrium), (2) abiotic conditions other than climate (i.e. soil type, disturbance regime) are not suitable, (3) biotic interactions preclude the colonization of the species, or (4) the species has undergone a niche shift and those conditions are no longer suitable.

The two aspects of the model that do not exactly fit the data are: (1) *C. melitensis* occurrences that fall outside areas of predicted habitat suitability; and (2) predicted suitable habitats that do not contain any *C. melitensis* occurrences. In the first case, if conditions in the biotic environment are different in the invasive range than in the native range, it is possible that different portions of the fundamental niche are being exploited in the invasive range. For example, species that are constrained by natural enemies in their native range can undergo phenotypic (Leger and Rice 2003) and demographic (Vilà et al. 2005) changes when introduced to regions where natural enemies are absent. Enemy release can be from interactions aboveground or belowground, and include interactions with predators, parasitoids, pathogens, or competitors. The climatic niche depicted in Spain resulting from the occurrence distribution may not be inclusive of the entire range of climatic physiological tolerance of the species (i.e., the fundamental niche as related to the climate), if enemies occur in areas of exclusion. Conversely, the enemies may be excluded from areas of occupation of *C. melitensis* because the climatic conditions are outside their range of physiological tolerance. Thus, if a mechanistic model were done for the fundamental climatic niche of *C. melitensis* based on physiological tolerances determined in the lab, it would potentially include all areas of occurrence records in California.

122

In the second case, where predicted suitable habitats do not contain any *C. melitensis* occurrences, either dispersal limitation or biotic interactions such as competitive exclusion from California native plants or attack from resident enemies, or founder effects might explain the absence. There are no occurrence records in the Mojave Desert, or on the eastern side of the Peninsular, Transverse, Sierra Nevada Ranges, or Cascade Ranges, which are areas that are predicted to be suitable. These areas are geographically separated from the known occurrences of *C. melitensis* by mountain ranges; thus, propagule pressure may not be high. The excluded areas contain different suites of native plants and animals than the areas of occurrence, which might serve to preclude the establishment of *C. melitensis*. These areas have much drier climatic conditions than much of the rest of the predicted range of suitable habitat in California. However, it is possible that founder effects might have excluded genotypes that are adapted to the drier conditions that occur in these areas; for example, genotypes that originated from the desert in Almería, which is an area of high suitability in the native range. In a previous study, invasive populations of *C. melitensis* were found to be genetically differentiated from native populations (Chapter 2). Whether these changes were caused by adaptation or founder effects is unknown, but either scenario could explain the shift in the niche.

Annual mean temperature was the most influential variable in the model trained in Spain, with the probability of the presence of *C. melitensis* increasing with annual mean temperature. For the model trained in California, the most important variable was isothermality. As this measure of temperature evenness increased, so did the probability of the presence of *C. melitensis*. The second most important variable in California was temperature, as in the model trained in Spain. Although both of these variables are related to temperature, they have different effects on predicted presences. For the model trained in Spain, occurrences tend to be in places

with more extreme temperatures, resulting in hotter and possibly more arid localities, while for the model trained in California, predictions shifted toward areas where temperature fluctuation is mediated, such as coastal areas, and away from drier, more extreme climate types. It may be that in the milder areas of Spain, biotic interactions exclude the presence of *C. melitensis*.

Despite the good performance of the models in their training ranges, as indicated by the high AUC values, the models were only moderately successful in predicting occurrence points in the projected ranges. The model trained in Spain excluded 12% of California occurrence points, even at the least conservative threshold, the minimum training presence threshold, while the model trained in California excluded 98% of Spain occurrence points at the most conservative threshold. This is not an indication of poor performance of the model. Rather, it indicates a difference in the niche that *C. melitensis* can exploit in each range. Furthermore, the success rate of the prediction depends on the threshold chosen.

Thresholds determine the cutoff point for suitable habitat to produce a binary outcome for success of prediction for each occurrence point. In other words, the model successfully predicts a locality if it falls into a space with predicted climatic suitability value equal to or greater than the threshold value. To evaluate the percentage of occurrence points successfully predicted by the model, I used two different thresholds representing the extremes of conservatism. The equal training sensitivity and specificity threshold is the most conservative threshold, while the minimum training presence threshold includes the maximum area of suitable habitats vulnerable to invasion. When the Spain-trained model was projected onto California, 57% of the occurrence points were successfully predicted under the equal training sensitivity and specificity threshold of 0.452 (most conservative), and 88% for the minimum training presence threshold of 0.096 (includes maximum area of suitability). When the California-trained model was projected onto

Spain, the percent of successfully predicted occurrence points in Spain were 2% for the equal training sensitivity and specificity threshold of 0.225, and 92% for the minimum training presence threshold of 0.005.

The results of this study have important management implications for *C. melitensis*. There are substantial areas of predicted suitability in California where no occurrences have been recorded. *Centaurea melitensis* was introduced to California just over 200 years ago (Hendry 1931), and thus may not be at equilibrium. The California Invasive Plant Council has determined that this species is actively spreading in range and that established populations are increasing in abundance (Cal-IPC 2006-2012). This, along with the predictive map of invasion produced in this study, suggests that *C. melitensis* is not yet at equilibrium, and might so far have been limited by dispersal. Managers of areas of high suitability must enact risk screening, early detection, and eradication plans for this species to prevent further spread of invasion (Lodge et al. 2006). Although eradication may be difficult to impossible in areas where *C. melitensis* has already established, managers must institute control and slow-the-spread programs, which have been shown to be cost effective (Lodge et al. 2006).

The occurrence of *C. melitensis* outside predicted suitable areas illustrates another use of predictive climate models for directing the prevention of invasive species spread. The model trained in California shows that most of the newly suitable areas occur along the coast. These results can focus directed study of the mechanisms of the niche shift by closer examination of the plants and environments that occur in coastal areas. For example, if the niche expansion into coastal areas is due to enemy release, then coastal populations of *C. melitensis* might be candidates for a biocontrol program. In this way, managers can direct efforts to limit range extensions by identifying the drivers of the niche shift using the bioclimatic niche models.

In conclusion, findings from this study provide evidence that invasive populations of *C. melitensis* have undergone a niche shift, occurring outside areas of predicted climatic suitability in California. By focusing future studies of trait differentiation on populations that occur outside suitable areas, the mechanisms of the niche shift, and of *C. melitensis* invasion more specifically, can be examined. Furthermore, the identification of areas that are vulnerable to invasion but do not yet support colonizers can direct management efforts to slow the spread of *C. melitensis* in California.

Table 6.1. Bioclimatic variables obtained from WorldClim Global Climate Data. Estimates of the relative contributions of the environmental variables to the species distribution model for *Centaurea meliteusis* when the model was trained in Spain, and when the model was trained in California.

		Percent contribution	
Variable	**Description**	**Model trained in Spain**	**Model trained in California**
BIO1	Annual mean temperature	60.7	31.7
BIO2	Mean diurnal range	16.5	12.2
BIO3	Isothermality	5	40
BIO4	Temperature seasonality	0.1	0
BIO5	Maximum temperature of the warmest month	0.3	0
BIO6	Minimum temperature of the coldest month	0.1	0
BIO7	Temperature annual range	0.1	0
BIO8	Mean temperature of the wettest quarter	0.2	0
BIO9	Mean temperature of the driest quarter	0	0
BIO10	Mean temperature of the warmest quarter	12.7	15.5
BIO11	Mean temperature of the coldest quarter	1.8	0.1
BIO12	Annual precipitation	0.5	0
BIO13	Precipitation of the wettest month	0.1	0.1
BIO14	Precipitation of the driest month	0.3	0.1
BIO15	Precipitation seasonality	1.1	0.1
BIO16	Precipitation of the wettest quarter	0.2	0.1
BIO17	Precipitation of the driest quarter	0	0.1
BIO18	Precipitation of the warmest quarter	0.1	0
BIO19	Precipitation of the coldest quarter	0.1	0

Table 6.2. Results of the application of two extreme thresholds on species distribution models for *Centaura melitensis* that were (a) trained in Spain and projected onto California, and (b) trained in California and projected onto Spain. The "minimum training presence" threshold includes the maximum area of suitable habitats, and the "equal training sensitivity and specificity" threshold is the most conservative. Percent predicted refers to the percentage of occurrence records in the projected range that fall within predicted suitable habitat. The fractional predicted area, training omission rate, and test omission rate refer to occurrence records in the training range. The P-values are one-tailed tests of the hypothesis that test points are predicted no better than by a random prediction with the same fractional area.

|	Logistic threshold	Description	Percent predicted	Fractional predicted area	Training omission rate	Test omission rate	P-value
Trained in Spain							
Spain test points	0.027	Minimum training presence		0.872	0	0.026	***
(averaged thresholds)	0.466	Equal training sensitivity and specificity		0.226	0.226	0.462	***
California test points	0.00961	Minimum training presence	88%				
(reclassified thresholds)	0.4519	Equal training sensitivity and specificity	57%				
Trained in California							
California test points	0.027	Minimum training presence		0.522	0	0.012	***
(averaged thresholds)	0.257	Equal training sensitivity and specificity		0.176	0.178	0.163	***
Spain test points	0.0048	Minimum training presence	92%				
(reclassified thresholds)	0.2535	Equal training sensitivity and specificity	2%				

***P<0.001

128
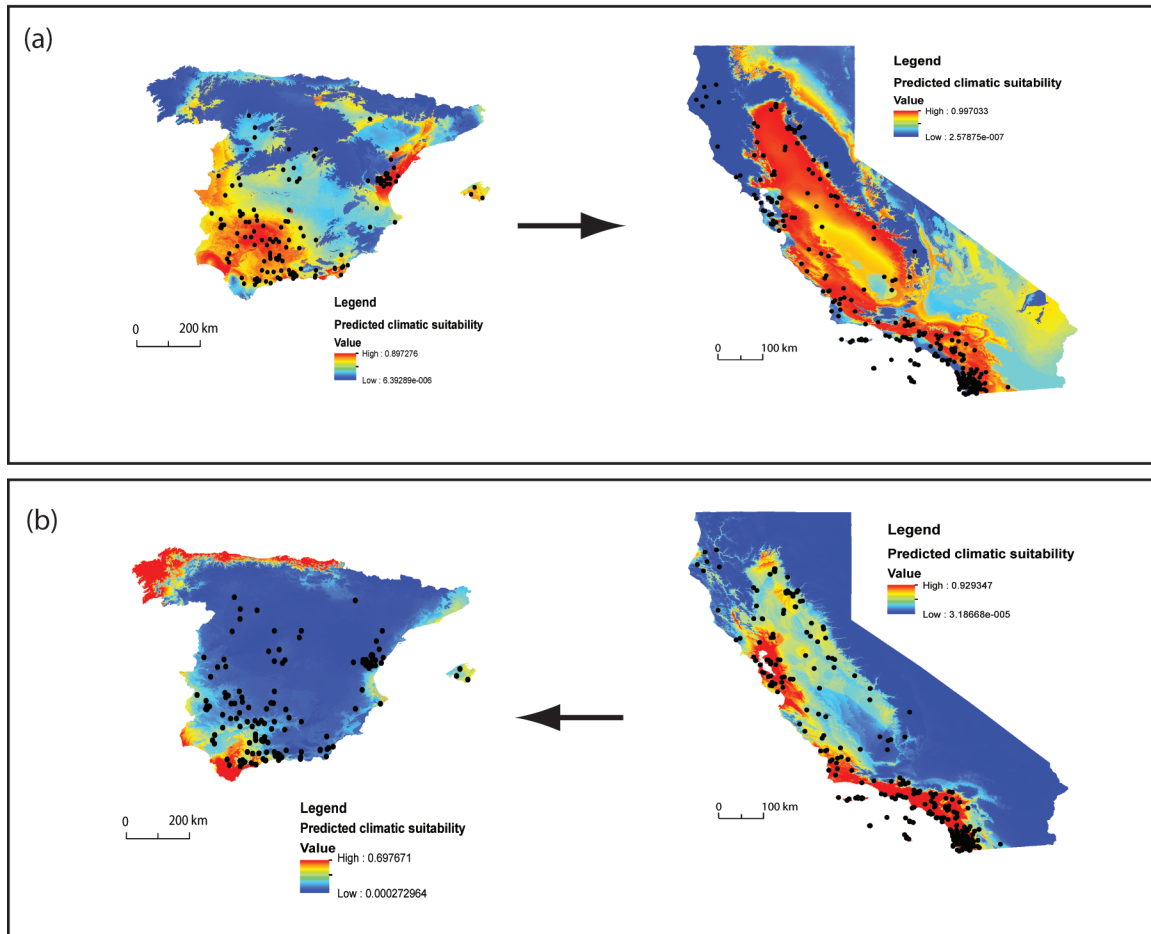
Figure 6.1. Representations of Maxent models of climatic suitability for *Centaurea melitensis*. The top row (a) shows the model trained on occurrence records in the native range, Spain, and projected onto the invasive range, California. The bottom row (b) shows the model trained on occurrence records in the invasive range, California, and projected onto the native range, Spain.

LITERATURE CITED

Araujo, M. B. and A. T. Peterson. 2012. Uses and misuses of bioclimatic envelope modeling. Ecology 93:1527-1539.

Bhagwat, S. A., E. Breman, T. Thekaekara, T. F. Thornton, and K. J. Willis. 2012. A Battle Lost? Report on Two Centuries of Invasion and Management of *Lantana camara* L. in Australia, India and South Africa. PLoS One 7.

Broennimann, O., U. A. Treier, H. Mueller-Schaerer, W. Thuiller, A. T. Peterson, and A. Guisan. 2007. Evidence of climatic niche shift during biological invasion. Ecology Letters 10:701-709.

Brooks, M. L. 2000. Competition between alien annual grasses and native annual plants in the Mojave Desert. American Midland Naturalist 144:92-108.

Cal-IPC. 2006-2012. *Centaurea melitensis* (Malta starthistle, tocalote) statewide maps.

Chun, Y. J., V. Le Corre, and F. Bretagnolle. 2011. Adaptive divergence for a fitness-related trait among invasive *Ambrosia artemisiifolia* populations in France. Molecular Ecology 20:1378-1388.

Dlugosch, K. M. and I. M. Parker. 2008. Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. Ecology Letters 11:1-9.

Finnoff, D., J. F. Shogren, B. Leung, and D. Lodge. 2007. Take a risk: Preferring prevention over control of biological invaders. Ecological Economics 62:216-222.

Gaertner, M., A. Den Breeyen, C. Hui, and D. M. Richardson. 2009. Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. Progress in Physical Geography 33:319-338.

Hendry, G. W. 1931. The adobe brick as a historical source. Agricultural History 5:110-127.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965-1978.

Hutchinson, G. E. 1957. Population studies - Animal ecology and demography - concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22:415-427.

Jackson, S. T. and J. T. Overpeck. 2000. Responses of plant populations and communities to environmental changes of the late Quaternary. Paleobiology 26:194-220.

Jeschke, J. M. and D. L. Strayer. 2008. Usefulness of bioclimatic models for studying climate change and invasive species. Pages 1-24 *in* R. S. Ostfeld and W. H. Schlesinger, editors. Annals of the New York Academy of Sciences.

Jimenez, A., A. Pauchard, L. A. Cavieres, A. Marticorena, and R. O. Bustamante. 2008. Do climatically similar regions contain similar alien floras? A comparison between the mediterranean areas of central Chile and California. Journal of Biogeography 35:614-624.

Leger, E. A. and K. J. Rice. 2003. Invasive California poppies (*Eschscholzia californica* Cham.) grow larger than native individuals under reduced competition. Ecology Letters 6:257-264.

Leung, B., D. M. Lodge, D. Finnoff, J. F. Shogren, M. A. Lewis, and G. Lamberti. 2002. An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proceedings of the Royal Society B-Biological Sciences 269:2407-2413.

Lodge, D. M., S. Williams, H. J. MacIsaac, K. R. Hayes, B. Leung, S. Reichard, R. N. Mack, P. B. Moyle, M. Smith, D. A. Andow, J. T. Carlton, and A. McMichael. 2006. Biological

invasions: Recommendations for US policy and management. Ecological Applications 16:2035-2054.

Mack, M. and C. M. D'Antonio. 1998. Impacts of biological invasions on disturbance regimes. Trends in Ecology & Evolution 13:195-198.

Maron, J. L., M. Vila, R. Bommarco, S. Elmendorf, and P. Beardsley. 2004. Rapid evolution of an invasive plant. Ecological Monographs 74:261-280.

Moroney, J. R. and P. W. Rundel. 2012. Abundance and dispersion of the invasive Mediterranean annual, *Centaurea melitensis* in its native and non-native ranges. Biological Invasions:10.1007/s10530-10012-10302-10531.

Mukherjee, A., D. A. Williams, G. S. Wheeler, J. P. Cuda, S. Pal, and W. A. Overholt. 2012. Brazilian peppertree (*Schinus terebinthifolius*) in Florida and South America: evidence of a possible niche shift driven by hybridization. Biological Invasions 14:1415-1430.

Olden, J. D., N. L. Poff, M. R. Douglas, M. E. Douglas, and K. D. Fausch. 2004. Ecological and evolutionary consequences of biotic homogenization. Trends in Ecology & Evolution 19:18-24.

Peterson, A. T. 2003. Predicting the geography of species' invasions via ecological niche modeling. Quarterly Review of Biology 78:419-433.

Peterson, A. T. and D. A. Vieglais. 2001. Predicting species invasions using ecological niche modeling: New approaches from bioinformatics attack a pressing problem. BioScience 51:363-371.

Petitpierre, B., C. Kueffer, O. Broennimann, C. Randin, C. Daehler, and A. Guisan. 2012. Climatic Niche Shifts Are Rare Among Terrestrial Plant Invaders. Science 335:1344-1348.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231-259.

Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O'Connell, E. Wong, L. Russel, J. Zern, T. Aquino, and T. Tsomondo. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. Agriculture Ecosystems & Environment 84:1-20.

Richardson, D. M. and P. Pyšek. 2006. Plant invasions: merging the concepts of species invasiveness and community invasibility. Progress in Physical Geography 30:409-431.

Sala, O. E., F. S. Chapin, 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. Biodiversity - Global biodiversity scenarios for the year 2100. Science 287:1770-1774.

Thuiller, W., D. M. Richardson, P. Pysek, G. F. Midgley, G. O. Hughes, and M. Rouget. 2005. Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. Global Change Biology 11:2234-2250.

Tutin, T. G., V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb, editors. 1976. Flora Europaea. Cambridge University Press, Cambridge, United Kingdom.

Vilà, M., J. L. Espinar, M. Hejda, P. E. Hulme, V. Jarosik, J. L. Maron, J. Pergl, U. Schaffner, Y. Sun, and P. Pysek. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters 14:702-708.

Vilà, M., J. L. Maron, and L. Marco. 2005. Evidence for the enemy release hypothesis in *Hypericum perforatum*. Oecologia 142:474-479.

Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to

    imperiled species in the United States. BioScience 48:607-615.

Xu, C. Y., M. H. Julien, M. Fatemi, C. Girod, R. D. Van Klinken, C. L. Gross, and S. J. Novak.

    2010. Phenotypic divergence during the invasion of *Phyla canescens* in Australia and

    France: evidence for selection-driven evolution. Ecology Letters 13:32-44.