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Biogeographical variation of herbivore response and competitive ability of *Centaurea* species

Dissertação de Mestrado em Biologia,
orientada pelo Professor Doutor João Castro Loureiro e pelo Doutor Daniel Montesinos,
e apresentada à Faculdade de Ciências e Tecnologia da Universidade de Coimbra

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia, realizada sob a orientação científica do Doutor João Castro Loureiro e do Doutor Daniel Montesinos (Universidade de Coimbra).

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ABSTRACT

Plant invasions have an important impact in the environment and the economy. Once introduced in their non-native range, exotic plants can experience rapid evolutionary processes which can be crucial for invasive success. Assessing trait-shifts between native and non-native ranges is an important step for understanding evolutionary changes occurring in the non-native ranges and, ultimately, for the development of management plans. *Centaurea solstitialis* and *Centaurea sulphurea* are annual herbs native to the Mediterranean Basin region that were introduced into California (USA) two centuries ago. While *C. sulphurea* has only naturalized into three known populations in California, *C. solstitialis* has become a noxious and widespread weed invasive. *C. solstitialis* has also been introduced into Chile, Argentina and Australia with different invasive success. The main objective of this thesis was to assess trait-shifts between native and non-native ranges of the two congeneric *Centaurea* species. Two main comparative approaches were developed in greenhouse studies: competitive ability of individuals from native and non-native ranges of *C. sulphurea,* and herbivory responses from a generalist herbivore species fed with leaves of both *Centaurea* species. For the assessment of competitive ability, we assessed total biomass of plants from both ranges, as well as inter-regional hybrids either growing alone in a control group, or in competition with the common competitor grass *Bromus hordeaceus*. Individuals from California were found to be larger and better competitors than individuals from Spain, whereas inter-regional hybrids from both regions showed competitive responses more similar to that of individuals from California. To assess for biogeographic differences on the response of herbivore, we fed a generalist and widespread herbivore (common snail *Helix aspersa)*, with plant leaves from both ranges in order to assess the snail's final weight. Snails fed with leaves from Spanish native populations of non-invasive *C. sulphurea* grew significantly less compared to snails fed with leaves from non-native California. For snails fed with the invasive *C. solstitialis,* significant differences were also found among regions, but the response was more complex, depending on population, with snails fed with Turkish and Australian plants presenting higher growth rates than the rest of the regions. Overall, these results deliver solid evidence for rapid differentiation between ranges, illustrated by phenotypic changes which might contribute for the invasive potential of exotic species, as exemplified by the observed differences in invasive success between the two studied congeneric species.

RESUMO

As invasões por plantas desempenham um papel fulcral para o ambiente e economia globais. Uma vez introduzidas em áreas não-nativas, as plantas exóticas podem sofrer rápidos processos evolucionários que por sua vez são decisivos para o sucesso dos invasores. Conhecer as mudanças nas características destas plantas entre as áreas nativas e não-nativas é um importante passo para perceber as alterações evolutivas que ocorrem na área colonizada e, em última instância, para o desenvolvimento de plano de gestão e controlo. *Centaurea solstitialis* e *Centaurea sulphurea* são plantas anuais nativas da Bacia Mediterrânea e introduzidas na Califórnia (EUA) há cerca de dois séculos. Enquanto *C. sulphurea* está naturalizada sem comportamento invasor e apresenta apenas três populações conhecidas na Califórnia, *C. solstitialis* é atualmente uma invasora agressiva e bastante disseminada. *C. solstitialis* está também introduzida no Chile, Argentina e Austrália com diferentes graus de sucesso invasor. O principal objetivo deste projeto foi explorar as possíveis alterações em características específicas de plantas entre a zona nativa e a zona não-nativa das mesmas. Dois estudos comparativos em ambiente controlado de estufa foram realizados, avaliando nomeadamente a habilidade competitiva entre indivíduos das áreas nativa e não-nativa de *C. sulphurea* e a resposta herbívora por parte de um herbívoro generalista alimentado com folhas de ambas as espécies de *Centaurea* em estudo. Para o estudo de habilidade competitiva foi registada a biomassa total das plantas de ambas as áreas, assim como de híbridos inter-regionais, a crescer sozinhas ou em competição com uma herbácea abundante – *Bromus hordeaceus*. As plantas da Califórnia provaram ser maiores e melhores competidores que os seus conspecíficos oriundos de Espanha, enquanto os híbridos inter-regionais de ambas as regiões apresentaram respostas competitivas semelhantes a indivíduos da Califórnia. Para o estudo de herbívora, um herbívoro generalista de elevada dispersão – caracol de jardim *Helix aspersa* – foi alimentado com folhas de ambas as áreas e o peso final destes indivíduos registado a fim de explorar possíveis diferenças significativas. Os caracóis alimentados com folhas de populações nativas de Espanha da não-invasora *C. sulphurea* cresceram significativamente menos quando comparados com caracóis alimentados com folhas das populações não-nativas da Califórnia. Para os caracóis alimentados com a invasora *C. solstitialis*, foram também registadas diferenças significativas, no entanto as respostas foram mais complexas, dependendo da população em causa, com caracóis alimentados por populações da Turquia e Austrália a registarem taxas de crescimento mais elevadas. Estes resultados introduzem evidências para a rápida diferenciação entre zonas, ilustrada pelas alterações fenotípicas aqui registadas e que por sua vez poderão contribuir para o potencial invasor de espécies exóticas, como exemplificados pelas diferenças previamente conhecidas no sucesso invasor das duas espécies congêneres em estudo.

KEY-WORDS:

Invasive plant; biogeography; competitive ability; herbivory; EICA; SDH; *Centaurea*.

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Ecological explosions differ from some of the rest by not making
such a loud noise and in taking longer to happen.
That is to say, they may develop slowly and they may die down slowly
but they can be very impressive in their effects,
and many people have been ruined by them,
or died,
or forced to emigrate.

Charles S. Elton, 1958

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Chapter 2, submitted to *Plant Ecology & Diversity*: (p.22)

Filipe, J.C.; Montesinos, D. (2016) Inter-regional hybrids of native and non-native *Centaurea sulphurea* inherit increased competitive ability from the non-natives. Plant Ecology and Diversity. *Under review*.

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Filipe, J.C.; Jorge, A; Eren, Ö.; Sotes, G; Hierro, J.; Montesinos, D. (2016) Invasive and non-invasive congeneric *Centaurea* show contrasting patterns of herbivory by snails. Plant Ecology and Evolution. *In press*.

[1] References and figures formatting in each chapter were kept according to the rules of journals where they were published/submitted.

Part I

Chapter 1
General Introduction

The following introduction is a brief state of the art of key questions and terminology topics on plant ecology and evolution, allopatry, biological invasions and exotic species characteristics and adaptations, focusing in some detailed issues related to the main body of this work, namely, chapters 2 and 3.

GREEN INVASIVES AS A STUDY MODEL: AN OVERVIEW

Biological invasions and humans.

In 1958, Charles Elton acknowledged biological invasions as “one of the great historical convulsions in the world’s fauna and flora”, perceiving this phenomenon as a path to worldwide biological homogenization. This complex process occurs when a species is transported from its native habitat range to a novel non-native region in which it is capable of establish populations after reproducing several generations and ultimately spread to neighboring areas (Richardson et al. 2000).

Trough human influence, an immense amount of plant species has been spread at a global scale, from Africa to the Americas, from the remote Australia to old Europe (Williams 1998, Tilman 2004, Lockwood et al. 2007). These so called exotics have occupied new habitats at an alarming rate (Reichard 1997, Kolar & Lodge 2001, Tilman 2004, Prentis 2008, Wilson et al. 2011, Lowry et al. 2012), striving severe impacts not only at natural ecosystems: competing with native organisms and changing the habitat structure, altering nutrient and energy flows, shaping food webs, disrupting pollination networks (Stohlgren et al. 1999, Davis et al. 2000, Sax et al. 2002, Hierro & Callaway 2003, Vivanco et al. 2004, Hawkes et al. 2005, Lockwood et al. 2007, Lowry et al. 2012, Marchante et al. 2015); but also at the human welfare, health and interests (e.g. damage to forestry, crops, urban areas and congestion in waterways) (Ewell 1999 et al., Brooks et al. 2004, Lockwood et al. 2007).

Controlling and eradicating plant invasions involves high efforts and costs (Pimentel et al. 2005, Eiswerth et al. 2005, Wilson et al. 2011). Even though, early research in plant ecology and evolution, as well as improving public awareness, might minimize future management expenditures and natural legacy loses, invasive species are nowadays ranked as one of the most dangerous threat for species endangerment and extinction (Pejchar & Mooney 2010). Therefore, assessing the causes of invasiveness and the agents involved in this phenomenon poses as a major step for plant ecology research and, ultimately, for managing biological invasions in a global context.

Invasiveness stages and success

When introduced into new regions, most plant species are expected to face a vast and complex set of barriers from different natures. Thus, a pivotal question in invasion ecology since its onset (Elton 1958, Baker & Stebbins 1965) has been distinguishing the minority of successful introduced species (i.e. invasives) from a massive majority of exotics which fail to establish, or from those which establish some populations but do not significantly expand (i.e. naturalized).

Richardson et al. (2000) suggested a basic model with three main phases for the plant invasion process - introduction, naturalization and invasion – each one based upon overcoming several barriers (Figure 1.1). Intentionally or accidently plant species may be transported across a major geographical barrier, thus occurring the introduction. Some introduced taxa only persist as casuals which depend on re-introductions over time to prevail, but fail to achieve consistent reproduction, and thus fail to establish self-sustaining populations.

However, those species who overcome the next level of barriers, both environmental and reproductive, might achieve the naturalized status. Naturalized plant species consistently produce descendants, and do not rely on re-introductions to do so. Once surpassing the last set of barriers, those related to regional dispersion and wider environmental (biotic and abiotic) obstacles, some species are able to produce vast reproductive outputs through extensive areas, at least some of them significantly distant from the introduction spots, in which case we can safely classify this species as invasive.

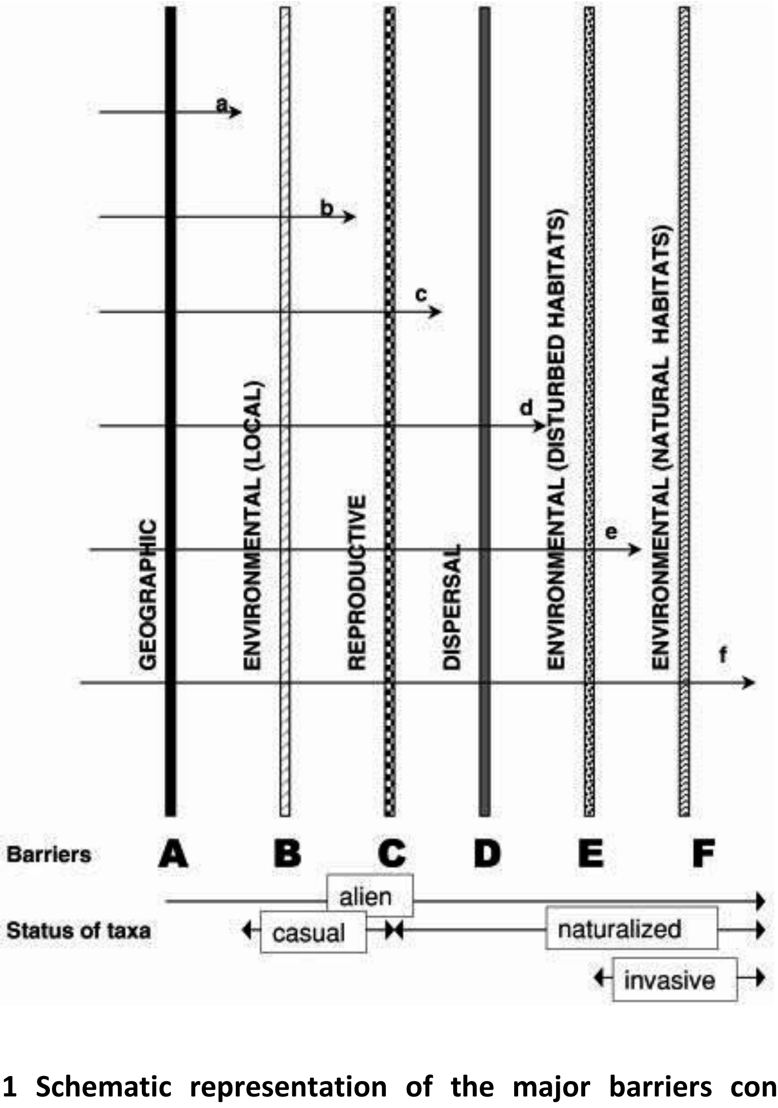

Figure 1.1 Schematic representation of the major barriers confining the spreading of exotic plants once introduced, listed from **A** to **F**. Arrows **a** to **f** indicate the direction followed by each taxa to achieve the different status, from introduced to invasive. (Richardson et. al 2000)

Following this concept, Marchante (2001) refined the idea with different key phases and terminology – introduction, naturalization, facilitation, increased distribution and stabilization. This model calls a particular attention for the trigger event occurring at some naturalized exotic species, stimulating the invasive process (Figure 1.2). This stimulus may be from anthropogenic disturbance as deforestation, or by casual nature such as climatic disturbing events, or adaptation to external selective pressures (i.e. seed dispersal vectors, herbivory, competition with natives). Subsequently, each species displays variable increased distributions depending on growth and reproduction rates, dispersal mechanisms and the invaded habitat' particular aspects. (Marchante 2001, Marchante 2008)

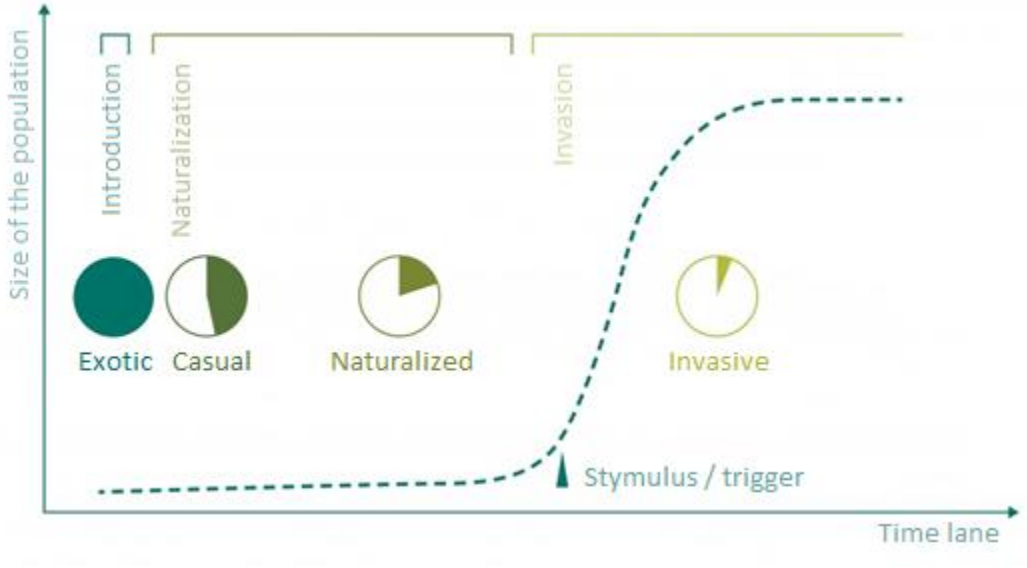

Figure 1.2 Biological invasions main stages. Each circle represents the whole of introduced species and dark areas within each circle show the % of species with potential to adapt into next category. The duration of each phase as well as the size of the population varies for each taxa (adapted from Marchante 2001).

Biogeography: assessing the model

Geographic isolation between populations of the same species is an initial and essential requirement for allopatric speciation; isolation significantly reduces or halts gene flow between populations, setting up the scene for genetic drift, mutations, and divergent selective pressures to act (Mayr 1963, Bush 1975, White 1978). Allopatry is then acknowledged as one of the most important and classic models of speciation (Mayr 1954, 1970, Lande 1980, Bossford et al. 2005, Hoskin et al. 2005, Montesinos et al. 2012, García et al. 2013, Noutsos et al. 2014, Colautti & Lau 2015, Filipe et al. 2016).

Understanding phenotypic and genetic diversity among populations has been vital for biogeography, evolution and ecology of almost all identified plant species. Current biological invasions thus provide a notable and resourceful context in which to explore and study biogeographic divergence of traits of species allopatrically distributed (Bossford et al. 2005, Sax et al. 2007).

Although there is no consistent pattern, traits of invasive plants differ between their native and non-native ranges (Thébaud and Simberloff 2001). Consequently, comparative studies between native and non-native populations of the same species allow us to understand how evolution acts on living organisms facing diverging selective pressures across distinct geographical regions. When assessing traits-shifts on populations from both native and non-native ranges, populations on the non-native range have been reported to produce more seeds, (Noble 1989, Montesinos et al 2012, García et al. 2013,) to grow faster and taller (Blossey and Notzold 1995, Graebner et al. 2012, García et al. 2013), to live longer (Rees and Paynter 1997), to flower earlier

(Eriksen et al. 2012) and to display improved herbivory defenses – both chemical (Sotes et al. 2015) and physical (i.e. spines length) (García et al. 2013).

Trait-shifts and evolution of invasiveness

Several intrinsic species traits, as well as many extrinsic factors, are crucial to promote the ability of a plant to invade a newly colonized region, and numerous hypotheses for exotic plant success consider some of the above-mentioned traits and their interactions, seizing a wide variety of mechanisms believed to be responsible for invasive success. Our work will focus in two well established hypotheses, the Evolution of Increased Competitive Ability, EICA (Blossey & Notzold 1995) and the Shifting Defence Hypothesis (SDH hereafter) (Joshi & Vrieling 2005). Both will be explained in detail in chapters 2 and 3 according to their relevance for the studies performed in each of these chapters. Both concepts follow a line of inquiry that focus on ecological interactions as main drivers for invasion success, greatly relying on the idea that escape from natural enemies acts as a prevalent role in the invasion process (Darwin 1859, Gillett 1962, Crawley 1987, Maron and Vilà 2001, Keane and Crawley 2002).

Cross-continental comparisons of plant species in their native and non-native ranges are then necessary to explain differences in performance of the species, which can be tested by assessing a number of variables across regions, including: competitive ability, population growth, increased plant vigour, herbivory resistance (Hierro et al. 2005)

In order to assess trait differences between introduced and native conspecific populations, plants from both ranges were grown in common garden environments using seed samples from several populations across each region. Finding phenotypic differences between native and non-native ranges growing under identical conditions is a strong evidence for trait-shifts and thus of genetic differentiation. We hereby focus in two main comparative assessments: competitive ability with other plant species, and the response of a generalist herbivore to leaf defences (Chapters 2 and 3, respectively).

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Part II

Chapter 2
Competitive Ability

ABSTRACT

Some exotic species can rapidly develop adaptations to their non-native regions, like increased size and competitive ability. Although those traits are believed to be responsible for invasive success, some non-invasive exotic species have them too, suggesting that they might be necessary but not sufficient to become a successful invader. Here, it is assessed size and competitive ability of the exotic non-invasive herb *C. sulphurea*, which presents overlapping distributions in the same native and non-native ranges that other closely related congeneric invasive species. It was experimentally produced a cohort of *C. sulphurea* individuals from their native range in Spain, non-native range of California, and hybrids from both regions. Then, plants were grown in pots in competition with the grass *Bromus hordeaceus,* or in control pots. Individuals from California were found to be larger and better competitors than individuals from Spain, whereas inter-regional hybrids from both regions showed competitive responses more similar to that of individuals from California. The results confirm that increased competitive ability might be more frequent than previously thought among introduced species regardless of their invasive success. They also shed light on the mechanisms by which locally adapted traits are conserved and spread in the non-native ranges of exotic species, and suggest that size and competitive ability are not directly associated in this species.

INTRODUCTION

Native and non-native populations of plant species are typically isolated in allopatric distributions, and geographic isolation among populations drastically reduces gene flow among them (Bateson 1909, Dobzhansky 1937, Muller 1940, Renaut et al. 2013, Noutsos et al. 2014). Comparative studies of trait-shifts between populations from native and non-native ranges of exotic species repeatedly find rapid evolutionary changes between them (Maron et al. 2004, Hierro et al. 2005, García et al. 2013); including differences in germination, growth rates, seed production and size, and plant size and competitive ability (Graebner et al. 2012, García et al. 2013). Local adaptations developed in the non-native ranges have proved to be crucial for the success of exotic species in their non-native ranges (Keller and Taylor 2010, Zenni et al. 2014). Most established exotic species naturalize in their non-native ranges but do not significantly expand from a few initial introduction points, however, a few of them are able to significantly spread and cause important damage to the environment and the economy (Richardson et al. 2000). One of the most important factors contributing to invasive success is the development of increased competitive ability (Reinhart & Callaway 2006, Ridenour et al., 2008; Graebner et al., 2012; Kaur et al., 2012; He et al., 2012; Shah et al., 2014).

The Evolution of Increased Competitive Ability (EICA) (Blossey & Nötzold 1995) poses that the escape from specialist herbivores present on the native range but not on the newly colonized regions favours the reallocation of resources devoted to herbivory defence in the non-native range to increased competitive ability in the non-native range, typically via increases in plant size and growth rates. Although several studies found no clear evidence of direct growth-defence trade-offs when increased competitive ability is reported (Joshi & Vrieling, 2005; Bossdorf et al., 2005), others did find reallocation of resources between defence and growth, a clear indicator of EICA (Siemann & Rogers, 2001, 2003; Huang et al, 2010, 2012; Carrillo et al, 2012), and yet others found that non-native populations were both better defended and better competitors (Ridenour et al. 2008). Nonetheless, increased competitive abilities of exotic species can result in strong impacts on native plant populations (Maddox 1985, Pennings & Callaway 1992, He et al. 2003, Eriksen et al. 2012, Dlugosch et al. 2015).

Comparison of invasive and non-invasive species has demonstrated to be a fruitful way to predict which traits might be responsible for invasive success, particularly when congeneric species with different invasive success are studied (Gerlach & Rice 2003). A step further consists in considering these congeneric species both in their native and non-native ranges (Graebner et al. 2012; Garcia et al. 2013). Interestingly, such studies regularly report that many traits which are found to be advantageous for invasive species are also present in their non-invasive congeneric exotics. Additionally, little is known about the inheritance and dominance of expression of locally adapted traits from isolated populations from the native and non-native ranges, and thus about the susceptibility of traits newly developed in the non-native ranges to be washed out by homogenizing gene flow from potential new reintroductions from the native range.

Centaurea sulphurea Lag. is an annual herb with a highly restricted native range in Spain and Morocco. It was introduced into California (USA) since at least 1923 (Muth & Pigliucci 2006) and it is currently naturalized there, occurring only in a few populations in this non-native area. It usually inhabits ruderal habitats and develop single bolting flower stems from basal rosettes. Several past studies showed relevant phenotypic disparities between individuals from the native and non-native regions of *C. sulphurea,* and between this species and the closely related, highly invasive*, C. solstitialis* (Graebner et al. 2012; García et al. 2013, Filipe et al. 2016).

Seeds were collected from both the native and non-native ranges of this annual herb and experimentally produced a cohort of inter-regional hybrid seeds by manual cross pollinations under common garden conditions (Montesinos et al. 2012). Then, a competition experiment was set up to compare competitive ability of individuals derived from seeds from native and non-native regions and for inter-regional hybrids. Specifically, we aimed to compare (1) the competitive ability of *C. sulphurea* individuals from its European native range, American non-native range, and their inter-regional hybrids, and (2) assess the inheritance of competitive ability on inter-regional hybrids.

MATERIAL AND METHODS

Experimental design

The trial used seeds collected in 2009 from seven different individuals from each of four different populations in Spain and three different populations in California (USA) (see Supplementary Table 1). The sampling covered the entire geographic distribution of the species in Spain, and included all current populations in California (intensive search in all other formerly known populations deemed no results). In early 2013 seven seeds from each population within each region were germinated and grown in common garden in 2 L pots and watered as needed; temperatures were kept between 15 and 30°C. Plants were grown until bolting, when we applied several manual cross-pollination treatments among individuals within and between regions, thus obtaining an F1 cohort of pure and inter-regional hybrid seeds with reduced maternal effects (see full description in Montesinos et al. 2012). In March 2015 seeds from ten different individuals from each of the seven populations, and seeds from 70 inter-regional hybrids between the two regions were germinated and grown under similar conditions. Albeit in this experiment, full-sibling plants were germinated and grown in 500 mL pots either alone in control pots, or in competition with one individual *Bromus hordeaceus*, a European native annual grass common to both ranges from where original *C. sulphurea* seeds were first collected ($N_{Centaurea}$=280; $N_{B.hordaceus}$=155). *Bromus hordeaceus* seeds were purchased from Emorsgate Seeds (UK). For *C. sulphurea*, descendants from exactly the same exact father and mother were used for each paired replication (i.e. competition and control pots) in order to reduce genetic and maternal effects. Each experimental group had ten control and ten competition duplicates. A control group of

B. hordeaceus was also seeded (N=15). Plants were grown for 80 days, after which they were harvested and dried at 70ºC for 48h, then weighed to the nearest mg.

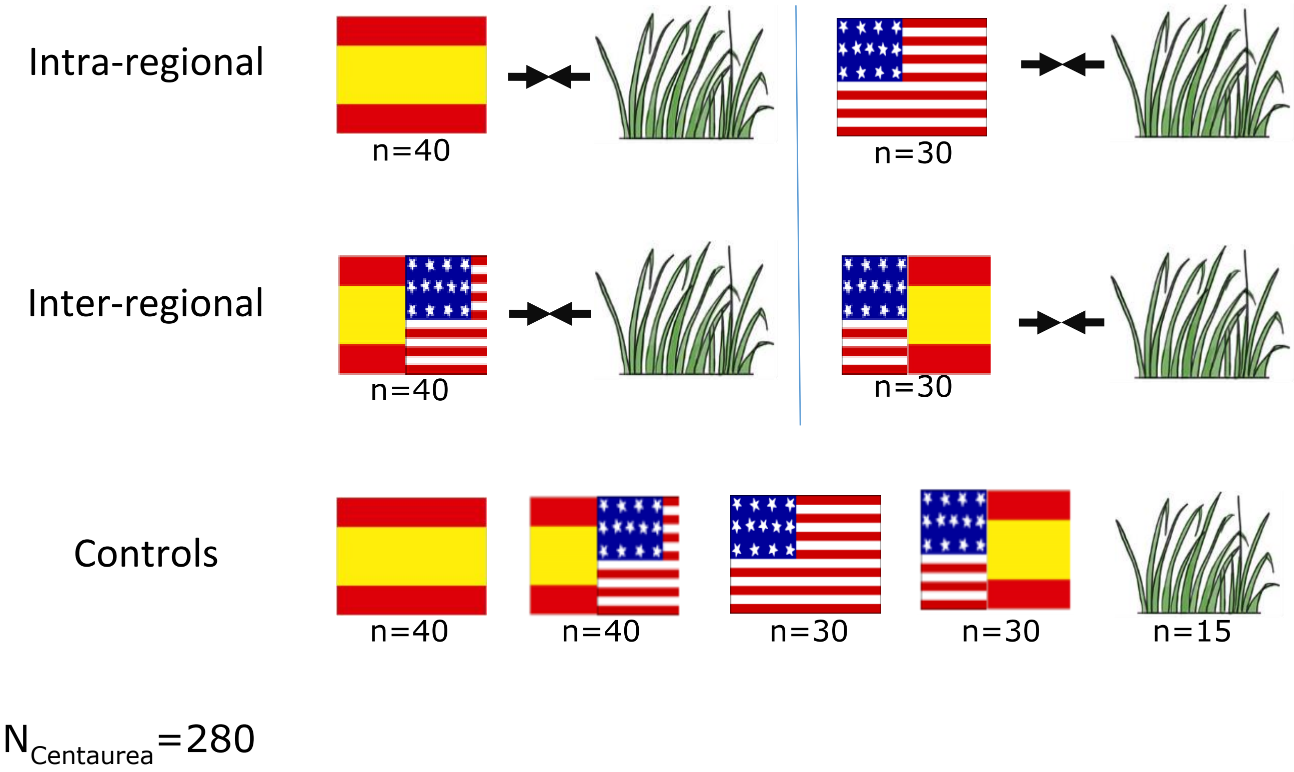

Figure 2.1 Schematic representation of the experimental design for the one-to-one competition experiment between *C. sulphurea* and *B. hordeaceus*. Flags indicate origin of the *C. sulphurea* plants (native Spain), non-native California-USA, inter-regional hybrids with Spanish mothers, or inter-regional hybrids with Californian mothers). Green grass figures indicate competition with *Bromus hordeaceus*.

Statistical analyses

Differences in total biomass among seed origins were tested with Linear Mixed Models in R 3.1.2 with the procedure *lme* in R's *multcomp* library (R Development Core Team 2010). For total biomass, plant dry-weight was used as the response variable, and each of the four possible combinations of *C. sulphurea* seed origin (pollen donor and pollen receiver) grown either in competition or control as a fixed factor with 8 groups. Maternal population of origin was used as a random effect nested within each treatment. Separate tests were run for *C. sulphurea* biomass, and for *B. hordeaceus* biomass. Since only one control group was available for *B. hordeaceus*, we used three dummy data-groups to respect the same data structure than that used for C. sulphurea, and results from those dummy groups were discarded during the interpretation of the post-hoc analyses. Tukey post-hoc tests were run when required by using the *glht* procedure.

Biomass data was then used to assess the strength of competitive interactions by calculating Relative Interaction Indices (RII) (Armas et al. 2004):

$$RII = (B_w - B_0) / (B_w + B_0),$$

in which B_0 corresponds to the biomass of a control individual, and B_w corresponds to the biomass of a full sibling individual grown in competition. RII has defined limits [−1, +1], presents approximately normal distribution, and is symmetrical around zero, with negative values indicating stronger competition effects and positive values indicating the strength of mutualistic interactions. RII values were then statistically compared by a similar procedure to that used with total biomass values, but with a fixed factor (RII) of four levels, corresponding to each of the four possible combinations of *C. sulphurea* seed origin.

RESULTS

Centaurea sulphurea total biomass was significantly different among groups ($F_{7,266} = 31.525$, $p < 0.001$). Post-hoc tests showed that descendants of Californian mothers and fathers were significantly larger ($p < 0.02$) than any other group in the control treatment. When in competition with *B. hordeaceus*, *C. sulphurea* descendants from Californian mothers were significantly larger than individuals from Spanish mothers. Individuals of *Bromus hordeaceus* competing with *C. sulphurea* also showed significant differences among groups ($F_{7,266} = 45.328$, $p < 0.001$). Post-hoc tests showed that grasses competing with descendants from Spanish mothers and fathers experienced lower competitive effects, and thus grew larger than the two groups with Californian ancestry ($p < 0.025$; Fig. 2.2).

Relative interaction indexes (RII) based on total biomass showed a coherent pattern. Significant differences were found among the effects of *B. hordeaceus* on *C. sulphurea* ($F_{3,130} = 22.285$, $p < 0,001$) (Fig. 2.3), although post-hoc tests showed that only individuals with both parents from Spain were significantly different from the other groups ($p < 0.001$), indicating that descendants of Spanish mothers and fathers were more negatively affected by competition than individuals of any Californian descent. For the effects of *C. sulphurea* on the competitor grass *B. hordeaceus*, differences among groups were only marginally significant ($F_{3,130} = 2.296$, $p = 0,081$), and post-hoc test did not indicated any significant difference among groups.

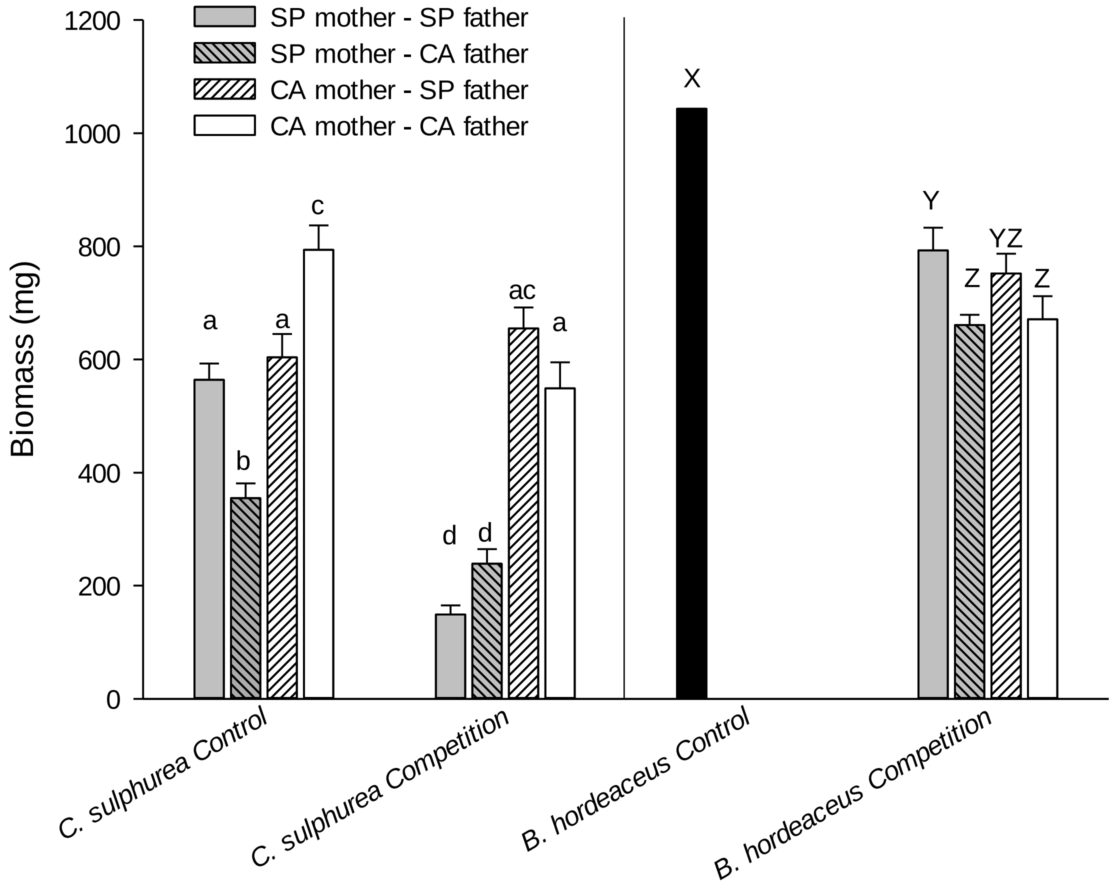

Figure 2.2. Total final biomass (mean ± SE; mg) for one-to-one competition experiments between *Bromus hordeaceus* and *C. sulphurea* from California (CA), Spain (SP), or inter-regional hybrids. Descendants of Spanish mothers are shown in grey bars and Californian mothers in white bars. Open bars stand for descendants of fathers and mothers from the same regions, and lined bars for descendants of parents from different regions. Means with different letters are significantly different ($p \leq 0.05$).

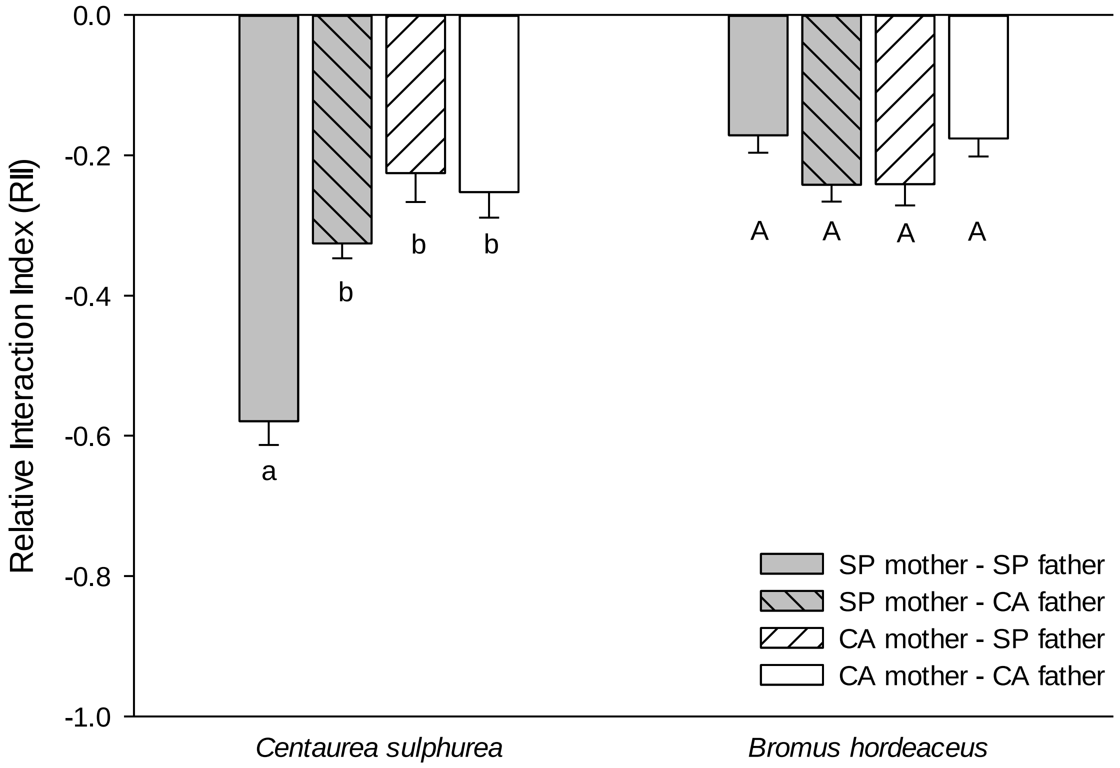

Figure 2.3 Relative Interaction Indexes (RII) for one-to-one competition experiments between *Bromus hordeaceus* and *C. sulphurea* from California (CA), Spain (SP), or inter-regional hybrids. Descendants of Spanish mothers are shown in grey bars and Californian mothers in white bars. Open bars stand for descendants of fathers and mothers from the same regions, and lined bars for descendants of parents from different regions. Means with different letters are significantly different ($p \leq 0.05$)

DISCUSSION

The results revealed clear differences between descendants of individuals from the native and the non-native ranges of *C. sulphurea,* with consistently larger individuals from California, and consistently smaller individuals from Spain. Relative interaction indexes (RII) also showed that descendants of Spanish fathers and mothers experienced significantly stronger competition than individuals of any Californian descent. Interestingly, inter-regional hybrid individuals with Californian mother and Spanish fathers not experiencing competition, tended to present sizes similar to pure Spanish plants, but when experiencing competition, these same inter-regional hybrids tended to present values more similar to pure Californian plants, a pattern also manifested by RII. This suggests that size and competitive ability might not be intimately bonded in this species, since some inter-regional hybrids tended to be smaller in the absence of competition, like their Spanish parents, but over-performed when in competition, like their Californian parents. Regardless, maternal ascents seemed to play a more important role, particularly when total biomass is observed, perhaps as the result of some residual maternal effects, even after one generation in common garden conditions.

A previous study (Graebner et al. 2012) reported increased competitive ability against the same grass competitor species for Californian individuals of *C. sulphurea* when compared to their Spanish counterparts. Although this previous study showed a similar trend, differences in total biomass were not deemed statistically significant, likely as a result of the smaller sample size used, of an experimental design that did not allow for the calculation of Relative Interaction Indexes, or perhaps because of the maternal effects inherent to plants grown from seeds that had been collected from the wild.

Consequently, these results provide the first evidence for a better competitive performance of the exotic populations of *C. sulphurea* when measured in one-to-one competition with a grass species. Although *C. sulphurea* is not considered an invasive species in California, it is closely related to the highly invasive *C. solstitialis* (García-Jacas et al. 2006), and the results are consistent with former studies showing how exotic species have developed increased competitive ability in their non-native ranges (Bais et al. 2003, Ridenour et al. 2008, Zou et al. 2008, Hahn et al. 2012, Graebner et al. 2012). Thus although increased competitive ability is deemed as crucial for invasive success (Blossey & Nötzold 1995, Jakobs et al. 2004, Brown & Eckert 2005, Keller and Taylor 2010, Tavares 2014, , Zenni et al. 2014), the results also indicate that increased competitive ability might be a necessary but not sufficient condition to become a successful invasive species, in line with previous works indicating that non-invasive exotic species are able to develop many of the same local adaptations that invasive species present (García et al. 2013).

Exploring if native and introduced populations react differently to competition with the same competitor is central to evaluate whether commonly observed phenotypic differences between native and non-native ranges do actually result into greater competitive ability in the non-native regions of invasive species. The study of experimentally produced inter-regional hybrids between native and non-native ranges might play an important role to understand the biological invasions. The observed divergence between native and non-native populations might be the result of founder events or of rapid post-introduction evolution, as suggested by other studies (Maron et al. 2004, Hoskin et al. 2005, Montesinos et al. 2012), or a combination of both. However, the present study is consistent with the EICA hypothesis (Blossey & Nötzold 1995), and recent studies with the same study species showed that snails fed with *C. sulphurea* leaves from California presented higher growth rates than snails fed with leaves from Spain, suggesting that individuals on the non-native range of California might present reduced defences against generalist herbivores than in native Spain (Filipe et al. 2016). A very similar combination of increased size and vulnerability to herbivory, consistent with EICA, was described for *Sapium sebiferum*, a native plant from China in which plants from the invasive range in North America attained larger size and experienced stronger herbivory than plants from native China in an experimental setup (Zou et al. 2008).

Two of the most remarkable conclusions that can be drawn are that (1) non-native populations of a non-invasive exotic species present increased levels of competitive ability, a trait which is often regarded as a sufficient condition for invasiveness; and (2) inter-regional hybrids of the native and non-native regions showed increased competitive abilities when compared to the pure native individuals, illustrating the way by which newly developed traits spread in the non-native regions of invasive species, even in the event of homogenizing gene flow from the native range. In fact, reintroductions are believed to be key to enrich the genetic diversity available in exotic species for natural selection to act (Eriksen et al. 2014), confirming the importance of avoiding new reintroductions of exotic species which have already developed some of the adaptations which are believed to be key for invasive success

(Blossey & Nötzold 1995, Jakobs et al. 2004, Brown & Eckert 2005, Tavares 2014, Keller & Taylor 2010, Zenni et al. 2014).

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APPENDIX

Supplementary Table 1 -- **Origin of *Centaurea sulphurea* seeds used.**

Coordinates are given in WGS84.

Region	*Location*	*Latitude*	*Longitude*
Spain	Malaga	36.840082	-3.981880
	Malaga	36.679348	-4.832505
	Malaga	36.907818	-4.118162
	Granada	37.172880	-3.579110
California (USA)	Sacramento	38.640605	-121.156370
	Santa Clara	37.245630	-122.110390
	Sacramento	38.684210	-121.180600

Chapter 3
Herbivore Response

ABSTRACT

Once introduced into new regions, exotic species often experience shifts in resource allocation in response to the different environmental conditions found in the introduction range. Plants naturally respond to specialist herbivores with quantitative defences, by producing large amounts of toxic and non-toxic compounds that typically difficult digestion (e.g. tannins, cellulose), and to generalist herbivores with qualitative defences, like specialized noxious chemicals (e.g. alkaloids). The Shifting Defence Hypothesis (SDH) poses that invasive plants decrease the production of defences against specialist herbivores in their introduction range, where specialist herbivores are usually absent, while boosting the production of defences against generalist herbivores. The aim of the following study was to assess the response of a generalist herbivore, the common garden snail (*Helix aspersa*), to feeding with leaves of the annual herb *Centaurea sulphurea*, native to Europe and naturalized in North America; and the congeneric species *C. solstitialis*, which is also native to Europe and invasive in the Americas. Snails fed with leaves from Spanish native populations of the non-invasive *C. sulphurea* grew significantly less compared to snails fed with leaves from non-native California. For snails fed with the invasive *C. solstitialis*, significant differences were also found among regions, but the response was more complex, depending on population, with snails fed with Turkish and Australian plants presenting higher growth rates than the rest of the regions. Overall, the following results stressed the importance of colonization history in shaping adaptive responses, and the stochasticity associated with colonization events of two closely related species, with contrasting invasive success and responses to herbivory.

INTRODUCTION

Plants are at the base of most terrestrial food webs, and herbivorous organisms play an important role in the consumption of the total vegetal biomass produced annually worldwide (Carmona et al. 2011). Plant-herbivore interactions often result in the development of diverse herbivore defence traits in plants in order to reduce the damage and impact produced by herbivory, and at the same time herbivores can evolve to respond to plant defences in an “arms race” (Berenbaum 1981).

Plant defences can be chemical (secondary metabolites; e.g. terpenoids, glucosinolates, tannins) and physical (e.g. latex, trichomes) (Ehrlich & Raven 1964). Defensive traits can also be classified according to how they act as either qualitative or quantitative (Müller-Schärer et al. 2004). Quantitative defences are meant to provide resistance against specialist herbivores, and are based on digestibility reducers from the primary (e.g. cellulose) or secondary plant metabolism (e.g. tannins) that typically occur at high concentrations inside plant tissues. Qualitative defences are aimed against generalist herbivores, and include secondary plant metabolites with poisonous effects even at low concentrations (e.g. sesquiterpene lactones; Müller-Schärer et al. 2004). Although secondary metabolites have been reported as important assets to defend plants from herbivores, a recent meta-analysis (Carmona et al. 2011) suggests that their importance for such trait is comparatively smaller than expected with other traits, such as genetic variation in life-history traits, leaf and stem morphology, and primary chemistry and physiology.

The success of some exotic species may be the result of a broad range of ecological and evolutionary factors, such as escape from enemies which are not present in non-native ranges (Keane & Crawley 2002). In their native ranges, exotic plants are the target of highly kinds of herbivores with whom they share a complex evolutionary history (Schaffner et al. 2011). However, in their non-native ranges, these plants are released of specialist herbivores but they can still be attacked by native generalist herbivores (Memmott et al. 2000). In this context, the Shifting Defence Hypothesis (SDH hereafter) proposes that, in their non-native range, exotic plants should reduce resource investments into specialized defences while increasing investments in defences aimed to generalist herbivores (Joshi &Vrieling 2005).

A recent study evaluated epicuticular morphological and chemical defences of invasive *Centaurea solstitialis* leaves from native and non-native ranges (Sotes et al. 2015). The results showed a significant production of sesquiterpene lactones, an important toxin with known activity against generalist herbivores (Amorim et al. 2013), that gradually increased in C. *solstitialis*' non-native regions (Sotes 2015). Other preceding works with *Centaurea* invasive species had also suggested that sesquiterpene lactones could play a significant role in their success in their non-native regions (Kelsey & Locken 1987, Stevens et al. 1990 and Landau et al. 1994). In order to experimentally assess for potential differences in SDH patterns, a generalist herbivore - *Helix aspersa* snail, was fed with leaves from the invasive *C. solstitialis*, and also with leaves of the exotic but non-invasive *C. sulphurea*.

Plants were grown in a common garden with seeds originating from native and non-native regions of each species and it was assessed how these potential defensive trait shifts affected the growth and survival of a generalist herbivore present across those same regions. Specifically, the aim was to check if snail growth and survival was related to: (1) the region origin of the leaves with which they were fed; (2) the species of the *Centaurea* plants used to feed them, and (3) the invasive success of each of the *Centaurea* species.

MATERIALS AND METHODS

Study species

Centaurea solstitialis L. is an annual herb native to Southern Europe and Turkey which was introduced into California since at least 1824 and where it has become an aggressive invader (Figure 3.1; Maddox et al. 1985). *Centaurea sulphurea* Lag. is an annual herb native to Southern Europe and northern Africa and it was also introduced into California (USA) since at least 1923 (Muthand Pigliucci 2006). *Centaurea solstitialis* occurs over broad native (Southern Europe) and non-native ranges (Americas and Australia), whereas *C. sulphurea* has a highly restricted native range in Spain and Morocco, and occurs at only a few populations in California (Gerlach and Rice 2003). Both species inhabit the same ruderal habitats, and develop single bolting flower stems from basal rosettes. Both species form large spines on their capitula, providing a common trait for which to compare allocation to defence (García at al. 2013).

Figure 3.1 Field invaded by *C. solstitialis*, California, USA. Photo credit: Daniel Montesinos.

Helix aspersa Müller, 1774 is a generalist herbivore snail native to the Mediterranean region and Western Europe, from northwest Africa and Iberia eastwards to Asia Minor, and northwards to the British islands. *H. aspersa* has been spread by humans, either deliberately or accidentally, and became naturalized in many regions with non-Mediterranean climates. It is present in Australia, New Zealand, and North and South America. It feeds on a variety of leaves, including a broad range of wild and domesticated plants (Pfleger and Chatfield 1983).

Experimental Design

For *C. sulphurea*, the seeds used were collected in 2009 from seven different individuals from each of four different populations in Spain and three different populations in California (USA). For *C. solstitialis*, seeds originated from seven different individuals from each of 48 different populations sampled among six different regions (Turkey, Spain, Chile, Argentina, U.S.A and Australia; see Supplementary table 1) and also collected in the same year. For *H. aspersa*, the snails were collected from the same cohort from a commercial snail farm. Snails had been grown in the same farm for more than 10 generations, and were regularly fed with flour for snails, thus preventing the used snails from being pre-adapted to any specific *Centaurea* seed origin. Snails were provided by the commercial snail farm *Casa às Costas* (Coimbra, Portugal), where a cohort of eight weeks old snails of similar size (young adults) was selected for the experiment.

In February 2013, seeds from each population were germinated and grown in 2.2 L pots with a 30:70 mix of grit sand and farm soil and watered as needed. Plants were grown in common garden greenhouse conditions in Coimbra, Portugal, and the experiment started when all of them had grown at least ten leaves.

Prior to the start of the experiment, snails' total biomass was determined to the nearest milligram in a laboratory scale, measurements were repeated weekly and the experiments finished after four weeks for *C. solstitialis*, and two weeks for *C. sulphurea*. The difference in experimental times was due to differences in the leaf-phenology of the two *Centaurea* species. Snails were put in transparent 0.3 L plastic flasks with the opening covered with a mesh fixed to the flask with an elastic rubber band and placed in a randomized design onto a table with controlled temperature and light conditions. Feeding was made by dropping leaves inside each flask every other day. After every feeding, fresh water was also sprayed inside of the flask in order to keep the humidity levels stable so the snails would not estivate due to dry conditions. Snails were fed *ad libitum* for the duration of the experiment, and each snail was always fed with leaves from the same individual plant. Since snails always had abundant fresh leaves to eat, potential differences in snail's growth would be more parsimoniously explained by differences in leaf herbivory defences that by differences in leaf nutrient content.

Statistical analyses

Growth rates were calculated as the absolute difference between final and initial snail biomass, divided by the number of days. Data from both plant study species was analysed with R 3.2.2. Growth rate (GR) was based on absolute values and analysed by means of linear-mixed effects models (procedure "lme") with region as a fixed factor, population within region as a random factor and GR as the study variable. Data was log-transformed for homoscedasticity. When necessary, Tukey's post-hoc was run (procedure "multcomp").

RESULTS

Snails fed with leaves of *C. sulphurea* of Californian origin presented GR values more than 80% higher than those fed with leaves of Spanish origin (L.Ratio3,4 = 7.040; logLik = -17.507; p = 0.008). The random factor “population” presented standard deviations (SD) and residuals nearing zero, thus indicating that its importance in structuring the data was likely low (SD = 3.290 10-7; Residual = 0.008) (fig. 3.2).

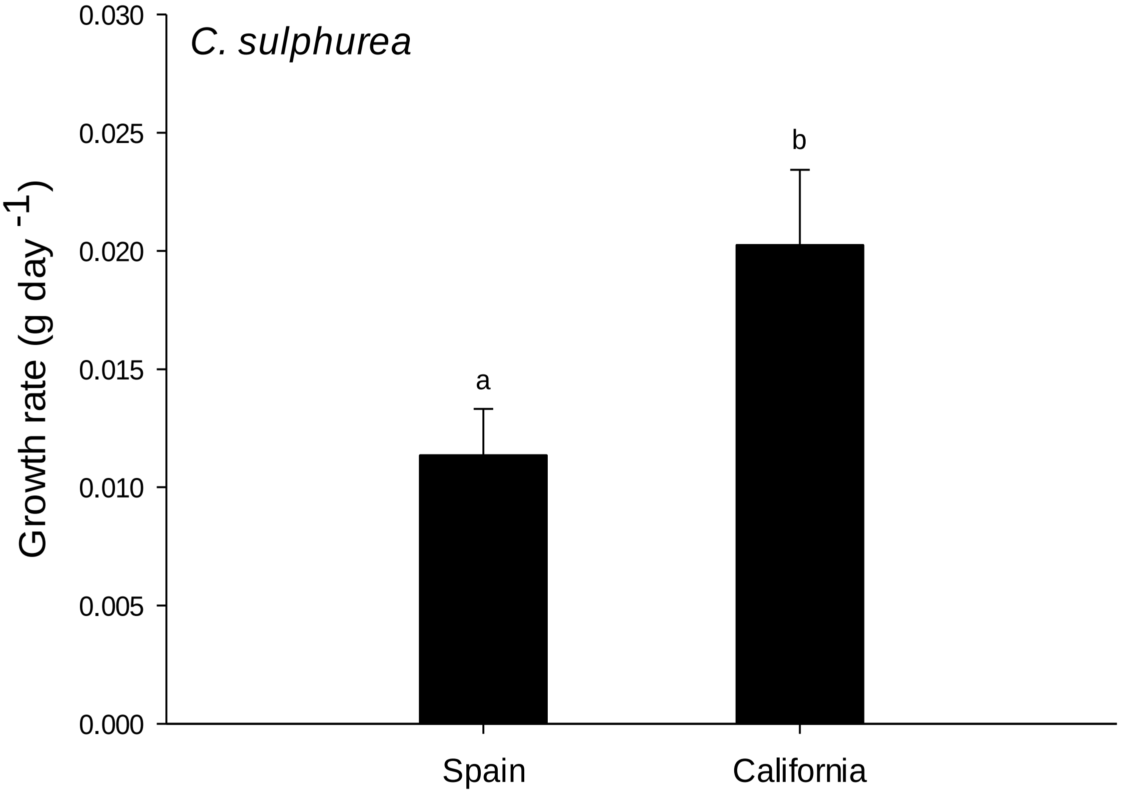

Figure 3.2 – *Helix aspersa* growth rate based on absolute mass gains per day (g; mean ± SE) when fed with *Centaurea sulphurea* leaves from plants from each region, native (Spain) and non-native (California). Means with different letters are significantly different (p = 0.008).

For *C. solstitialis*, significantly different GRs among some regions were found (L.Ratio3,8 = 18.880; logLik = 639.913; p = 0.002). The random factor "population" presented SD also nearing zero, and although it presented slightly higher residuals its importance in structuring the data was also likely low (SD = 6.635 10-6; Residual = 0.348). Post-hoc analyses showed that Turkish populations were significantly different (p < 0.05) from every other region except for Australia (z = -0.453; p = 0.997) and marginally different from Chile (z = -2.753; p = 0.0615) (Fig. 3.3). Net mass gain for each treatment paralleled RGR results, and can be viewed on Table 1.

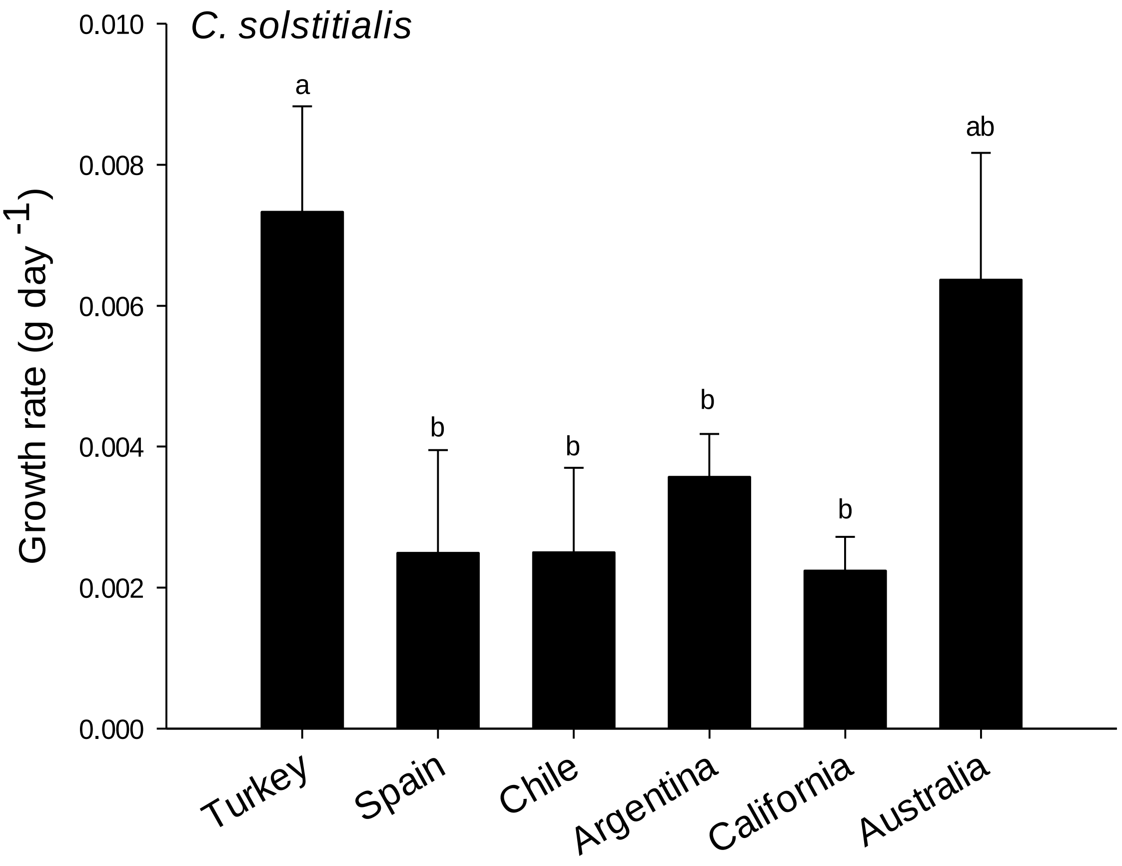

Figure 3.3 – ***Helix aspersa* growth rate based on absolute mass gains per day (g; mean ± SE) when fed with *Centaurea solstitialis* leaves from plants from each region, native (Turkey, Spain) and non-native (Chile, Argentina, California and Australia).** Means with different letters are significantly different (p ≤ 0.05).

Table 1 – Total biomass gain by snails for each *Centaurea* species by region. The feeding with leaves from both *C. solstitialis* and *C. sulphurea* finished after 28 and 14 days respectively. Prior to the start of the experiment, a set of snails with similar size was selected and their total initial biomass was determined to the nearest milligram in a laboratory scale.

Species	Region	Mean biomass gain (g)	SE
Centaurea solstitialis	Turkey	0.170	0.039
	Spain	0.095	0.025
	Chile	0.118	0.039
	Argentina	0.130	0.018
	California	0.094	0.011
	Australia	0.174	0.070
Centaurea sulphurea	Spain	0.158	0.027
	California	0.283	0.044

DISCUSSION

For the invasive plant species *C. solstitialis*, snails fed with leaves from Spain, Argentina, Chile and California showed lower growth rates than those fed with leaves from Turkey, with Australia showing intermediate values between the two groups. This suggests that plants from Spain, Argentina and California could be investing more resources into defence against generalist herbivores than plants from Turkey and Australia. These results partially support the SDH hypothesis, and are in agreement with previous chemical studies showing increasing amounts of sesquiterpene lactones from Turkey (53%), to Spain (69%), Chile (72%), and California (75%) (Sotes et al. 2015); in a coherent inverse trend with the growth rate values found on this study for snails fed with *C. solstitialis* leaves, in which higher reported concentrations of sesquiterpene lactones correspond to lower snail growth rates.

The results were also consistent with previous genetic studies describing the migration route of the species. Initially, *C. solstitialis* spread with the expansion of agriculture from Turkey through the Mediterranean to Spain. Later, it was introduced into Argentina, Chile and California (Eriksen et al. 2014). In this context, is thus interesting to ponder the similarity between the values found for Turkey and Australia, which point towards a potential Turkish origin of seeds invading Australia, although genetic data for Australian populations is currently missing.

Interestingly, snails fed with leaves from the non-native range of the non-invasive *C. sulphurea* presented higher growth rates than snails fed with leaves from the native Spain, i.e. exactly the opposite to what was found for *C. solstitialis*, suggesting that different trait-shifts in herbivory defence might be involved in the different invasive success of each species in California, where *C. sulphurea* might be more vulnerable to generalist herbivores.

Inter-regional trait-shifts among plants from native and non-native regions, such are the ones presented here, typically include traits like higher germination rates, reproductive outputs, and size (Ridenour 2008, Jakobs 2004). Some of these traits could be the result of resource trade-offs with either generalist or specialist herbivore defences (Willis 1999, Maron 2004), which could be interpreted as an evolutionary trade-off in the context of the hypothesis of Evolution of Increased Competitive Ability, EICA (Blossey 1995). These results indicate that herbivory and herbivory defence might underlie the contrasting invasive success of these two species, although a recent meta-analysis suggests that in fact secondary metabolites might play secondary role for the anti-herbivore defence of plants when compared with other traits (Carmona et al. 2011).

In conclusion, the results are partially supportive of the Shifting Defence Hypothesis (SDH), with the invasive species *C. solstitialis* showing a tendency towards reducing growth of generalist herbivores in its American non-native range, and the non-invasive *C. sulphurea* showing the opposite pattern. However, further studies are needed to confirm this pattern, and to test it with other herbivores (e.g. aphids). In any case, these results should be interpreted with caution, since some trait-shifts are known to occur both in invasive and non-invasive species, and might not be necessarily responsible for invasive success, which could be better explained by a complex combination of traits (García et al. 2013).

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APPENDIX

Supplementary Table 2-- **Origin of *Centaurea* seeds used**.
Coordinates are given in WGS84.

Species	Region	Location	Latitude	Longitude
C. solstitialis	Turkey	Pınarkent	37.802833	29.195250
		Dazkırı	37.950833	29.840333
		Isparta	37.898444	30.438278
		Burdur	37.616083	30.146167
		Serinhisar	37.531556	29.300861
		Dinar to Çay	38.153917	30.234167
		Beydağ	38.085861	28.215472
		Bozdağ	38.301361	28.049861
		Akçaköy	37.952917	28.029972
		Geyre	37.712306	28.692694
	Spain	Cuenca	40.241590	-2.687453
		Teruel	40.461633	-1.615508
		Tarragona	41.068820	0.334305
		Lleida	41.504990	1.005857
		Lleida	41.877755	0.778373
		Cuenca	39.847187	-2.501784
		Salamanca	41.026140	-5.605175
		Burgos	41.728801	-3.834349
	Argentina	Paraje El Tropezón	-36.709000	-64.831055
		El Durazno	-36.700077	-65.391416
		Rucanelo	-36.708944	-64.830833
		Victorica	-36.250111	-65.454250
		Winifreda	-36.223972	-64.281333
		Santa Rosa	-36.616583	-64.256250
		Trenel	-35.724583	-64.269277
		Quehué	-37.121611	-64.286611
		Unanue	-37.559666	-64.291500
		Lonquimay	-36.565527	-63.664666
	Chile	Talagante	-33.570833	-70.855277
		Talagante	-33.689444	-71.055277
		Santiago	-33.538888	-70.851111
		Santiago	-33.524722	-70.751666
		Santiago	-33.370000	-70.429722
	California (USA)	Sacramento	38.642150	-121.175960
		Sacramento	38.682934	-121.181127
		Santa Clara	37.242980	-122.870740
		Marin	38.156220	-122.692620
		Solano	38.203570	-122.156220
		Solano	38.209540	-122.146310
		Sonoma	38.224558	-122.534050
		Sonoma	38.236430	-122.563780
		Napa	38.339041	-122.154670
		Solano	38.410590	-121.934338
		Napa	38.453530	-122.152875
	Australia	Colbinabbin	-36.595489	144.736346
		Holbrook	-36.677787	147.369684
		Gundagai	-35.067197	148.108528

Species	Region	Location	Latitude	Longitude
C. sulphurea	Spain	Malaga	36.840082	-3.981880
		Malaga	36.679348	-4.832505
		Malaga	36.907818	-4.118162
		Granada	37.172880	-3.579110
	California (USA)	Sacramento	38.640605	-121.156370
		Santa Clara	37.245630	-122.110390
		Sacramento	38.684210	-121.180600

PART III

Chapter 4
General Discussion and Conclusions

CONCLUSIONS

Overall, these results suggest:

(1) Non-native populations of the non-invasive exotic *C. sulphurea* presented increased levels of competitive ability, a trait which has been frequently regarded as a sufficient condition for invasiveness.

(2) Inter-regional hybrids of the native and non-native regions of *C. sulphurea* showed increased competitive abilities when compared to native Spanish individuals, shedding light on the mechanisms by which newly developed traits spread in the non-native regions of invasive species, even in the event of homogenizing gene flow from the native range.

(3) For the invasive plant species *C. solstitialis*, snails fed with leaves from Spain, Argentina, Chile and California showed lower growth rates than those fed with leaves from Turkey, with Australia showing intermediate values between the two groups. This proposes that plants from Spain, Argentina and California could be investing more resources into defence against generalist herbivores than plants from Turkey and Australia.

(4) Snails fed with leaves originating from seeds from the non-native range of the non-invasive *C. sulphurea* presented higher growth rates than snails fed with leaves from the native Spain, a different pattern to what was found for *C. solstitialis*, suggesting that different trait-shifts in herbivory defence might be potentially responsible for the different invasive success of each species in California, where *C. sulphurea* might be more vulnerable to generalist herbivores.

(5) Partial support of the Shifting Defence Hypothesis (SDH) was found, with the invasive species *C. solstitialis* showing a tendency towards reducing growth of generalist herbivores in Spain, but also in all the American non-native regions, when compared to native Turkey, while the non-invasive *C. sulphurea* showed the exact opposite.

Both ecological and evolutionary differences between native and non-native populations may have significant effects on the different invasion ability of the studied species. The ensemble of results clearly exposes a convincing essay for genetic based differences in life-history traits between the native and non-native populations of both *Centaurea* species.

Of course it is difficult to generalize from a limited study on only two congeneric taxa, but we can however conclude that invasive plants can exhibit a broad range of adjustments in traits due to a wide variety of changes of different nature: abiotic conditions, competition, and lack of specialist herbivores; all of these with significant consequences for population dynamics. Our results are consistent with the escape from herbivores hypothesis. However, only a great number of studies on a broad array of species could assess the relative importance of this factor in determining size differences or herbivory patterns between regions of origin and introduction.

Future research

Previous studies, like Willis et al. (1999), found that individuals of *Lythrum salicaria* (purple loose-strife) from introduced populations were taller than those of native populations; although they found no differences in herbivore resistance in individuals from any introduced populations when compared to the native populations. Like many studies previously cited, our results once more suggest that seeking a single hypothesis or trait to explain invasive success is inappropriate, and that the specific combination of species, biotic and abiotic conditions, and history, seem to be unique for each invasion.

The present work indicates that the different invasive success observed between *C. solstitialis* and *C sulphurea* may be driven by their specific patterns of local adaptation. This system may thus be a good candidate to look for further evidence of morphological and ecological differentiation in order to gain understanding of the extent to which local adaptation occurs in invasive species across broad biogeographic regions. Further studies about which traits contribute to the disparate invasive success of these two closely related congeneric species are desirable, and may set the ground for the possible future development of management strategies and control methods (Müller-Schärer et al. 2004).

Final remarks

Apart from ecology itself, invasion science is constantly broadening its scope into more diverse and interdisciplinary areas that range from human history and geography to immunology, epidemiology, and palaeontology (Kueffer & Hirsch Hadorn, 2008; Richardson, 2011). Globalization has been driving tremendous rates of invasions (Ricciardi, 2007), thus one of the principal goals of the field, if not the main, should be predicting the potential of introduced species to become noxious invaders with deep impact in societies and the environment, posing a true challenge for any management assessment. To answer global climate change, new concepts and tools are being incorporated into sophisticated technologies as molecular-genetic methods and remote sensing, in a daring race against time to understand and manage biodiversity (Schweiger et al., 2010). Recent studies consider of the assessment of novel ecosystems (Richardson & Gaertner, 2013), managed relocation (Ricciardi & Simberloff, 2009), and methods of risk estimation for rising threats (Leung et al., 2012; Dick et al., 2014).

Decades of peer-reviewed science confirm biological invasions as a first rate global threat for biodiversity conservation. It is thus crucial to improve investigation on this field. The study of plant traits might contribute at least partially to predict future plant invasions, thus being a valuable step for the development of management policies. With the knowledge we have gained, we need to move forward and keep the ecosystems balanced and sustainable.

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