Clinal variation in life-history traits of the invasive plant species *Echium plantagineum* L.

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Certificate of Authorship/Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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Abstract

Range expansion during the invasion of a novel environment requires that invading species adapt to geographical variation in climate and maintain positive population growth in the face of environmental heterogeneity. Thus, invasive species are expected to undergo adaptive evolutionary changes as they encounter novel selection pressures. The aim of this thesis was to identify adaptive changes in plant growth and reproductive traits throughout the lifecycle of a model invasive species to determine which traits are vital to the success of invasive species encountering novel environments.

The model species used in this study was the widespread European invader, *Echium plantagineum,* which has invaded over 33 million ha across Australia, causing ~$30 million (AUD) damage per annum. I investigated geographic variation in life-history traits of 34 populations of *E. plantagineum* across a 1,000 km arid-mesic gradient throughout south-eastern NSW, Australia. Seeds were collected for each population along the arid-mesic gradient, germinated in the laboratory and grown in the glasshouse in a common environment.

I found that *E. plantagineum* has rapidly adapted to environmental selection pressures throughout its range, resulting in two major clines linked to plant flowering time and seed size of progeny. Compared with populations from mesic habitats along the arid-mesic gradient, plant populations from arid environments had significantly higher relative growth rate and leaf production which was associated with much earlier flowering time and reduced time between stem production and flower production. Plants from arid regions also produced significantly larger seeds compared with plants from mesic habitats. Interestingly, seeds from all *E.*

plantagineum populations along the arid-mesic gradient germinated rapidly (within 48 hours of water exposure) allowing them to quickly and opportunistically take advantage of available resources. Considered together, these adaptations allow *E. plantagineum* to grow rapidly, reproduce and produce progeny before conditions become unsuitable.

The findings in this thesis provide compelling evidence for the rapid development, within 150 years, of clines in reproductive strategies linked to flowering and seed size evolution. My results support the notion that the successful invasive spread of species can be increased through genetic divergence of populations along arid-mesic climatic gradients. The climate of south-eastern Australia is predicted to change to become hotter and drier inducing many species to adapt or perish. The range and distribution of *E. plantagineum* is unlikely to be altered by these climatic changes as pre-adapted genotypes currently exist in the range margins and have persisted in arid regions for over 100 years. Consequently, further work is required to investigate the evolutionary capacity of other native and invasive species to determine how ecosystem dynamics and composition may change in the future.

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Acronyms

Δ	Carbon isotope discrimination
AAGB	Average above ground biomass
ACT	Australian Capital Territory
AGB	Above ground biomass
AI	Aridity index (AI = P/PET)
ANCA	Automated Nitrogen Carbon Analysis
AVH	Australian Virtual Herbarium
AWB	Atmospheric water balance (AWB = P - PET)
B	Unstandardised regression coefficient
B_{leaf}	Leaf biomass
BHC	Broken Hill Complex bioregion
BGB	Below ground biomass
C	Carbon
COAST	Coastal bioregion including south-east corner and Sydney basin bioregions
EICA	Evolution of increased competitive ability
FI_{time}	Time to first flower in days
FIV	Flower initiation variance
IBRA	Interim Biogeographic Regionalisation for Australia – a frame work for the grouping of regions by vegetation, climate and soil characteristics
L	Largest leaf length
LAR	Leaf area ratio
LMA	Leaf mass area
LMM	Linear mixed model

MDD	Murray Darling depression bioregion
N	Nitrogen
NPK	Nitrogen, phosphorous and potassium
NSW	New South Wales
P	Precipitation
P_{area}	Photosynthetic area
PC1	Principal component one representative of climate
PC2	Principal component two representative of edaphic factors
PET	Potential evapotranspiration
RGR	Relative growth rate
RIV	Riverina bioregion
RSR	Root:shoot ratio
SE	Standard error
SEH	South-eastern highlands bioregion
SF_{time}	Time between stem initiation and flower initiation
SILO	Climate database that contains Australian climate data from 1889 to current, hosted by the the State of Queensland Department of Environment and Resource Management 2012.
SI_{time}	Time to first stem in days
SIV	Stem initiation variance
SLA	Specific leaf area
SWS	NSW south-west slopes bioregion
t	Time in days
WUE	Water use efficiency
W	Total water use

Chapter 1: Introduction

1.1 Biological invasions

The introduction of exotic species to new geographical regions through human activities is a global phenomenon (Vitousek *et al.* 1996; Chown *et al.* 2012). Internationally, there has been growing concern about such introductions given the role that invasive exotic species play as drivers of unprecedented environmental change (Wilcove *et al.* 1998; Mack *et al*. 2000; Pimentel *et al*. 2001; 2005). The invasive spread of exotic species can lead to a range of detrimental ecological and costly economic impacts (Sinden *et al.* 2004; Pimentel *et al*. 2005; Hulme 2007; Hejda *et al*. 2009; Vilà *et al.* 2010). Indeed, it has been suggested that invasive species pose the second greatest threat to global biodiversity after habitat fragmentation (Walker & Steffen 1997; Allendorf & Lundquist 2003). In this context, the growth and development of the field of invasion ecology, which seeks to determine the patterns and processes of biological invasions, has been timely (Richardson 2011).

Exotic species can be introduced to new regions as a result of intentional transport (e.g. for the horticultural or agricultural industries; Keller *et al.* 2011) or unintentional release (e.g. from contaminated farm or transport equipment; Hulme 2009). Importantly, however, only a subset of introduced exotic species become established (naturalised) and even fewer proceed to spread invasively causing adverse effects on the invaded habitat (Mack *et al*. 2000; Richardson & Pyšek 2006; Hulme 2012). Traversing the introduction-to-invasion pathway requires a species to overcome several barriers (Richardson *et al.* 2000; Blackburn *et al.* 2011) and arguably the most important is the barrier between naturalisation and invasion (Phillips *et al.* 2010). To overcome this barrier, species must spread throughout the

new geographical region (Lachmuth *et al.* 2010). Such invasive spread generally leads to the exposure of populations to a wide range of environmental conditions with their associated selection pressures (Montague *et al*. 2008; Prentis *et al.* 2008; Colautti *et al.* 2010).

The invasive spread of an exotic species across a range of environments provides a rare opportunity not only to study the invasion process but also to examine broader ecological and evolutionary processes (Sax *et al.* 2007). This is because successful invasion of novel environments requires complex interactions between the characteristics of the recipient ecosystem and the life-history traits of the invading species (Closset-Kopp *et al*. 2007). Adding to this already complex situation is the fact that exotic species can invade areas in the introduced range that are outside their predicted climatic envelopes (Broennimann *et al*. 2007; Fitzpatrick *et al*. 2007; Beaumont *et al*. 2009). Adaptation during range expansion is thus an essential component of invasive spread (Reznick & Ghalambor 2001; Sakai *et al.* 2001). Importantly, invasive spread across broad climatic gradients is expected to involve adaptive differentiation of populations (Cox 2004), with such differentiation often expected to produce clines (e.g. Montague *et al.* 2008; Monty & Mahy 2009) as a range of climatic factors vary predictably along geographic gradients (Endler 1977).

1.2 Clinal differentiation of populations during invasion in response to climate

1.2.1 Clinal patterns

A clinal pattern is a gradual change in a trait among the populations of a species over a geographical area (e.g. plant height or flowering time, Endler 1977). Given that

climate has been recognised for a long time as one of the most important abiotic factors related to the distribution of species (Monty & Mahy 2009), there are many examples of clinal patterns in response to climatic gradients (See Table 1.1). Since coping with environmental heterogeneity is vital for an invasive species to overcome the naturalisation to invasion barrier, there are clear advantages for invasive spread. For example, if a species possesses a strategy such as the ability to adapt to a spatially variable environment resulting in the formation of a cline in life-history traits among populations, this may enhance reproductive success and population survival.

Table 1.1 Examples of evidence of clinal variation in native and invasive species in response to climate throughout different life stages.

Species	Clinal pattern	Invasive status	Location	Reference
Verbascum thapsus	Time to flowering production and seed quantity increased with increasing latitude	Invasive	North America	Reinartz 1984
Daucus carota	Age at reproduction increased with increasing latitude	Native	North America	Lacey 1988
Quercus lyrata	Larger acorn size with increasing latitude	Native	North America	Azien & Woodcock 1992
Prunella vulgaris	Flower number and time to first flower increase with decreasing latitude	Native	North America	Winn & Gross 1993
Arabidopsis thaliana	Seed size, rosette size, plant size, leaf number, leaf area and relative growth rate decreased with increasing latitude	Native	Europe	Li *et al.* 1998
Solidago altissima *Solidago gigantean*	Plant size increased and time to first flower decreased with decreasing latitude	Invasive	Europe	Weber & Schmid 1998
Clarkia unguiculata	Flower morphology (petal, style and stamen lengths) increased and physiological traits (photosynthetic rate and stomatal conductance) decreased with increasing latitude	Native	North America	Jonas & Geber 1999
Phragmites australis	Growth rate and time to first flower increase with increasing latitude	Native	Europe	Clevering *et al.* 2001
Lythrum salicaria	Growth initiation, time to first flower, flowering period, plant size decreased, bud size and quantity increased with increasing latitude	Native	Europe	Olsson & Ågren 2002
Poa bulbosa	Time to summer dormancy, age at summer dormancy and flowering capacity decrease with increasing aridity	Native	Middle East	Ofir & Kigel 2003
34 *Glycine* species	Seed size decreases and seed mass increases with decreasing latitude	Native	Australia	Murray *et al.* 2003; 2004
Chamaecrista fasciculata	Time to reproduction, leaf number and leaf thickness increased with decreasing latitude	Native	North America	Etterson 2004

Table 1.1 Examples of evidence of clinal variation in native and invasive species in response to climate throughout different life stages.

Species	Clinal pattern	Invasive status	Location	Reference
Impatiens glandulifera	Biomass, height and basal diameter decreased and time to first flower increased with increasing latitude	Invasive	Europe	Kollmann & Bañuelos 2004
Hypericum perforatum	Plant size, leaf size and fecundity increase with increasing latitude	Native & invasive	Europe & North America	Maron *et al.* 2004
Eschscholzia californica	Plant size and fecundity increases with rainfall	Invasive	South America	Leger & Rice 2007
Solidago altissima	Height, leaf number, leaf length and width, stem diameter increased with increasing latitude	Native & invasive	North America & Japan	Etterson *et al.* 2008
Populus deltoides	Cold hardiness increases with increasing latitude	Native	North America	Friedman *et al.* 2008
Arabidopsis thaliana	Erect leaf stature and longer petiole with decreasing latitude	Native	Europe	Hopkins *et al.* 2008
Lythrum salicaria	Time to first flowering time & size at first flower decreased with increasing latitude	Invasive	North America	Montague *et al.* 2008
Four native Four invasive	Flower size and flower quantity decreases and seed size increases with increasing altitude	Native & invasive	Europe & North America	Alexander *et al*. 2009
Silene vulgaris *Silene latifolia*	Plant size (produced more leaves and stems) and reproductive effort increased with increasing latitude and cooler temperatures	Invasive	Europe	Keller *et al.* 2009
Silene vulgaris *Silene latifolia*	Plant size and flower number decreased with increasing latitude	Native	North America	Keller *et al.* 2009
Senecio inaequidens	Plant height and biomass increased with decreasing altitude	Invasive	Europe	Monty *et al*. 2009
Arabidopsis thaliana	Flowering time increases with decreasing longitude	Native	North America	Samis *et al*. 2012

1.2.2 Evolutionary underpinnings of clinal patterns

There are a range of processes that may encourage population divergence and the development of clinal patterns in life-history traits. For example, one process is 'climate matching' that involves the introduction of pre-adapted genotypes to climates similar to the native range (Maron *et al*. 2004). Multiple introductions also play a role in population divergence as they can increase genetic diversity and reduce the impact of invasion limitations such as founder effects and genetic bottlenecks caused through low genetic diversity (Bossdorf *et al.* 2005; Dlugosch & Parker 2008). It is arguably more likely in the case of invasive species that natural selection and selection pressures during range expansion in the invaded range have an important influence on changes in life-history traits that promote population divergence (Montague *et al.* 2008). While there are many possible processes generating clinal patterns in invading populations, these processes are not mutually exclusive (Montague *et al.* 2008; Monty & Mahy 2009). Nevertheless, the first step in identifying the adaptive capacity of an invading species is to identify clinal patterns in life-history traits among populations in relation to climatic and environmental heterogeneity.

Future changes in climate are predicted to influence ecosystem dynamics (Walther *et al.* 2002; Hellmann *et al.* 2008) and favour the spread of invasive species (Thuiller *et al.* 2007; Vilà *et al.* 2007). The identification of current clinal patterns can allow the prediction of future patterns of selection (Etterson 2004) and changes in population distribution as pre-adapted genotypes move into novel environments (Kirkpatrick & Barton 1997; Etterson *et al*. 2008). In the future, pre-adapted genotypes will provide a buffer against changes in climate as these genotypes can disperse to other areas along the range replacing populations or increasing genetic diversity through admixture of genetic variation (Bossdorf *et al*. 2005; Montague *et*

al. 2008). High stress environments, such as arid and semi-arid ecosystems, are vulnerable to future changes in climate (Smith *et al*. 2000). Therefore, an understanding of plant responses to abiotic stress in such environments will assist in the prediction of the ecological consequences of such changes (Maestre *et al.* 2005).

1.2.3 Clinal patterns in a vulnerable environment: Arid environments

Arid environments contain many novel challenges to invaders as water (Fischer & Turner 1978; Armas & Pugnaire 2005) and nutrients (Maestre *et al*. 2005) are frequently limiting factors. Water and nutrient availability have been found to influence plant species at all life stages resulting in the development of clines. For example, Ofir & Kigel (2003) found that when grown in a common environment, the morphological, phenological and life-history traits of a native species, *Poa bulbosa*, clinally varied in relation to variation in rainfall patterns across the collection sites. As a result, *P. bulbosa* populations from arid environments compared to more mesic environments displayed changes in leaf colour (grey compared to green), differences in leaf shape (curved compared to straight), reduced flowering capacity, shorter time until summer dormancy and younger onset of summer dormancy.

Similar clinal patterns have been found in the invasive species *Eschscholzia californica* in North America. For example, under consistent glasshouse conditions, Leger & Rice (2007) observed clinal population differentiation in size and fecundity in relation to a rainfall gradient, resulting in larger and more fecund *E. californica* populations from arid environments (see Table 1.1 for more examples). Surprisingly, however, few studies have assessed adaptive variation in invasive species in arid environments or along arid-mesic gradients. The response of invasive species to harsh

conditions such as those found in arid environments can provide an insight into their adaptive capacity and potential response to future changes in climate.

The primary limitation in arid environments, reduced water availability, can affect plant morphology and physiology throughout the entire plant lifecycle. For example, plants in arid environments display rapid germination, high seed dormancy (Clauss & Venable 2000), small leaf size, short leaf lifespan, low photosynthetic area (Wright & Westoby 2002), reduced relative growth rate (Lambers & Poorter 1992), high water use-efficiency, early maturity and early flowering (Franks *et al.* 2007; Kolář & Seňková 2008). The importance of growth and reproductive traits during range expansion of invasive species is well documented, and indeed a small number of studies have identified clinal patterns in one or both of these types of traits (e.g. Olsson & Ågren 2002; Kollmann & Bañuelos 2004; See Table 1.1 for more examples). In contrast, very few studies have examined clinal patterns in seed germination or seed traits in invasive species (Table 1.1). Given that germination and seed traits are fundamental components of the invasion process and are predicted to be under strong selection pressure during invasion (Venable & Lawlor 1980; Hierro *et al.* 2009; Mason *et al.* 2008 respectively), the paucity of research into clinal patterns in this area is conspicuous.

In this thesis, I suggest that considering that various plant life-history stages are intimately linked due to trade-offs and dependences on resources collected during previous life stages, there is a desperate need to examine clinal patterns across multiple integrated life-history stages in invasive species that have invaded arid environments. The investigation of multiple life-history traits is vital to provide a comprehensive understanding of the adaptive capacity of invasive species. In the two empirical examples that I discussed previously (Ofir & Kigel 2003; Leger & Rice

2007), each study used a 'common garden' approach to identify clinal variation. Such a method for observing population responses to variability in environmental conditions is critical for the analysis of clinal patterns as a consistent setting is provided where local variability and idiosyncrasies in environmental conditions are removed.

1.2.4 An experimental approach for determining clinal patterns: the 'common garden'

The use of a 'common garden' approach is invaluable in the study of clinal variation in life-history traits. Critically, this approach involves raising individuals in a common environment that allows one to differentiate between adaptive and plastic responses to divergent environments (Ballentine & Greenberg 2010). For example, in a situation where plants from a range of populations of a species are grown under the same conditions in the glasshouse, the observation of variation in life-history traits among populations of a species, that correspond with variation in the environmental conditions among the sites from which the populations originated, is evidence that natural selection has influenced the observed pattern through local adaptation (Endler 1986; Leger & Rice 2007; Montague *et al.* 2008). Thus, the common garden approach is a widely used, robust method for quantifying phenotypic differentiation among populations (Leger & Rice 2007; Colautti *et al*. 2009). The common garden approach is a well-established procedure that has been employed to identify population differentiation in a range of plant traits including germination (e.g. Clauss & Venable 2000; Qaderi & Cavers 2002), basic plant morphology (e.g. Clevering *et al.* 2001; Hopkins *et al.* 2008), growth (e.g. Weber & Schmid 1998; Maron *et al*. 2004; Monty & Mahy 2009; Keller *et al*. 2009), phenology (e.g. Reinartz 1984; Kollmann &

Bañuelos 2004; Leger & Rice 2007; Montague *et al*. 2008) and seed traits (e.g. Vaughton & Ramsey 2001; Münzbergová & Plačková 2010).

1.3 Invasive exotic plant species in Australia

1.3.1 Background

Australia is a small isolated continent with a total land mass of approximately 7.7 million km^2 and a human population of just over 22 million. The distribution of vegetation in Australia is skewed towards the coastal and northern areas of the continent with almost 20% of the continent classified as desert. Australia is characterised by its low variable rainfall and nutrient poor soils.

In the past, poor agricultural practices have led to the clearing and degradation of large areas of agricultural land, resulting in a decline in soil quality and creating pathways for invasion. For example, during the first 130 years of European settlement, 35.3 million ha were partially cleared primarily for agricultural use (Reed 1990; Benson 1991). Since European settlement in 1788, over 26,000 exotic plant species have been transported to Australia, both intentionally and unintentionally, with 2,739 species becoming naturalized and 130 species known to be invasive (Randall 2007). Despite the relatively small number of species that have become invasive, the cost to the agricultural industry is over $3.5 billion (AUD) annually in lost production and control costs (Sinden *et al.* 2004; Randall 2007). In the Australian agricultural industry, $1 from every $7 profit is re-invested into invasive species management (Sinden *et al.* 2004).

The ecological impacts of invasive species on native species in Australia include habitat disturbance, competition for resources and even native species

extinction (Leigh & Briggs 1992; Grice 2004). Many of Australia's worst invasive species were intentionally introduced as garden plants that escaped and formed wild populations, such as one of Australia's worst invasive species, *Echium plantagineum*.

1.3.2 *Echium plantagineum* (Paterson's Curse): model study species

Echium plantagineum is an invasive species in Australia originally from Europe and the Mediterranean region. The species occurs across over 33 million ha of Australia (Piggin & Sheppard 1995; Nordblom 2003), is estimated to cause upwards of $30 million (AUD) in damage per annum (Shea *et al.* 2000) and has reached noxious weed status in all states of Australia (Parsons & Cuthbertson 1992). *Echium plantagineum* was selected for use in this study as it is a serious invasive species globally (Piggin & Sheppard 1995), widespread within Australia (Parsons & Cuthbertson 1992), with a high genetic diversity throughout its invaded range (Brown & Burdon 1983), multiple introduction points (Forcella *et al*. 1986) and most importantly, it spans a broad climatic gradient across eastern Australia.

1.3.3 General biology of *Echium plantagineum*

Echium plantagineum is a winter annual or biennial forb that is part of the Boraginaceae family (Piggin & Sheppard 1995). The growth form of this species is a large flat rosette (Fig. 1.1a) that stores reserves in a large tap root (Piggin 1977; Piggin & Sheppard 1995). In spring, the plant produces multiple paniculate inflorescences of numerous branched cymes and these flowers are usually 20–30 mm long in a range of colours including blue, purple, pink or white with blue-grey pollen (Fig. 1.1b; Piggin & Sheppard 1995). *Echium plantagineum* grows to a height of between 1.2–2.0 m tall (Fig. 1.1c; Piggin & Sheppard 1995). A single plant can

produce up to 10,000 seeds with seed rains of 30,000 per m^2 which can remain dormant in the soil for over six years (Piggin 1976a; Shea *et al.* 2000; Blood 2001).

1.3.4 History of introduction of *Echium plantagineum* to Australia

Echium plantagineum is a native species of annual grassland in the western Mediterranean basin with the evolutionary centre of the genus in Morocco, Portugal and Spain (Fernández Alés *et al*. 1993; Piggin & Sheppard 1995). It has spread widely throughout the world and has been recorded from southern Africa, the Americas, Australia and New Zealand (Piggin 1976a; Piggin & Sheppard 1995). It was first introduced intentionally in the 1850s as a garden plant and could be found in nursery catalogues and growing in botanic gardens (Piggin 1977; Fig. 1.2). From the analysis of herbarium specimens, Forcella *et al*. (1986) has predicted that there were at least four isolated points of successful introduction in Australia with the current distribution representing an amalgamation of the fronts spreading from these points. *Echium plantagineum* was listed as naturalised in New South Wales (NSW), and Victoria (VIC) before 1860 (Piggin & Sheppard 1995) and has become a common invasive species of degraded, neglected and disturbed agricultural and roadside areas (Piggin & Sheppard 1995; Parsons & Cuthbertson 1992).

(a)

(b)

(c)

Figure 1.1 *Echium plantagineum*, the model study species: (a) juvenile rosette, (b) fully-developed flowers and (c) adult plant.

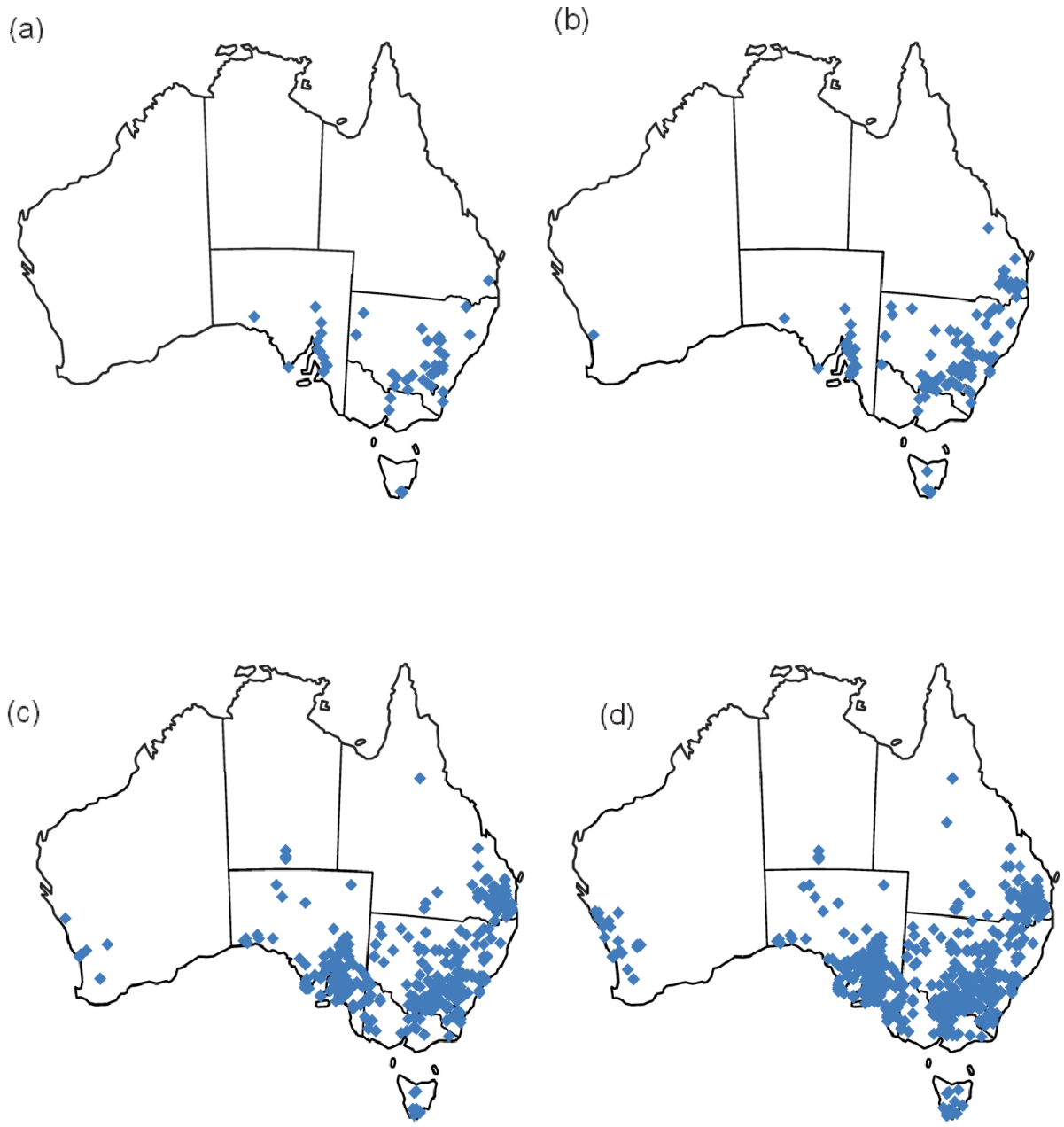

Figure 1.2 Distribution of *Echium plantagineum* over four time intervals: (a) 1880-1920, (b) 1920-1950, (c) 1950-1980 and (d) 1980-2010. Based on data collected from the Australian Virtual Herbarium (AVH; Copyright 2009; Council of Heads of Australasia herbaria Inc).

1.4 Research Approach

In this thesis, I examine clinal variation in life-history traits of the invasive exotic plant species *E. plantagineum*. This study provides an advance on our current understanding of clinal patterns in invasive species, as it investigates rapid population divergence along an arid-mesic gradient across the entire lifecycle of the serious invasive species *E. plantagineum*. This gradient includes an environment where very little work has been done, arid environments, and provides an insight into the ability of this species to overcome the introduction-to-invasion pathway and successfully adapt to local environmental conditions. I perform a series of experiments that traverse the life-history stages of the plant including seed germination, seedling growth, phenology and seed traits to bring together a full life-cycle analysis of the adaptive capacity of a serious invasive species. The critical issue facing the invasive spread of *E. plantagineum* is survival in arid conditions where temperatures are high and critically, water is limiting. Therefore, I focus my predictions for clinal variation in relation to variation in life-history traits along an arid-mesic gradient.

1.4.1 Thesis Structure

The thesis is structured with the following chapter progression:

Chapter 2 describes the first stage of the lifecycle, seed germination. This chapter addresses the challenges of seed germination in arid environments. In particular, I address predictions centred on the idea that populations from arid environments will germinate faster and reserve a greater portion of seeds for germination at a later date (i.e. 'bet-hedging') compared to populations from mesic environments.

In chapter 3, I present an experimental investigation of early plant growth strategies using a common garden glasshouse design. Here, I asked whether three important features including growth (e.g. growth rate), stature (e.g. plant size) and water use (e.g. water use efficiency) traits vary among populations along an arid-mesic gradient. In this chapter, I target the prediction that plants from arid environments have slower growth, smaller stature and higher water use efficiency compared to plants from mesic populations.

Chapter 4 describes the reproductive phase under common garden glasshouse conditions. This chapter identifies if and how stem and flower production differ among populations from arid and mesic environments. This chapter is intimately linked with chapter 3 as reproductive capacity is heavily dependent on resource collection during the growth phase. Therefore, I test a specific prediction that plants from arid environments produce stems and flowers earlier and in higher quantities than plants from mesic populations.

Chapter 5 explores the influence of climate on the mass and size of progeny seed through open pollination with European honey bees (*Apis mellifera*) during a common garden glasshouse experiment. This chapter addresses the prediction that larger seeds are produced in arid environments compared to mesic environments.

Chapter 6, the final chapter, provides an overall synthesis of the findings obtained within each chapter and how these further our understanding of the adaptive capacity of invasive species in novel environments.

Chapter 2: A comparison of seed germination strategies among populations of *Echium plantagineum* along an arid-mesic gradient

2.1 Introduction

The ability of an exotic plant species to respond optimally to spatial and temporal variation in environmental features in a novel geographic range will contribute to its invasion potential (Barrett *et al.* 2008; Childs *et al.* 2010). Novel environments and new selection pressures may induce evolutionary responses in invading species across a range of important life-history stages (Barrett *et al.* 2008; Monty *et al.* 2009). Such responses can be found across the whole plant life-history spectrum ranging from plant germination (e.g. Erfmeier & Bruelheide 2005; Kudoh *et al.* 2007; Hierro *et al.* 2009) to growth (e.g. Weber & Schmid 1998; Maron *et al.* 2004; Kollmann & Bañuelos 2004) and reproduction (e.g. Leger & Rice 2007; Colautti *et al.* 2010; Ridley & Ellstrand 2010). Identifying which plant traits are most responsive to selection pressures during the invasion process is a key goal in the management of invasive species (Sakai *et al*. 2001).

Germination traits are predicted to be under strong selection pressure during invasion due to their consequences for plant fitness (Venable & Lawlor 1980; Allen & Meyer 2002; Zheng *et al.* 2005; Hierro *et al.* 2009). Selection pressures on germination may include, for instance, reduced water availability (e.g. Meyer & Allen 1999; El-Keblawy & Al-Ansari 2000; Zheng *et al.* 2005) and unsuitable temperatures (e.g. Piggin *et al.* 1973, 1976a; Wulff 1995; Jensen & Eriksen 2001) that may result in either seed death or reduced plant health in seedlings emerging in unfavourable

environmental conditions (Piggin & Sheppard 1995; Baskin & Baskin 1998; El-Keblawy& Al-Rawai 2006; Donohue *et al.* 2010).

Two key adaptive strategies have been proposed for seed germination in unpredictable and variable arid ecosystems (Cohen 1966; Venable & Lawlor 1980; Venable & Brown 1988; Clauss & Venable 2000; Mandák 2003). These include rapid seed germination to maximise available resources and seed dormancy where a fraction of the seed pool does not germinate under favourable conditions (Bewley 1997; Donohue *et al.* 2010). Rapid germination allows progeny to make the most of available resources before other plants have a chance to germinate and compete for limited resources (Brown & Venable 1986). Rapid germination is a common adaptation in arid environments in response to the rapid drying of the surface soil layer (El-Keblawy *et al*. 2009) and can also reduce seed predation and mortality in the seed bank by allowing seeds to germinate rather than waiting for large rainfall events that may not arrive while seeds are still viable (Brown & Venable 1991; Donohue *et al.* 2010).

At the same time, however, risk-spreading through seed dormancy can be an ideal bet-hedging strategy for populations growing in unpredictable environments (Cohen 1966; Venable & Lawlor 1980; Brown & Venable 1986; Venable & Brown 1988; Philippi 1993; Venable 2007). Under this strategy, a plant produces seeds, a fraction of which delay germination (i.e. remain dormant) as a buffer against unfavourable conditions (Evans *et al.* 2007; Venable 2007; Childs *et al.* 2010). Bet-hedging reduces the risk of seedling death of an entire cohort from a ‘false break’, that is, mortality during tough environmental conditions that follow a short watering event. Studies have found that seeds produced in a maternal environment with high temperatures and low water availability had lower germination percentages (i.e.

higher dormancy) than those produced at lower temperatures and favourable moisture conditions (Philippi 1993; Hume 1994; Clauss & Venable 2000; Venable 2007).

Previous studies have found that native plant species in arid environments utilise rapid germination strategies to make the most of unpredictable rainfall and to increase establishment success (Clauss & Venable 2000; Evans *et al.* 2007). For example, Mott (1972) observed rapid germination (initiation within two days) and a short germination period (within six days) after a single rainfall event under laboratory conditions in three native Australian species from arid environments. In relation to seed dormancy and bet-hedging, several studies have found that native plant species under stress (such as reduced rainfall) in arid environments demonstrate low seed germination fractions (i.e. high fractions of seeds remaining dormant; Philippi 1993; Evans *et al*. 2007; Venable 2007; but see El-Keblawy & Al-Rawai 2006). Importantly, however, most previous studies, that focus on seed germination strategies have been confined either within arid regions (e.g. Philippi 1993; Clauss & Venable 2000; Adondakis & Venable 2004; Evans *et al*. 2007; Venable 2007) or mesic regions (e.g. Alexander & Wulff 1985; Gonźalez-Astorga & Núñez-Farfán 2000). In arid environments, seed dormancy has been the focus of many germination studies involving exotic species while there has been comparatively less on germination velocity and period which are also vital for survival in tough conditions. There are very few studies that have compared germination strategies among populations of a given native species where populations occur in both arid and mesic environments (e.g. Sadeh *et al.* 2009) or along an environmental gradient (e.g. Meyer *et al*. 1989; Meyer 1992).

In the case of exotic plant species, rapid germination has been linked to increased invasion success (Baker 1974; Radford & Cousens 2000; Kudoh *et al.*

2007). For example, Grice (1996) observed comparatively rapid germination in the invasive species *Cryptostegia grandifiora*, with more than 90% of seeds germinating within ten days of a 'rainfall event' under laboratory conditions. In relation to seed dormancy, Hume (1994) investigated among-population differences in the invasive species *Thlaspi arvense* finding lower germination fractions, (i.e. increased dormancy) with increasing temperature. Previous work on germination strategies in invasive species has tended to focus on differences between native and introduced ranges (e.g. Erfmeier & Bruelheide 2005; Kudoh *et al.* 2007; Hierro *et al.* 2009) and among species comparisons with native and other invasives (e.g. Grice 1996; Mandák 2003; Gardarin *et al*. 2011). Very few studies have examined among population differences in germination speed or dormancy in invasive species (e.g. Hume 1994; Li & Feng 2009). The challenges that face an exotic species during invasion of a novel environment vary depending on the environmental variables and selection pressures present. Arid and mesic environments require vastly different germination responses and many studies look at single populations within arid or mesic environments (e.g. Qaderi *et al.* 2003; Mandák 2003; El-Keblawy & Al-Rawai 2006). Allen & Meyer (2002) is one of few examples that investigate seed dormancy among populations of an exotic species within arid and mesic environments however I was unable to find any studies that investigated germination traits along an environmental cline.

2.1.1 Chapter aim and approach

In this study, I performed a common-garden experiment to compare germination strategies of *E. plantagineum* among populations along an arid-mesic environmental gradient in its introduced range in south-eastern Australia. The focus of this study was on evidence for local adaptation. Local adaptation is when natural selection favours individuals within a species due to phenological or morphological traits that allow

better fitness and performance (eg. Leger & Rice 2007). Although plant life-history traits might exhibit phenotypic plasticity to cope with novel environments (e.g. Davidson *et al.* 2011), here I am interested in determining the role of differential evolution among populations. First, I tested the prediction that seeds from populations that have established in arid environments germinate more rapidly in the first few days of imbibition than seeds from populations found in more mesic habitats. Such a response would indicate local adaptation of *E. plantagineum* populations in response to arid conditions, an evolutionary response linked to increased invasive potential under conditions of low and unpredictable rainfall. Second, I predicted that a smaller fraction of seeds would germinate during a simulated rainfall event from populations in arid environments compared with mesic environments following a single imbibition event, providing a larger viable seed bank that can respond to later rainfall events. Such an arid-adapted strategy would indicate local adaptation via a bet-hedging strategy in this annual species to counter the potentially increased risk of seedling death from 'false breaks' following short watering events, a counter that will increase the likelihood of invasion success.

2.2 Methods

2.2.1 Site selection and seed collection

Seeds were collected from 34 sites within six bioregions defined by the Interim Biogeographic Regionalisation for Australia (IBRA) framework during the 2009 productive season (October - December) along a broad rainfall gradient. The IBRA framework was developed in 1993 as a management tool by the Australian government to identify land for conservation. This framework divides Australia in to 89 bioregions based on similarities in climate, geology, landform, native flora and

native fauna (environment.gov.au). The seven bioregions used in my study are found in the state of New South Wales and form an aridity gradient from the mesic coast to the arid interior of the continent (See Table 2.1; Fig. 2.1). Note that for all analyses Sydney Basin (SB) and South East corner (SEC) were combined into a single coastal bioregion (COAST). I collected seeds from fifty haphazardly selected individual plants at each site within each bioregion and transported them to the laboratory in paper bags.

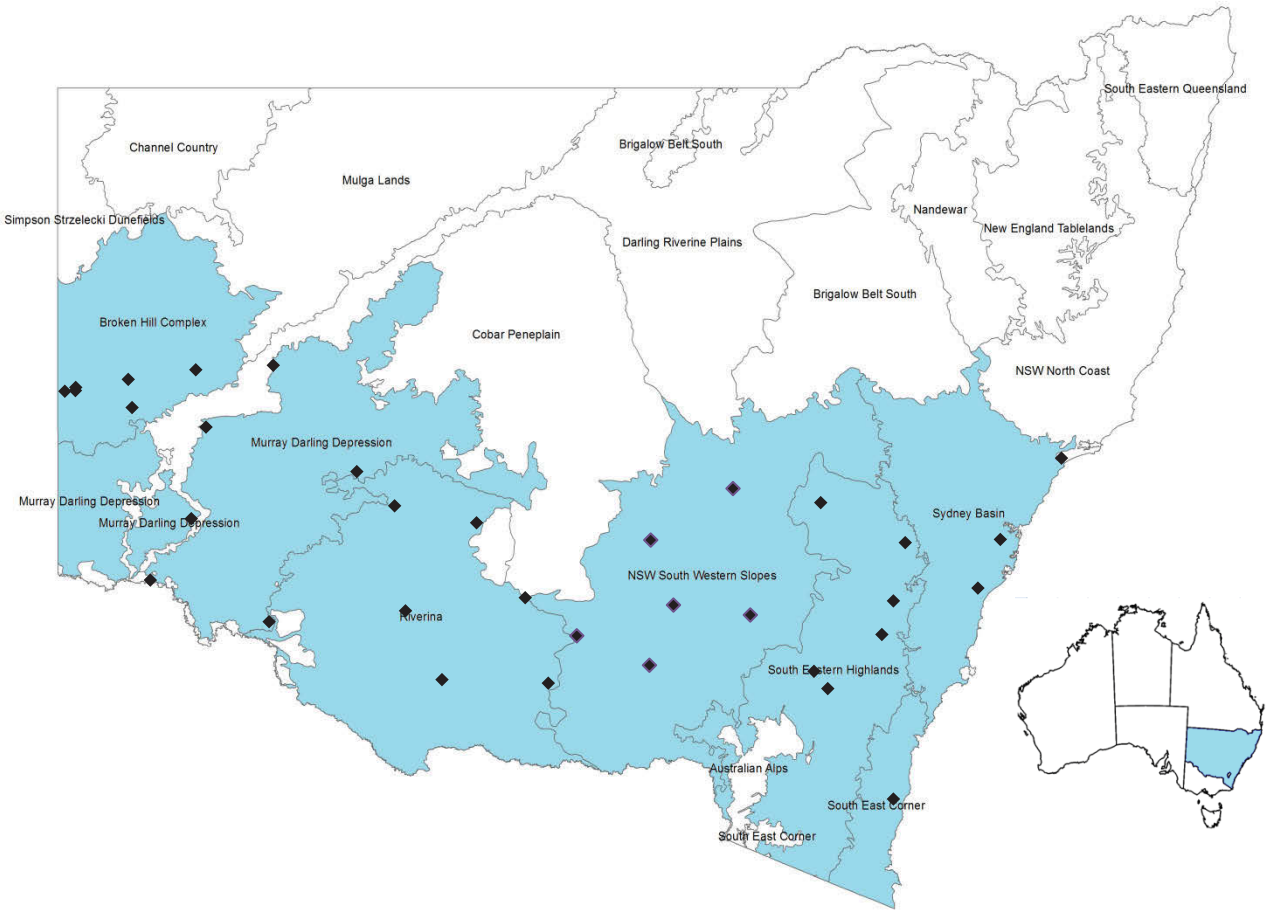

Figure 2.1 Distribution of study sites (depicted by black diamonds) across south-eastern Australia, grouped by Interim Biogeographic Regionalisation for Australia (IBRA) framework (see 2.1.1). Bioregions used in this thesis are coloured blue. Broken Hill Complex (BHC), Murray Darling Depression (MDD), Riverina (RIV), NSW South-Western Slopes (SWS), South-Eastern Highlands (SEH), Sydney Basin (SB), South-East Corner (SEC). Note that for all analyses SB and SEC were combined into a single coastal bioregion (COAST).

Table 2.1 Bioregion profiles for bioregions used in this study. Data were obtained from the NSW Department of Environment and Heritage (www.environment.nsw.gov.au; 2012) and the SILO enhanced meteorological dataset and datadrill procedure (see 2.2.5 for details). Abbreviations: Pvar = annual precipitation variability; Pav = mean annual precipitation; Tav = mean annual temperature.

Bioregion	Size	Topography	Soil	Main vegetation types	T_{av} (°C)	P_{av} (mm)	P_{var} (%)
Broken Hill Complex (BHC)	3,811,697 ha 4.8% of NSW	Rocky Hills and desert plains	Shallow stony soils and deep red sand	Mulga communities (*Acacia aneura*), chenopod shrub land (composed of saltbush and bluebush communities)	17-20	137-257	47.3
Murray Darling Depression (MDD)	8,026,167 ha 10% of NSW	Dunefields, sandplains, undulating plains. Lakes, swamps and depressions	Red, brown and yellow calcareous sands (Dunefields), sandy rises (Mallee vegetation), gradational or mixed texture profiles (Sandplains). Lakes and depressions all have clay floors	Dunefields support mallee (*Eucalyptus* sp.) communities and mixed shrubs. Sandplain species include rosewood (*Heterodendrum oleifolium*), white cypress pine (*Callitris glaucophylla*), narrow-leaf hopbush (*Dodonea viscosa*), punty bush (*Cassia eremophila*), belah, copperburrs (*Sclerolaena* sp.), black bluebush (*Maireana pyramidata*)	16-19	210-408	40.8
Riverina (RIV)	7,090,008 ha 8.9% of NSW	Alluvial fans, flood plains and river channels	Sandy soil and heavy grey-brown clay	River red gum, (*Eucalyptus camaldulensis*) and river cooba (*Acacia stenophylla*) communities along river channels. Black box (*Eucalyptus largiflorens*) and yellow box (*Eucalyptus melliodora*) woodlands with an understory of saltbush, grasses and dasies.	15-18	238-617	34.1
NSW South West Slope (SWS)	8,070,608 ha 10% of NSW	Foothills and ranges	Shallow stony soils at the tops of ridges. Mixed texture soils, alluvial sands and loams further down the slope. Poorly drained soils in the valleys. Dry land salinity is widespread	Open woodlands of white box (*Eucalyptus albens*) are dominant in the west with grey box (*Eucalyptus microcarpa*) and white cypress pine (*Callitris glaucophylla*) in the north.	11-17	360-1266	29.2
South Eastern Highlands (SEH)	4,888,633 ha 6.1% of NSW	Ranges and plateaus of the great dividing range	Mottled red and yellow mixed texture soils, deep coarse sands and shallow red-brown to black stony loams	Yellow box (*Eucalyptus melliodora*), red box (*Eucalyptus polyanthemos*) and Blakely's red gum (*Eucalyptus blakelyi*), and white box (*Eucalyptus albens*) in low lying areas.	6-16	460-1883	26.8
South East Corner and Sydney Basin (COAST)	4,926,149 ha 6.2 % of NSW	Steep hills, gorges and plateaus (SEC) geological basin with sandstone and shales (SB)	Large variety of soil types including dunes, sandy soils, basalts, mixed texture profiles and coarse granite soils	Very diverse vegetation types due to variety of soils and topography in the region including dunes; rainforest; tall forest; sand stones plateaus; estuaries; mangroves; riparian and swamp; heath; Red bloodwood and spotted gum (*Eucalyptus maculata*) forests and coastal forest characterised by gum trees.	7-17	507-2395	31.2

2.2.2 Seed collection and measurement

Prior to the commencement of the study, seed viability was determined by visual inspection and a squeeze test. Forceps were lightly pressed on either side of the seed and light pressure was imposed on the seed. Seeds were considered viable if the seed coat did not crack or deform (Piggin 1976a). Experience with germination of field seed indicated that seeds were viable if the seed coat did not crack or deform under light pressure (i.e. the seed was filled). Only healthy viable seeds were used in assessing germination (Fig. 2.2).

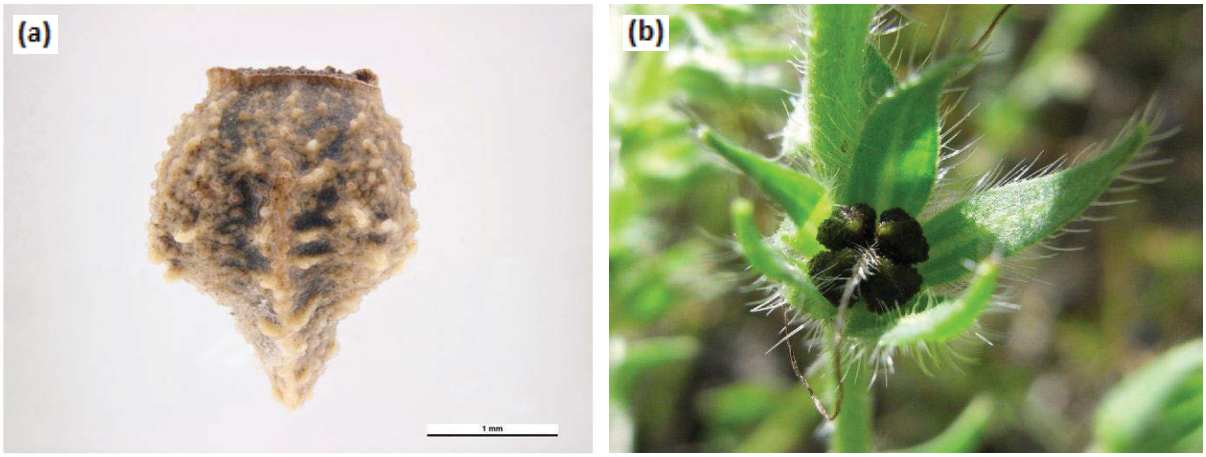

Figure 2.2 *Echium plantagineum* seed is (a) 1-3 mm long and brown to black in colour and (b) found in groups of four in the seed head.

Seeds were initially measured for mass and dimensions (length and width). Seed mass was measured as the dry weight of randomly selected seeds that were dried for one week at a temperature of 80ºC. I assessed differences in seed mass and dimensions among bioregions and sites using three separate linear mixed models, each with one of the three seed characteristics as a response variable. Bioregion was a fixed explanatory variable and site within bioregion was a random nested explanatory variable in models to control for variation in seed mass among sites. Seed mass and dimensions were found not to vary significantly among bioregions after accounting

for variation among sites (mass $F_{5,28} = 0.15$, $P = 0.98$; length $F_{5,28} = 0.15$, $P = 0.86$; width $F_{5,28} = 0.38$, $P = 0.86$). For the present study, I standardized seed selection from all sites, with individual seed mass within a narrow range of 2.0 – 5.0 mg.

2.2.3 Seed germination

Germination was assessed using ten seeds from each of ten plants from each of the 34 sites (n = 3400 total). The ten seeds from each plant were placed in separate petri dishes on filter paper (Whatman Inc, Clifton, NJ) and moistened with de-ionised water to simulate an artificial rainfall event. Dishes were sealed with Parafilm to prevent desiccation and placed in dark conditions at room temperature (23ºC ±1ºC). Germination was assessed every 12 hours during the first five days and then every 24 hours until two days passed with no further seed germination (a ten day period in total). Germination was recorded as having occurred when the radicle protruded through the seed coat. Any seeds that had not germinated after this time were examined using visual inspection and a squeeze test for viability. *Echium plantagineum* has coat-enhanced dormancy, where germination is restricted by the seed coat (Piggin 1976a). Germination can be artificially promoted by chipping or removal of the seed coat (Piggin 1976a; Bewley 1997), seeds that had not germinated at this point in time were scarified and subjected to the same germination treatment described above to further estimate viability of the remaining seeds.

Rapid germination was assessed using two variables. Time in days for the first seed to germinate represented how fast seeds are able to emerge after a rainfall event. Germination period, measured as the number of days from first to last seed germination, represented the overall ability of all germinating seeds to do so rapidly. The fraction of seeds that germinated was assessed using two variables to provide a

measure of dormancy after a single simulated rainfall event. The proportion of seeds that had germinated was assessed at both 24 hours as well as at the end of the experiment (ten days). The first proportion gave an indication of the fraction of seeds germinating in initially moist conditions. Under field conditions, soil may arguably start to dry out after the first 24 hours following rainfall (El-Keblawy *et al*. 2009). Hence, I felt that this first measure of the fraction of seeds that remained dormant provided an indication of germination prior to a decline in water availability, simulating field conditions. The number of viable seeds remaining dormant out of the total number of viable seeds per dish after ten days provided an overall estimate of the fraction of seeds remaining dormant after a prolonged period of water availability for seed germination.

2.2.4 Soil collection and nutrient analysis

A representative soil core, 10 cm^2 and 10 cm deep, was collected at each field site, sealed in a plastic bag, and stored at room temperature in the laboratory. A representative subsample (five grams) was ground to a fine powder using a tissuelyser at a frequency of 30.1 rpm for ten minutes, and analysed for percentage carbon (C) and percentage nitrogen (N) using a Europa 20-20 isotope ratio mass spectrometer with an automated Nitrogen Carbon Analysis preparation system (Dumas 1981). Soil electrical conductivity and soil pH were measured as per the method in Rayment & Higginson (1992).

2.2.5 Climate data and site conditions

Climate data for both the year of collection (2009) and the historic time frame (1910-2010) were obtained for all study sites using a portable global positioning system (GPS) device and the SILO enhanced meteorological dataset and datadrill procedure

hosted by the State of Queensland Department of Environment and Resource Management 2012 (Jeffrey *et al*. 2001 see http://www.longpaddock.qld.gov.au/silo/). We then derived five key climatological variables for each site, focusing on the time frame of May to October during which *E. plantagineum* completes its life-cyle (Piggin 1976a): total precipitation (P), mean maximum temperature, mean minimum temperature, mean temperature, and total potential evapotranspiration (FAO56). We also derived a measure of aridity for each site; the annual atmospheric water balance (AWB; Rasmusson 1968), defined as AWB = P- FAO56.

2.2.6 Statistical analysis

I used two approaches to determine whether the predicted variation in germination traits was observed along the arid-mesic gradient. First, linear mixed models (LMM; SPSS Inc, version 19, IBM) were used to determine differences in germination traits among bioregions. Second, linear regressions combined with principal components analysis were used to examine variation in the germination traits as a function of continuous environmental measures of the arid-mesic gradient.

For the LMMs, separate models were built treating each germination trait as a single response variable with the factor 'bioregion' (one of the six bioregions) as a fixed explanatory variable. The random factor 'site' (nested within bioregion) was included in models as a control variable, thus allowing a focus on determining significant variation in the germination traits at the bioregion level, over and above the effects of site variation (Quinn & Keough 2002). The variables time to first seed germination and germination period were in the form of count data and did not require transformation however, the number of seed germinated at 24 hours and ten days were converted to proportions and transformed by using the Arcsine transformation.

The relationship between climatic conditions and germination traits were quantified using standard linear regression. Principle component analysis with a varimax rotation was used to reduce ten correlated environmental variables describing the sites (Table 2.2) to two components that explained 82% of the total variance. The first component (PC1) explained 58% of the variation in the data and was strongly associated with climatic variables (Table 2.3). The second component (PC2) explained 24% of the variation and primarily reflected site-level soil characteristics (Table 2.3). The first component (PC1) was most strongly associated with annual evapotranspiration, temperature, rainfall, and the derived variable atmospheric water balance (Table 2.3), due to the strong east-west climatic and topographic gradient that dominates the study region. The strong relationship between soil pH and PC1 is indicative of the general tendency for soil acidity to increase from the western to eastern parts of the study area (Scott *et al.* 2000). The second component (PC2) was most strongly associated with soil N and C levels, which tended to increase at lower elevations (Table 2.3). These components were then used as independent variables in linear regressions with the reproductive traits as dependent variables (Sokal & Rohlf 1995; Dytham 2011). Mean scores on PC1 and PC2 for each bioregion are shown in Fig. 2.3. Scores on PC1 decrease from the arid (BHC) to mesic (SEH) bioregions, with coastal (COAST), slopes (SWS) and Riverina (RIV) regions having intermediate scores (Fig. 2.3a). PC2 primarily distinguished between coastal and highland bioregions (Fig. 2.3b), with low-elevation coastal areas having higher soil fertility (Table 2.2). No data transformations were required for the regression analyses.

Table 2.2 Environmental characteristics of the six IBRA bioregions sampled within the study region during the time period May to October 1910 – 2010 and soil variables (pH, EC, %N and %C) were collected during 2010. Means are provided for based on site-level data in each bioregion. Abbreviations denote the individual bioregions, See Fig. 2.1 for full names. Tmax = maximum temperature; Tmin = minimum temperature; Tav = average temperature; FAO56 = potential evapotranspiration; Atmospheric water balance (AWB) = Rainfall – FAO56 (Rasmusson 1968); EC = electrical conductivity. Data were obtained for all study sites using GPS locations and the SILO enhanced meteorological dataset and datadrill procedure (see section 2.2.5 for details).

Bioregion	Precipitation (mm)	Tmax (ºC)	Tmin (ºC)	Tav (ºC)	FAO56 (mm)	AWB (mm)	pH (CaCl2)	EC	Nitrogen %	Carbon %
BHC	109.6	19.7	7.5	13.6	513	-805.9	8.1	142	0.10	2.3
MDD	141.5	19.9	6.9	13.4	470	-656.1	7.9	130	0.16	3
RIV	202.9	18.4	6.1	12.2	429	-452.4	6.8	176	0.22	2.6
SWS	273.7	17.1	5.1	11.1	386	-224.3	6.0	192	0.16	1.9
SEH	383.5	13.5	2.7	8.1	338	91.6	6.2	103	0.17	2.2
COAST	358.5	18.9	7.1	13.0	419	-121.8	5.6	168	0.48	6.3

Table 2.3 Principal component loadings (Varimax rotation) from ten variables measured between May to October 1910-2010. Data were obtained for all study sites using GPS locations and the SILO enhanced meteorological dataset and datadrill procedure (see section 2.2.5 for details). Atmospheric water balance (AWB) was calculated by AWB = rainfall-evapotranspiration. Component loadings above 0.400 are shown in bold.

	Eigenvectors	**PC 1**	**PC 2**
Eigenvalue		5.8	2.4
% variation explained		58.4	24.4
	Atmospheric water balance	**-.972**	.047
	Evapotranspiration	**.957**	.065
	Average temperature	**-.931**	.175
	Max temperature	**.924**	.290
	Min temperature	**.923**	.317
	Rainfall	**.895**	.345
	Soil pH	**.763**	**-.406**
	Soil nitrogen	-.070	**.957**
	Soil carbon	.062	**.891**
	Soil electrical conductivity	.100	**.474**

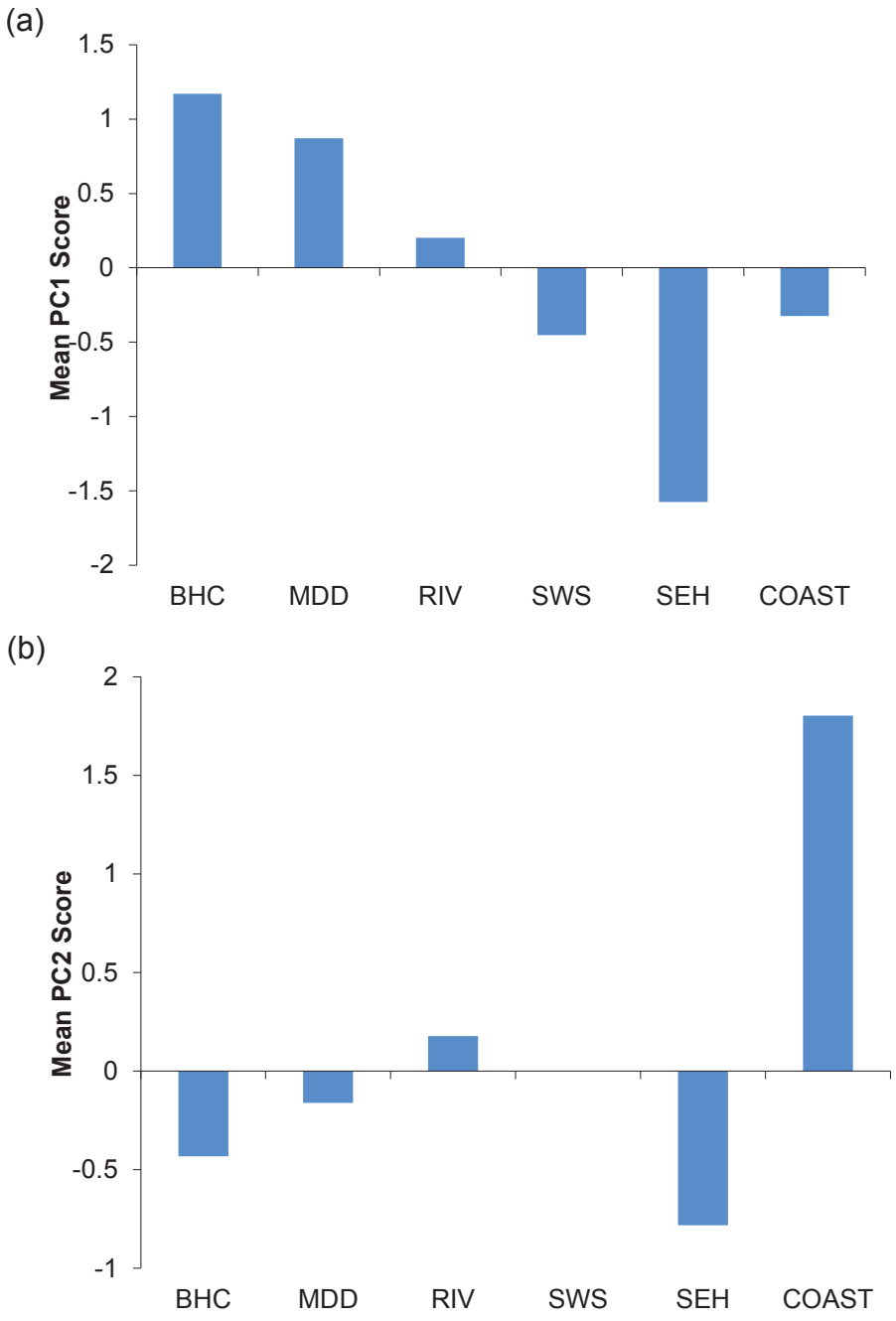

Figure 2.3 Mean scores for each bioregion on first (PC1) and second (PC2) principle components derived from principle component analysis of data from ten climatological and soil-related variables collected at each of the 34 study sites (see methods). a) Mean scores on PC1. b) Mean scores on PC2. Abbreviations denote the individual bioregions, See Fig. 2.1 for full names. Bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).

2.3 Results

There were no significant differences among bioregions in time to first seed germination ($F_{5,28}$ = 0.86, P = 0.52; Fig. 2.4a), germination period ($F_{5,27}$ = 1.87, P = 0.13; Fig. 2.4b), the fraction of seeds that had germinated after the first 24 hours of the experiment ($F_{5,28}$ = 0.71, P = 0.62; Fig. 2.5a) and after the ten-day experimental period ($F_{5,28}$ = 0.30, P = 0.91; Fig. 2.5b). Across all bioregions, mean time to first seed germination occurred within the first two days following imbibition (Fig. 2.4a) and the mean germination period was less than four days (Fig. 2.4b). Substantially larger fractions of seeds had germinated after ten days than after the first 24 hours, with mean total seed germination proportions ranging between 0.7 and 0.8 across the bioregions (Fig. 2.5 a, b). There were similar trajectories across time in germination rates across the bioregions (Fig. 2.6). Most notably, rapid increases in germination consistently occurred during the 24 to 48 hour period (Fig. 2.6).

In regression analyses, PC1 did not explain any significant variation in any of the four germination traits (time to first seed germination R = 0.09, P = 0.62; germination period R = 0.28, P = 0.12; germination at 24 hours R = 0.05, P = 0.79; germination at ten days R = 0.10, P = 0.56). Similarly, PC2 did not explain significant variation in any of the germination traits (time to first seed germination R = 0.19, P = 0.29; germination period R = 0.12, P = 0.50; germination at ten days R = 0.23, P = 0.20) with the exception of germination at 24 hours which was marginally explained by soil characteristics (PC2) R = 0.33, P = 0.06.

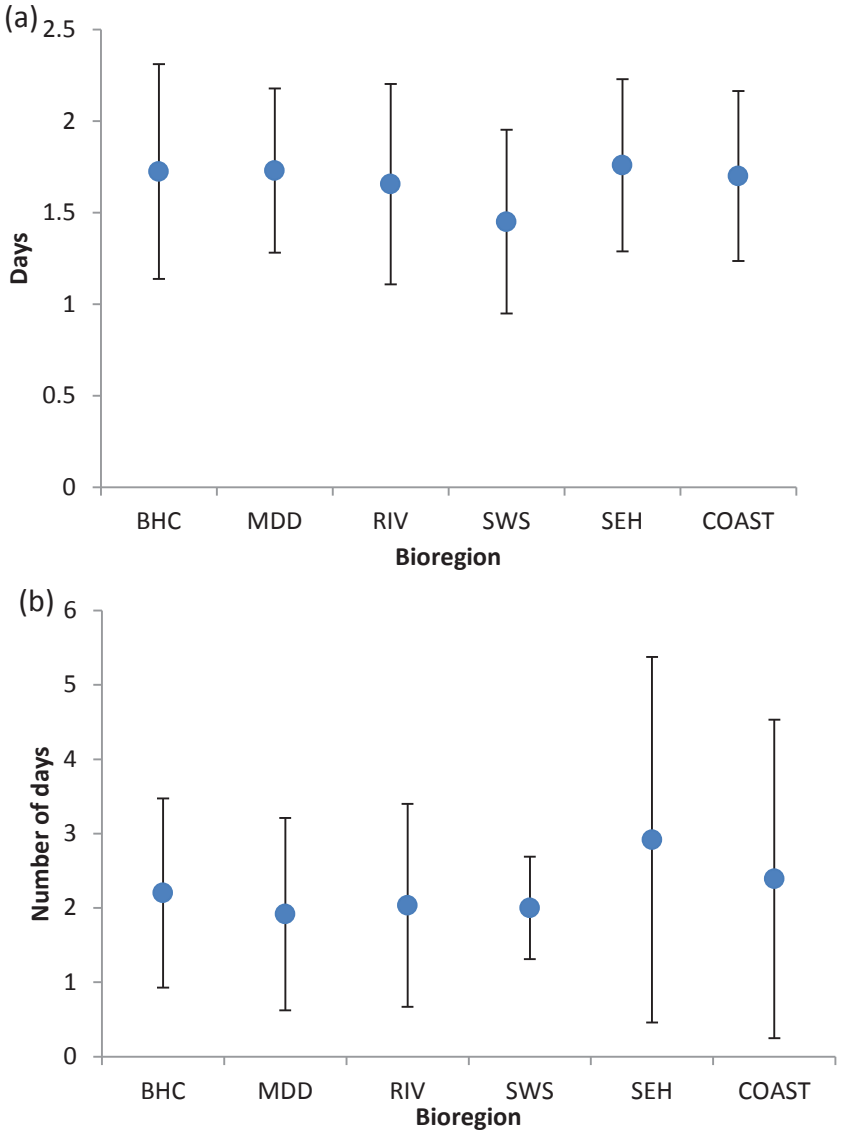

Figure 2.4 *Echium plantagineum* (a) Time to first seed germination (mean ± SD per bioregion) and (b) Germination period (mean ± SD per bioregion). Abbreviations denote the individual bioregions, See Fig. 2.1 for full names. Bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).

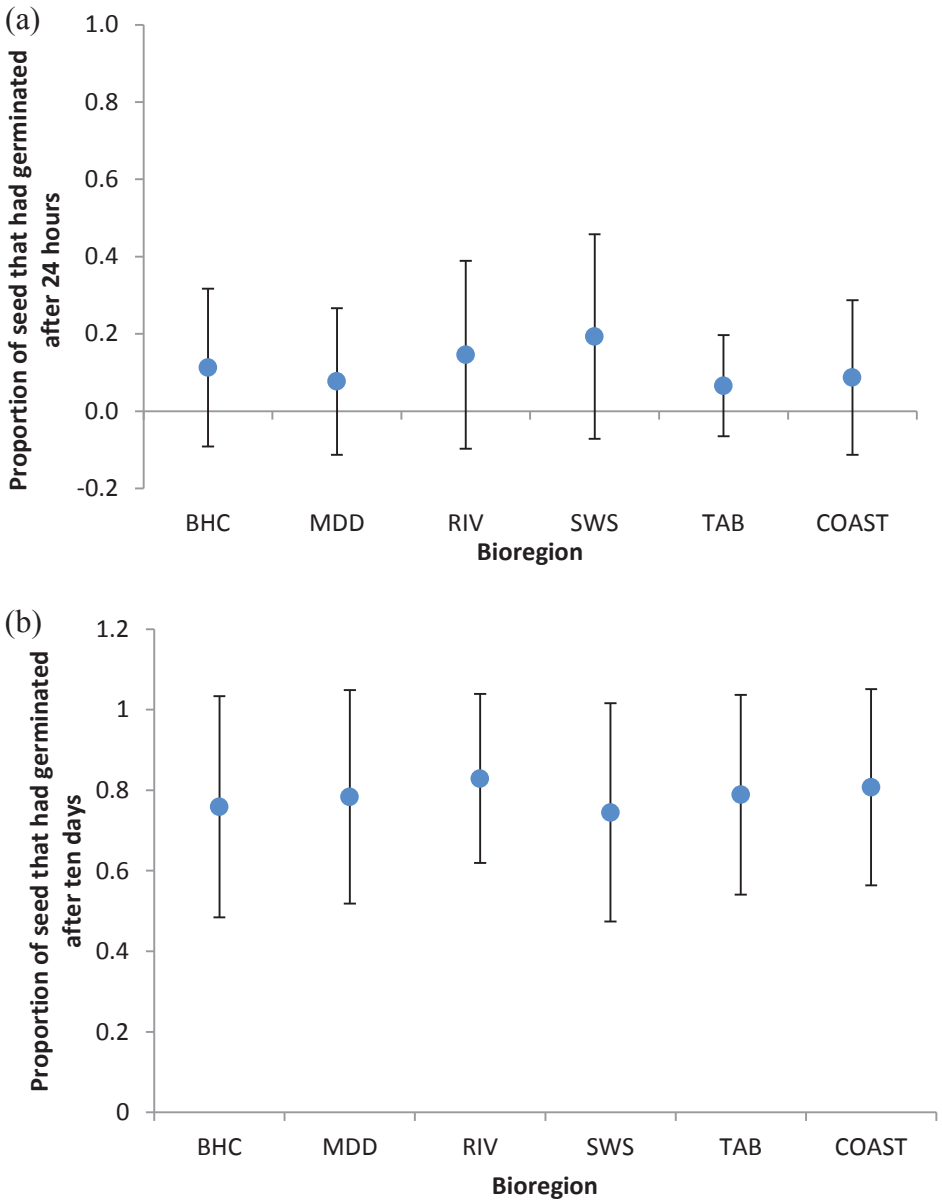

Figure 2.5 Fraction of *Echium plantagineum* seeds (mean ± SD per bioregion) that had germinated after (a) the first 24hrs and (b) ten days. Abbreviations denote the individual bioregions, See Fig. 2.1 for full names. Bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).

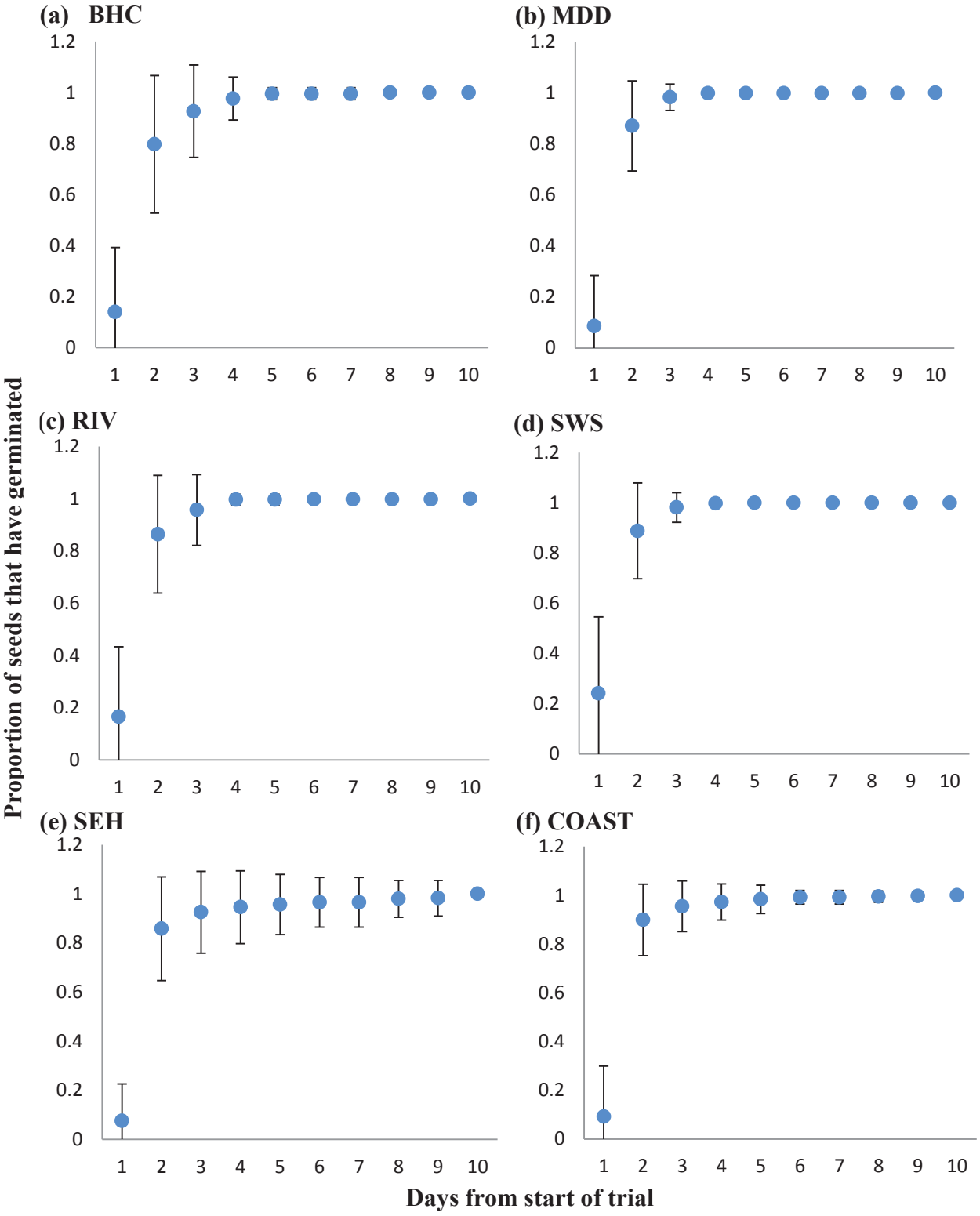

Figure 2.6 Mean ± SD proportion of seeds germinating over time for the bioregions (a) BHC, (b) MDD, (c) RIV, (d) SWS, (e) SEH and (f) COAST. Abbreviations denote the individual bioregions, See Fig. 2.1 for full names.

2.4 Discussion

Contrary to initial predictions, there was no significant variation in any of the germination traits along the arid-mesic gradient. Rather, I found that seeds of *E. plantagineum* from both arid and mesic bioregions germinate equally rapidly, over the same short period of time and with very low levels of dormancy. The rapid germination findings are consistent with previous work by Trumble (1937), Ballard (1970), Piggin (1976a) and Forcella *et al*. (1986), all of which also found rapid germination trends in *E. plantagineum.* For example, Forcella *et al.* (1986) found that *E. plantagineum* germinates very rapidly, with up to 50% germination within the first two days at temperatures between 10ºC to 36ºC. Forcella *et al*. (1986) also found that up to 70% of *E. plantagineum* seed germinated at 23ºC after a ten day period, consistent with my study. However, it is not known how much of the remaining seed in Forcella *et al*. (1986) was still viable, thus, the percentage of seed germinating out of those viable could be much higher.

Given that I did not find the expected shifts to more rapid germination and increased dormancy (at the ten-day stage) in populations of *E. plantagineum* from arid bioregions, what might explain the common germination strategies exhibited by both arid and mesic populations? I discuss reasons below for the observed commonality in germination strategies. The pace at which seeds of *E. plantagineum* germinate, regardless of habitat, is incredibly fast. Previous work has shown that a rapid germination strategy is common among invasive species irrespective of maternal environment (Erfmeier & Bruelheide 2005; Hierro *et al.* 2009). Notably, in comparison with eight other exotic naturalized plant species in Australia, time to first germination and germination period for *E. plantagineum* are among the fastest (Table

2.4). A comparatively high proportion of seeds germinating over a short period of time provide a competitive advantage for light and resources for newly emerging seedlings. Furthermore, because the germination period of this species is very short, it can establish and utilise resources before other species have started to germinate (Brown & Venable 1986). This is a valuable adaptation in environments with high temporal variability. As this species produces a prolific amount of seeds, having a reduced germination period allows rapid establishment of multiple cohorts in a season that are able to prevent other plants from establishing, reducing competition from other species. In arid locations, favourable sites such as roadsides with a source of run-off are sparse and need to be retained for the next generation.

It is interesting to note that the germination response was uniform across the arid-mesic gradient after the simulated rainfall event. All populations exhibited high dormancy within the first 24 hours, however, after five days the dormancy was reduced to low levels across the range. This difference over time suggests that dormancy levels of this species are related to the immediate availability of soil moisture, irrespective of the arid-mesic gradient. Soil moisture for a period of five days or longer is a signal for good conditions that induce seeds to germinate and take advantage of available resources. In arid environments, soil moisture will rapidly drain and evaporate from the soil, which is represented somewhat by the 24-hour period of this study. In contrast, in mesic conditions, populations are more likely to experience several consecutive days of rain, represented in this study by the five to ten day period. This study indicates that all populations, regardless of geographic origin along the gradient, will retain a small portion of dormant seeds after a short rain event.

The annual rainfall along the arid-mesic gradient in the field ranges from 209 mm to 914 mm with growing season (May to October) rainfall from 95 mm to 458 mm (average per site over 1910-2010; collected by GPS co-ordinates from SILO enhanced meteorological dataset and datadrill procedure hosted by the State of Queensland Department of Environment and Resource Management 2012; Jeffrey *et al* 2001). This indicates that sites with very low rainfall, such as the six sites in the Broken Hill Complex with an average of 230 mm annually, will not have extended periods of soil moisture, however, they will have a large quantity of *E. plantagineum* seed that can remain dormant in the soil for over six years (Piggin 1976a) until conditions are suitable for germination. The response of species to multiple rainfall events and rainfall variability is out of the scope of this project but may provide a greater insight into the influence of soil moisture on germination traits in species invading arid areas such as *E. plantagineum*.

The incredibly high seed output of *E. plantagineum* provides a buffer for the species, such that per plant, *E. plantagineum* is capable of producing up to 10 000 seeds with a population seed rain of 30 000 seeds m^2 (Piggin & Sheppard 1995; Shea *et al.* 2000). Rapid germination may also reduce the risk of seed bank depletion by predators (Brown & Venable 1991; Donohue *et al.* 2010) such as ants which were seen harvesting seeds in the field (T. Konarzewski, personal observation). Considerable research on the natural enemies of *E. plantagineum* has determined that it has few seedling predators or pathogens (Wapshere 1985) indicating that it may be safer for seeds to germinate than remain in the seed bank (Donohue *et al.* 2010). Seed predators such as ants can dramatically reduce the seed bank resulting in fewer opportunities for recruitment (Andrew 1986; Brown & Venable 1991). Although *E. plantagineum* seeds are capable of remaining in the soil for over six years (Piggin

1976a), most seeds germinate within the first 12 months (Muyt 2001). Our study suggests that a large portion of the seeds in the soil will germinate with the first sufficient rainfall event, a temporal pattern seen in other invasive species that also produce large quantities of seeds (Grice 1996). In terms of overall seed numbers in the soil, due to high seed production of *E. plantagineum* populations, there will still be a huge amount of seeds in the soil for germination at a later date. The vast quantity of seeds produced and the rapid germination of this species indicates that it is able to invade and establish very quickly in novel environments (Kudoh *et al*. 2007).

Previous work has found that *E. plantagineum s*eedlings are highly resistant to moisture stress (Piggin 1976b). Thus, rapid germination under 'false break' conditions would not have such a deleterious effect in this species compared to a species whose seedlings were more susceptible to water stress. *Echium plantagineum s*eedlings would have a better chance of establishment in arid environments, even in tough years with low water availability. After the ten day germination trial all populations were found to have a fraction of mechanically dormant seeds as a bet-hedging strategy against the environmental variation encountered by previously germinated seeds which may die before reaching reproductive maturity (Venable 2007). This pattern of delayed germination is consistent with among year bet-hedging predictions and has been observed in a number of annual desert species (Clauss & Venable 2000; Evans *et al.* 2007). Maternal factors known to alter seed dormancy include drought stress, warm night time temperatures during maturation, nutrient status, light and the presence of predators (Bewley 1997; Meyer & Allen 1999). These factors are not likely to be the cause of the result in our study, as plants were collected from a range of sites with varying maternal environmental variables and seeds were standardised by seed size and weight.

My study observed that after a simulated rainfall event in the laboratory, rapid germination occurred within the first 48 hours and was completed with a short germination period of two days. In a comparison to 12 other introduced invasive and non-invasive species *E. plantagineum* was found to germinate faster than all but one (Table 2.4) even when most species seeds had been treated to increase germination. This indicates that in a variable environment *E. plantagineum* would be able to germinate and establish as a drought resistant rosette, faster than any of the species listed giving it a substantial advantage over any other species in the vicinity. This strategy may allow this species to use available resources rapidly before other species are able to take advantage of them (Brown & Venable 1986). Three of the species listed, two of which are highly invasive, have the same strategy of rapid germination within 2-4 days indicating that this technique may be a key strategy in the invasion of novel environments.

Concentrated germination periods have also been noted for native, invasive and introduced species, for example Mott (1972) found that three annual native species from arid maternal environments had germination periods of between three and eight days, while Phillips (unpublished) has found that six invasive species across a range of maternal environments have germination periods of between 12 – 29 days and six introduced non-invasive species have germination periods of between 14 – 25 days (data not shown).

Table 2.4 Comparisons of germination traits among exotic plant species (invasive and non-invasive, M. Phillips unpublished data). Time to germination refers to the number of days between the simulated rainfall event and the first seed to germinate. Proportion of dormant viable seeds remaining at end of trial refers to the proportion of viable dormant seeds remaining after two days has passed with no germination.

Plant species	Common name	Invasive status	Scarification technique	Time to germination (days)	Proportion of dormant viable seeds remaining at end of trial (%)
Echium plantagineum	Paterson's curse	1A - 2A - 3A - 4A - 5	No	2	20
Aristolochia elegans	Dutchman's pipe	1A - 2A - 3 - 4A - 5A	Distilled water 48 hours	9	14.2
Aristolochia grandiflora	Pelican flower	-	Distilled water 48 hours	11	5
Coreopsis lanceolata	Lanceleaf tickseed	1A - 2A - 3A - 5A	No	4	17.5
Coreopsis grandiflora	large-flowered tickseed	2 – 3	No	6	10.8
Lonicera japonica	Japanese Honeysuckle	1A - 2A - 3A - 4A - 5A	Warm and cold stratification	15	26.7
Lonicera fragrantissima	Winter Honeysuckle	1A - 2A – 5	Warm and cold stratification	12	17.5
Passiflora foetida	Stinking passionfruit	1A - 2A - 3 - 5A	Distilled water 24 hours	6	22.5
Passiflora coccinea	Scarlet passion flower	1A - 2A	Distilled water 24 hours	7	18.3
Salvia coccinea	Texas sage	1A - 2A - 3 - 5A	No	2	1.6
Salvia splendens	Scarlet sage	2	No	3	2.5
Solanum torvum	Turkey berry	1A - 2A - 3A - 4 - 5A	Pre-chilled (5ºC) 24 hours	11	26.7
Solanum betaceum	Tree tomato	2	Pre-chilled (5ºC) 24 hours	8	14.1

* Invasive status refers to the listed invasive categories of Randall (2007). These numbers indicate that this plant 1 – is a weed of the natural environment, 2 – has escaped from cultivation, 3 – is a weed of agriculture, 4 – has been declared as a noxious weed under some form of legislation, 5 – has been recorded as an invasive species. Capital A signifies that it has reached this status in Australia.

Seed dormancy was predicted to vary across bioregions as different environmental conditions require different survival strategies, however this is not the case for *E. plantagineum*. The amount of dormant seeds remaining at the end of the trial did not vary across bioregions and when compared to the dormancy rate of other invasive and non-invasive species *E. plantagineum* has an intermediate level of seed dormancy with 20% of viable seed remaining in the soil until another rainfall event (Table 2.4). *Salvia* and *Coreopsis* species have a rapid germination rate similar to *E. plantagineum* and low seed dormancy without the assistance of a dormancy breaking treatments. This may act as a successful invasion strategy as two of these species, *S. coccinea and C. lanceolata,* are highly invasive. All *E. plantagineum* populations were found to have a fraction of mechanically dormant seed as a bet-hedging strategy against environmental variation, however, when compared to other species (Table 2.4) there is a comparatively large quantity of seed left in the soil after a single rainfall event. The ability to spread germination over a longer period of time combined with the drought resistant capability of the rosette (Parsons & Cuthbertson 1992) allows this species to cope with the unpredictable conditions in arid environments. As these populations have been recorded at these locations at multiple times over the last 100 years this strategy must be suitable for these locations (See Fig. 1.2 for distribution in Australia).

The germination strategy of *E. plantagineum* across its range is to rapidly germinate immediately after a rainfall event while leaving a portion of viable seed in the soil for the next rainfall event. This strategy allows this species to take advantage of available resources while allowing a portion of seed to remain dormant in the soil as an insurance policy against false breaks and early season rainfall. Germination is clearly an important component of the invasion strategy of *E. plantagineum* but how

do the growth characteristics that follow contribute to the success of this species? The next chapter of the thesis will look at the growth characteristics of *E. plantagineum* and how they contribute to the invasion strategy of this successful invasive species.

Chapter 3: An examination of growth trait variation among populations of *Echium plantagineum* along an arid-mesic gradient

3.1 Introduction

Invasion success in novel environments can be linked to morphological and physiological growth traits that are crucial for plant survival and reproductive success (Merilä & Sheldon 1999; Garnier *et al*. 2001; van Kleunen *et al*. 2010; Castellanos & Verdú 2012). Many studies, however, have found contradictory patterns regarding the significance of plant functional traits related to growth, such as growth rate and biomass allocation, for invasion success as they vary substantially among species and environments (Macel *et al*. 2007).

Plant growth traits are likely to vary among species, their populations, and environments as they are highly plastic, have high genetic variance and are constantly exposed to selection pressures (Pattison *et al*. 1998, DeWalt *et al*. 2004). Climatic adaptation often produces clinal variation in life-history traits among populations of species (Barrett *et al.* 2008), with studies observing clinal variation in growth traits in response to selection pressures such as rainfall (e.g. Pattison *et al*. 1998; Schulze *et al*. 2006; Hierro *et al*. 2009), temperature (e.g. Atkin *et al*. 2006) and nutrient availability (e.g. Austin *et al*. 1985; Wright & Westoby 1999). Surprisingly, few studies have examined growth trait variation in arid environments (but see Angert *et al*. 2007 and Donovan *et al*. 2007) and none (that I am aware of) compare growth trait variation among populations of an invasive species that has extended its range into arid environments. Life-history trait adaptation in response to climate is vital to survival

and range expansion in novel environments, especially when resource availability fluctuates, such as in arid environments. In this chapter, I test three predictions about growth trait variation among populations of *Echium plantagineum*.

In the same way that particular growth traits are important for invasion success, they are essential for survival and range expansion in arid environments. Water is one of the most limiting factors in arid environments (Armas & Pugnaire 2005), followed by nutrient availability (Maestre *et al*. 2005), and a combination of growth traits is required to cope with this variability (Fonseca *et al*. 2000; Peperkorn *et al*. 2005). Growth traits are heavily influenced by environmental factors and in arid environments traits that facilitate slower growth may increase survival. I suggest lower relative growth rate (RGR) in populations of an invasive plant species invading arid habitats may contribute to increased invasion success. In addition, as photosynthetic capacity is critical for plant growth, variation in leaf shape is predicted to reflect selection pressures on function (Nicotra *et al.* 2011), inducing the production of many small leaves in plants in arid environments. Smaller leaves are advantageous in hot, dry, high light and low nutrient environments due to their smaller surface area, lower transpiration rate and high heat exchange capacity (Castro-Díez *et al*. 1997; M^{c}Donald *et al*. 2003; Kleiman & Aarssen 2007). This reduction in leaf size in arid areas has been observed by McDonald *et al*. (2003) in 690 native plant species across 47 sites spread through-out south-east Australia.

Growth traits that are likely to assist invasiveness (Siemann & Rogers 2001), including traits associated with return on investment and resource use, are likely to be influenced by water availability. For example, traits such as SLA, leaf area ratio (LAR) and leaf life span (LL) are strongly linked to rainfall, with drier sites having reduced investment in leaf structure per resource input than higher rainfall sites

(Lambers & Pooter 1992; Reich *et al.* 1997; Cunningham *et al*. 1999; Wright & Westoby 2002; Wright *et al*. 2004). Moreover, the efficient utilization of available nutrients in arid environments may assist range expansion in invasive species as leaf nutrient content can be used to take advantage of resources (Cunningham *et al*. 1999). For example, increasing leaf nitrogen (N) content with decreasing rainfall, as observed by Cunningham *et al*. (1999) in ten native Australian species, may be an adaptation to exploit high light availability in arid environments to allow more efficient photosynthesis. Slow growth rates in arid environments are likely to increase invasion success and survival, however, they are also likely to cause a reduction in plant size and stature.

Plant size and stature do not only influence resource collection and plant storage but they can also influence invasion success (Maron *et al*. 2004). Plants from arid environments have been observed to allocate a greater proportion of their resources to reproduction than vegetative growth (Aronson *et al*. 1993) resulting in shorter plants with less above ground biomass (AGB) compared to plants from mesic environments (e.g. Archibald & Bond 2003; Peperkorn *et al*. 2005). In addition, plants can be subject to selection for increased resource collection capacity through below ground traits, such as biomass, which are positively related to efficient nutrient extraction (Lloret *et al*. 1999). The 'functional equilibrium theory' of Brouwer (1962; 1963) states that above ground biomass is favoured when light and CO_2 are limited and below ground growth is favoured when water and nutrients are limited. As a result, plants in variable arid environments are predicted to have larger below ground biomass (BGB), larger root:shoot ratios (RSR) and increased initial rooting depths compared to plants from mesic environments (Chapin *et al*. 1993; Schenk & Jackson 2002, Peperkorn *et al*. 2005). For example, Lloret *et al*. (1999) observed increased

seedling survival with increased allocation to BGB in 12 native Mediterranean shrubs during summer drought conditions. In addition, soil moisture at greater depth is less variable and dependent on long-term weather patterns (Lloret *et al*. 1999) making it a more reliable resource for invading species.

Efficient resource use is critical to plant survival when resources are scarce and vital for species expanding their range into arid environments (Brock & Galen 2005). For adaptation to occur in resource use traits there must be a fitness benefit and a compromise between water conservation and resource collection (Nicotra & Davidson 2010). In addition, efficient resource use may facilitate higher fitness when the growing season is short or disturbance is frequent (Arntz & Delph 2001; Ebdon & Kopp 2004) and thus is predicted to be under natural selection in arid environments (Heschel *et al*. 2002; 2005; Donovan *et al*. 2007; Nicotra & Davidson 2010). Consequently, plants from arid environments can benefit from lower stomatal conductance, lower carbon isotope discrimination (Δ; e.g. Ehleringer 1993) and higher water use efficiency (WUE; e.g. Donovan *et al*. 2007).

Carbon isotope discrimination (Δ) is predicted to vary between arid and mesic environments as it is a measure of biochemical and structural factors that relates CO_2 uptake and water loss in plants (photosynthetic gas exchange; Ehleringer 1993). Furthermore, in C_3 plants, Δ values are determined by the ratio of CO_2 concentrations in the leaf intercellular spaces compared to that of the atmosphere (Ehleringer 1993) and low values indicate individuals have high WUE. In addition, WUE is associated with changes in plant growth, fitness and climate (Heschel *et al*. 2002; 2005; Donovan *et al*. 2007; Nicotra & Davidson 2010). For example, Heschel *et al*. (2002) observed higher WUE in arid environments compared to mesic environments in 28 native North American populations of *Impatiens capensis*. Similarly, Donovan *et al*.

(2007) observed the same pattern of increased WUE in arid environments for two native species of desert sunflower (*Helianthus anomalus* and *Helianthus deserticola*). In contrast, very few studies have assessed physiological traits in invasive species (e.g. see Brock & Galen 2005). Collectively, invasive species benefit from a combination of resource use traits which may contribute to increased invasiveness and better success when invading a novel environment (Siemann & Rogers 2001).

3.1.1 Chapter aim and approach

In this chapter, I compared growth trait variation among populations of the invasive annual forb *E. plantagineum* to test predictions for clinal shifts along an arid-mesic gradient. Seeds were collected from field populations of *E. plantagineum* along the gradient, germinated in the laboratory and transferred to a uniform glasshouse environment as seedlings for periodic growth trait measurements until reaching the reproductive phase (approximately 15 weeks). The aim of this study was to test three predictions concerning the invasive spread of populations of *E. plantagineum*. Compared to populations from mesic environments, arid populations should demonstrate:

(1) Slower growth (RGR) with an associated suite of traits linked to slow growth including smaller leaf size, higher leaf number, lower specific leaf area (SLA), lower leaf area ratio (LAR) and higher nutrient uptake (leaf nitrogen, carbon and chlorophyll);

(2) Smaller stature including shorter plant height, reduced petiole length, less photosynthetic area (P_{area}), smaller above-ground biomass (AGB), larger root:shoot ratio (RSR) and larger below-ground biomass (BGB); and

(3) Efficient water use including lower stomatal conductance, lower carbon isotope discrimination (Δ) and higher water use efficiency (WUE).

These responses would indicate that populations from arid environments have adapted to low, unpredictable rainfall and low nutrient availability in arid environments, allowing them to accumulate resources, mature and reproduce efficiently under comparatively harsh growing conditions.

3.2 Methods

3.2.1 Seed germination and glasshouse conditions

Echium plantagineum seeds were collected during the 2009 reproductive season (October to December; see seed collection method in 2.2.1) from a total of 34 sites randomly selected within six IBRA bioregions (see 2.2.1 and Fig. 2.1 for a description of bioregions). Seeds were collected from ten individual plants at each site, extracted from the mature fruit using a rubbing board (consisting of two flat rubber pads) and stored in paper bags in the laboratory at room temperature. Seed viability was determined by seed weight, visual inspection and a squeeze test (as described in 2.2.2). Seeds used in these experiments were of a similar seed mass, seed size, germination rate and dormancy, which minimised the possibility of maternal effects impacting on growth of glasshouse-grown plants (See 2.2.2 for more details). Seeds were germinated in the laboratory in darkness on moist filter paper in petri dishes. Five days after germination, two groups of plants were planted into biodegradable pots (Jiffypot®) with standard, high nutrient compost potting soil (consisting of a mix of calcium carbonate lime, dolomite lime, blood and bone, and NPK fertiliser; pH =

6.5), transported to the glasshouse, transplanted to 10cm pots after two weeks and then to 20cm pots after six weeks. Pots were fertilised with Aquasol® Soluble Fertiliser (Yates, Australia) fortnightly or as required. Plants were grown from April to December 2010 under a photoperiod governed by natural sunlight and a targeted day/night temperature regime of 25/15ºC with an average of 20°C. Temperatures were logged from August to October and followed the targeted regime reasonably closely, with daily averages of 16-20ºC, although spot temperatures as high as 27ºC and as low as 12ºC were observed.

Group one consisted of one germinant from each of ten plants per site (n = 340) used to measure growth and stature traits. These plants were arranged in a randomised block design of five groups of three benches and separated from the sides of the glasshouse to minimise edge effects. Two plants from each site were randomly selected and periodically destructively sampled at the time intervals four, six, eight and 12 weeks from germination. Group two consisted of one germinant from two randomly selected plants per site (n = 68), used to measure water use traits, leaf chlorophyll, leaf nitrogen and leaf carbon content. These plants were distributed on three benches, in block one, in between plants marked for harvest until all plants reached the un-reproductive adult phase (15 weeks from germination) and the water use trial commenced (see 3.2.4 below).

3.2.2 Growth traits

Growth traits measured in this study include relative growth rate (RGR), leaf number, leaf dimensions including largest leaf length and largest leaf width, leaf accumulation rate, specific leaf area (SLA; leaf area of one side of a single leaf divided by the dry mass; Weiher *et al*. 1999), leaf area ratio (LAR; total plant leaf area divided by total

plant leaf dry mass; de la Bandera *et al*. 2008), plant height, leaf chlorophyll and leaf nutrient content (carbon and nitrogen). RGRs were calculated through biomass measurements collected by destructively sampling two plants from each site at the time intervals four, six, eight and 12 weeks from germination, and the following equation (1.1; Oesterheld & McNaughton 1988).

$$RGR = \frac{\ln(W_2) - \ln(W_1)}{(t_2 - t_1)} \qquad 1.1$$

where W_x and t_x are total dry weight and time in days respectively, and the subscripts indicate the harvest number. The number of days (t) varied between each harvest (17, 16 and 33 days respectively).

Leaf traits including leaf number, leaf dimensions and leaf accumulation rate were measured on two plants per site during each harvest. SLA was calculated by estimating leaf photosynthetic area (P_{area}) and leaf biomass (B_{leaf}) through allometric relationships with length on three leaves from two plants per site. Individual leaf P_{area} measurements were measured on 12-16 leaves per site per harvest conducted at four, six and eight weeks from germination. These leaves were then traced on to cardboard and measured for leaf P_{area} using a flat-bed leaf scanner (Licor Leaf Area Meter LI-3100C). Leaves were collected over a four week period to ensure that a range of large and small leaves could be collected. The equation used to calculate P_{area} (1.2) is based on the graphed measurements of these leaves ($R^2 = 0.91$; Fig. 3.1a).

$$P_{area} = 0.1905 (\text{leaf length})^2 + 2.3331 (\text{leaf length}) - 8.5921 \qquad 1.2$$

where P_{area} and *L* represent leaf photosynthetic area and the largest leaf length, respectively. Leaf biomass (B_{leaf}) was measured using the same leaves as leaf P_{area}, dried in an oven at 70ºC for 48 hours (Peperkorn *et al*. 2005; Zou *et al*. 2007). The equation used to calculate B_{leaf} (1.3) is based on the graphed measurements of leaf dry weight and hydrated leaf length ($R^2 = 0.84$; Fig. 3.1b).

$$B_{leaf} = 0.0018 (\text{leaf length})^2 - 0.004 (\text{leaf length}) + 0.1658 \qquad 1.3$$

where B_{leaf} and *L* are representative of leaf biomass and largest leaf length, respectively. This approach, using allometric relationships, is adapted from DeWalt *et al.* (2004). Variables such as leaf number and leaf width were assessed as surrogates for leaf length however leaf length is the best indicator of leaf P_{area} and B_{leaf}. LAR was calculated as the sum of the whole plant P_{area} divided by the sum of the whole plant leaf dry biomass.

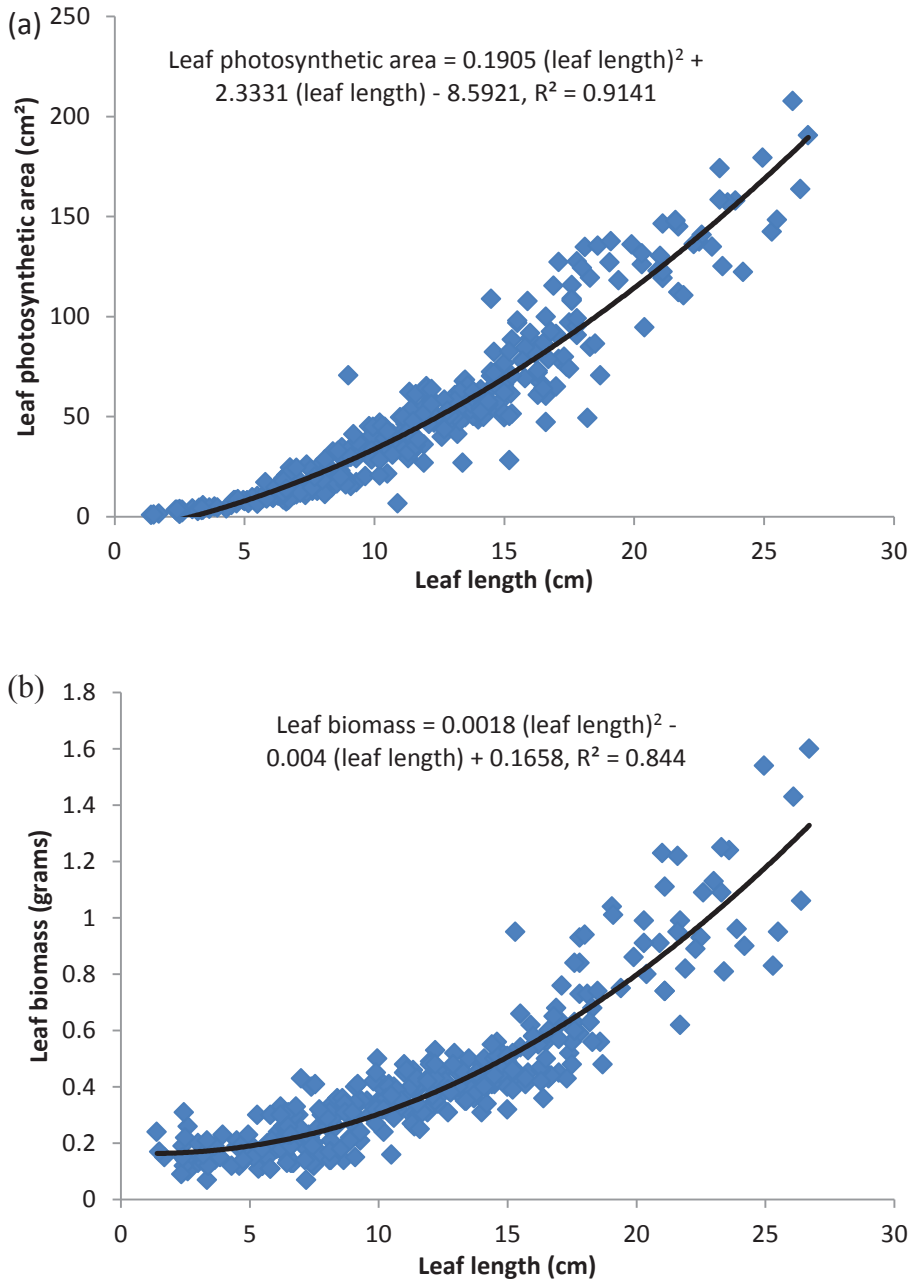

Figure 3.1 Allometric relationships between leaf length and (a) leaf photosynthetic area and (b) leaf biomass using three leaves collected from one *Echium plantagineum* plant per site over three time intervals spanning four weeks.

Leaf chlorophyll, nitrogen (N) and carbon (C) content were measured on plants from group two. Leaf chlorophyll was measured once during the experiment on three leaves per plant on two representative plants per bioregion, 16 weeks from germination, using a SPAD chlorophyll meter (SPAD meter 5020, Japan, Konica Minolta sensing inc). Percentage leaf N and C content were measured once during the experiment, at 20 weeks from germination. Three leaf samples were collected from a representative plant from each bioregion, ground to a fine powder using a tissuelyser at a frequency of 30.1 rpm for ten minutes and analysed using a Europa 20-20 isotope ratio mass spectrometer with an Automated Nitrogen Carbon Analysis (ANCA) preparation system (Dumas 1981).

3.2.3 Stature traits

Stature traits measured during the trial include photosynthetic area (P_{area}), above ground biomass (AGB; dry shoot weight), below ground biomass (BGB; dry root weight), root:shoot ratio (RSR), petiole length of an averaged sized leaf and plant height. Although many of the traits measured in this chapter are highly correlated, individually they are surrogates for measures of plant growth: for example height and AGB are correlated however AGB can also provide an estimate of plant fecundity, which makes both traits important (Weiher *et al*. 1999). P_{area} of the whole plant was measured as the sum of the photosynthetic area of all leaves determined through the hydrated length of each leaf and equation 1.2. Above ground biomass (AGB), including all plant material above the ground level, and BGB, including all plant material below the ground level, were washed and biomass obtained by weight after 48 hours of drying at 70ºC (Peperkorn *et al*. 2005; Zou *et al*. 2007). Measurements of final plant size included height, width, stem length and final above ground biomass did not differ significantly among bioregions and therefore are not considered further.

3.2.4 Water use strategy

Individual plants of group two were evaluated for the following water use traits: transpiration rate, carbon isotope discrimination (Δ) and physical water use per unit biomass. Transpiration rate was determined using porometer (Delta-T AP4 Porometer) readings on the adaxial (upper surface of the leaf) and abaxial (underside of the leaf) sides of a single leaf on two plants per site, at 20 weeks from germination, to measure instantaneous gas exchange. Porometer readings were collected when the plants were fully hydrated. Δ samples were collected at 16 weeks from mature leaf tissue, dried at 70ºC for 48 hours (Peperkorn *et al*. 2005; Zou *et al*. 2007), allowed to cool and ground in a puck mill to ensure sample homogeneity. A sample size of between 2.0 - 2.5 mg was weighed into a tin foil capsule and the weight recorded. The analysis was then performed using a Europa 20-20 isotope ratio mass spectrometer with an ANCA preparation system (Analytical Services, Black Mountain Laboratories, ACT Australia; Dumas 1981).

Physical water use was measured over two weeks during the 15th and 16th week from germination. Water use efficiency (WUE) was tested by watering the plants to capacity, recording their weight to get an initial saturation measurement, sealing the base of the pots with plastic and adding 100 grams of polyethylene beads (Pacific West Corporation, NSW; Fig. 3.2a, b) to the top to prevent drainage and evaporation, respectively. For a period of two weeks a known volume of water was added daily and the weight of the pots was recorded (Fig. 3.2b). After two weeks the plants were watered back to capacity. Four control pots filled with soil, covered with polyethylene beads and sealed at the base with plastic were distributed amongst the experimental plants to ensure that the polyethylene beads were effective in preventing water loss. Throughout the trial the control pots lost between 3.5 and 5.2 mls of water,

with the average value (4.49 mls) removed from the total plant water use of the experimental plants. WUE was determined as the daily volume of water that was lost from the pots divided by the average plant biomass (1.4).

$$WUE = (W/t)/AAGB \qquad 1.4$$

where W represents total water use, t represents time in number of days and $AAGB$ represents the average estimated total above-ground dry biomass estimated using equation 1.3.

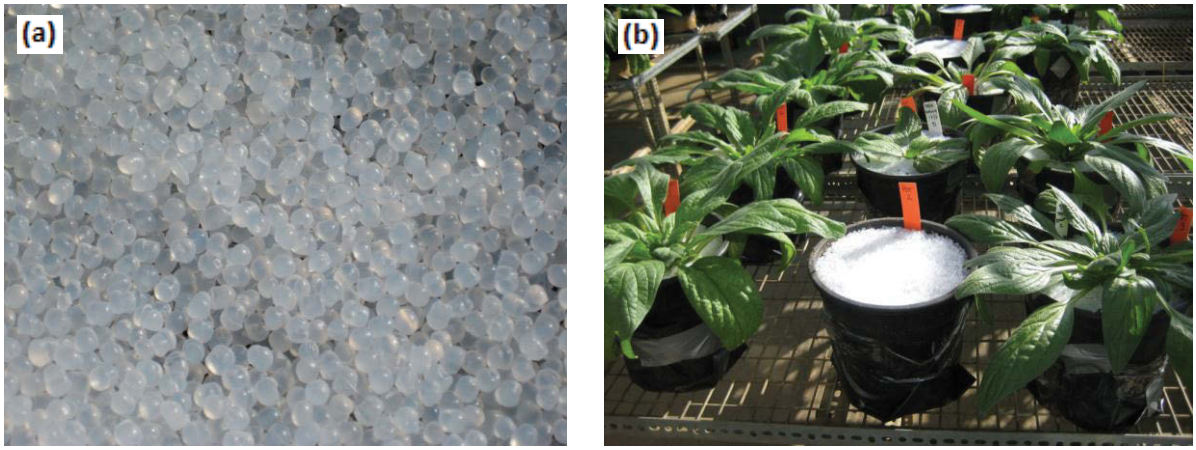

Figure 3.2 Water use efficiency trial (a) Polyethylene beads added to the surface of pots to prevent water loss, (b) water use trial set-up with control pots covered in beads in between experimental plants.

3.2.5 Climate, spatial and edaphic data collection

Climate data were collected from SILO enhanced meteorological dataset and datadrill procedure hosted by the State of Queensland Department of Environment and Resource Management 2012 (Jeffrey *et al.* 2001; See 2.2.5). Spatial data were

collected by GPS. A representative soil core 10 cm^2 and 10 cm deep, was collected at each field site, sealed in a plastic bag, and stored at room temperature in the laboratory and soil characteristics measured as outlined in Rayment & Higginson (1992; electrical conductivity and pH) and Dumas (1981; nitrogen content, carbon content; See 2.2.4).

3.2.6 Statistical analysis

Each trait, during each harvest, was analysed individually using a linear mixed model (LMM) with bioregion as a fixed explanatory variable and site within bioregion as a random explanatory variable. Normality was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests and very few variables required transformation. These variables were transformed using logarithmic (base-10) transformations to normalise the data and meet the assumptions of the mixed model analysis. This approach has previously been used for the measurement of leaf traits (Reich *et al*. 1991, 1992, 1997). The dependent variables included in this analysis are split into three categories; (1) growth traits including RGR, leaf number, largest leaf length, largest leaf width, SLA, LAR, leaf accumulation rate, leaf N, leaf C and leaf chlorophyll; (2) stature traits including P_{area}, AGB, BGB, RSR, petiole length, plant height; and (3) water use traits including WUE, carbon isotope ratios (Δ) and porometer readings for the adaxial and abaxial sides of the leaves. Simple linear regression was used on untransformed data to compare each trait to two representative environmental components (1) climatic factors (PC1; See Fig. 2.3a; 2.2.6) and (2) edaphic factors (PC2; See Fig. 2.3b; See 2.2.6; Table 2.3). No data transformations were required for the regression analyses. All analyses include site within bioregion as a random variable however very few variables were significant at the site level and as site level

data are not the focus of this study, site level data are not shown. All data were analysed using SPSS (SPSS Inc, version 19, IBM).

3.3 Results

3.3.1 Growth traits

LMM analyses showed that RGR varied among bioregions during harvest intervals 1-2, 2-3 and 3-4 (Fig. 3.3a; Table 3.1). Simple linear regressions revealed that RGR was highly positively correlated with climate with plants in arid bioregions growing faster than plants in mesic bioregions (Fig. 3.3b, c; Table 3.1). Leaf number varied among bioregions during the first and third harvest, and marginally at the fourth harvest (Table 3.1; Fig. 3.4a). Simple linear regressions indicated that these differences were highly positively associated with climatic factors (Table 3.1; Fig. 3.4b, c) with plants from arid maternal bioregions producing more leaves than plants from mesic coastal bioregions. Largest leaf length differed among bioregions during the third and fourth harvest, however, this was not linked to climatic factors or edaphic characteristics (Table 3.1; Fig. 3.5a). Largest leaf width varied during the fourth harvest and was positively correlated with edaphic factors (Fig. 3.5b, c) with high nutrient sites for example within the SWS bioregion having wider leaves than low nutrient sites such as sites within the BHC bioregion. LAR and SLA varied among bioregions (Table 3.1; Fig. 3.6a, b), however, while SLA, during the third harvest, was marginally correlated with edaphic variables (Fig. 3.6c) LAR was not correlated with climatic or edaphic variables. Leaf accumulation rate, leaf carbon and leaf nitrogen did not vary among bioregions at any point during the experiment ($P>>0.05$; Table 3.1; Fig. 3.7, 3.8a, b).

Leaf chlorophyll content was found to differ among bioregions however this was not linked to climatic factors or edaphic conditions (Table 3.1; Fig. 3.8c).

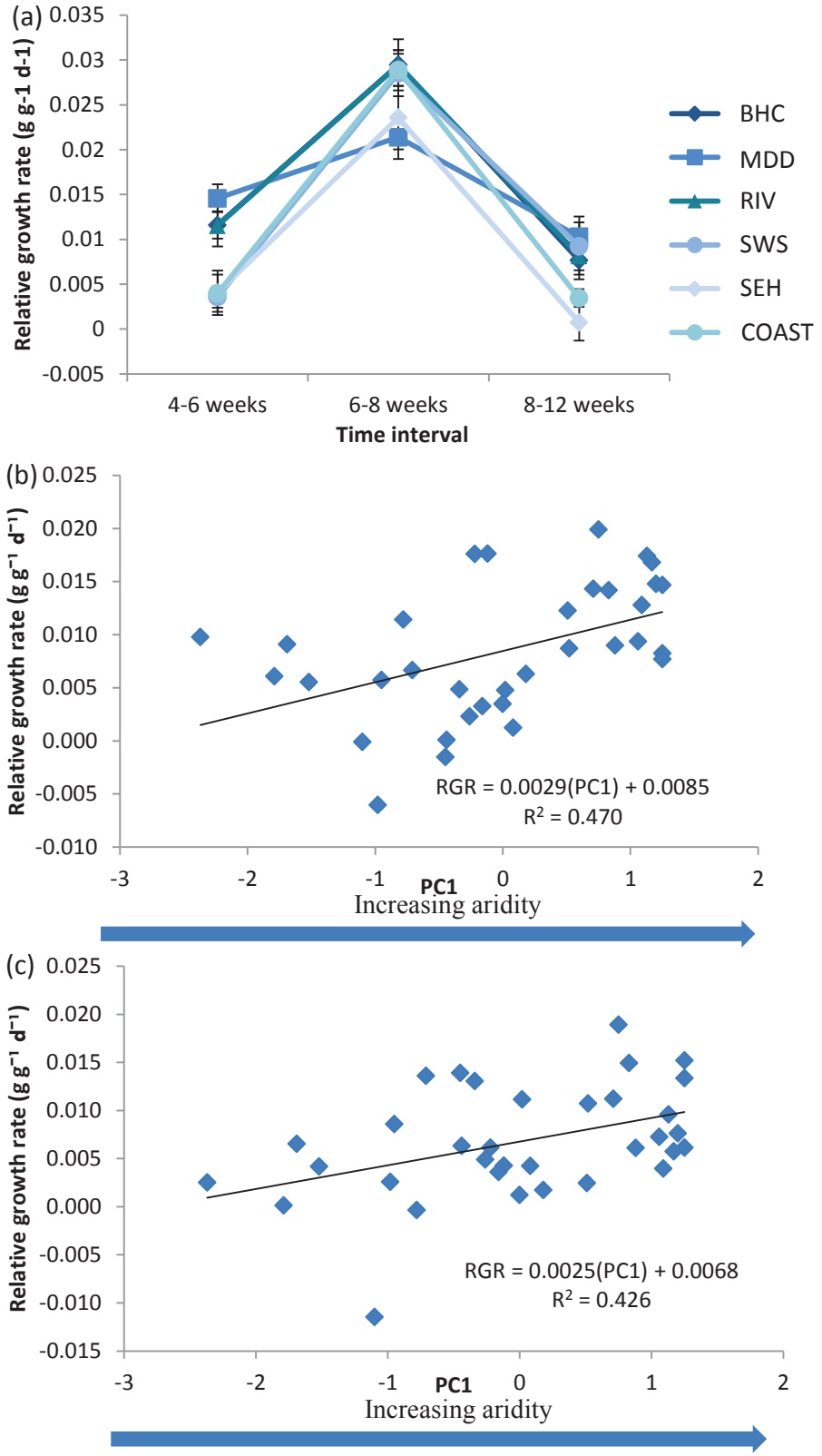

Figure 3.3 Relative growth rate measured over three time intervals 4-6, 6-8 and 8-12 weeks from germination during a common garden glasshouse study; (a) actual means ± SE; (b) RGR first harvest site level scores on PC1 and (c) RGR third harvest site level scores on PC1. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

Table 3.1 Results of linear mixed models and linear regressions of growth traits.

Plant trait	Time frame*	Linear mixed model		Regression			
		Bioregion		PC1		PC2	
		F	*P*	R^2	*P*	R^2	*P*
Relative growth rate (RGR)	1-2	$F_{5,28} = 6.236$	**0.001**	0.470	**0.005**	0.154	0.386
	2-3	$F_{5,28} = 3.196$	**0.021**	0.135	0.445	0.137	0.440
	3-4	$F_{5,28} = 2.988$	**0.028**	0.426	**0.012**	0.014	0.938
Leaf Number	1	$F_{5,28} = 3.469$	**0.015**	0.409	**0.016**	0.219	0.213
	2	$F_{5,28} = 1.603$	0.192	0.414	**0.015**	0.209	0.235
	3	$F_{5,28} = 3.441$	**0.015**	0.419	**0.003**	0.073	0.682
	4	$F_{5,28} = 2.119$	0.093	0.393	**0.021**	0.324	0.061
Largest leaf length	1	$F_{5,28} = 0.457$	0.804	0.030	0.867	0.163	0.358
	2	$F_{5,28} = 1.105$	0.380	0.163	0.358	0.029	0.873
	3	$F_{5,28} = 4.088$	**0.007**	0.109	0.541	0.114	0.522
	4	$F_{5,28} = 3.766$	**0.010**	0.017	0.924	0.144	0.416
Largest leaf width	1	$F_{5,28} = 1.108$	0.379	0.161	0.363	0.078	0.659
	2	$F_{5,28} = 0.560$	0.729	0.140	0.428	0.199	0.260
	3	$F_{5,28} = 0.912$	0.487	0.052	0.770	0.047	0.794
	4	$F_{5,28} = 2.566$	**0.049**	0.096	0.589	0.398	**0.020**
Specific leaf area (SLA)	1	$F_{5,28} = 0.312$	0.902	0.010	0.953	0.132	0.456
	2	$F_{5,28} = 3.285$	**0.019**	0.245	0.163	0.142	0.424
	3	$F_{5,28} = 4.856$	**0.003**	0.175	0.321	0.399	**0.019**
	4	$F_{5,28} = 2.093$	0.096	0.082	0.647	0.028	0.875
Leaf area ratio (LAR)	1	$F_{5,28} = 1.054$	0.407	0.031	0.863	0.022	0.901
	2	$F_{5,28} = 2.981$	**0.028**	0.260	0.138	0.012	0.948
	3	$F_{5,28} = 3.511$	**0.014**	0.126	0.478	0.175	0.322
	4	$F_{5,28} = 2.320$	**0.070**	0.121	0.495	0.173	0.327
Leaf accumulation	1-2	$F_{5,28} = 0.672$	0.648	0.291	0.095	0.143	0.418
	2-3	$F_{5,28} = 0.807$	0.555	0.193	0.274	0.079	0.658
	3-4	$F_{5,28} = 0.577$	0.717	0.079	0.656	0.285	0.102
Leaf chlorophyll	5	$F_{5,28} = 3.069$	**0.025**	0.037	0.837	0.007	0.968
Leaf carbon	6	$F_{5,28} = 1.359$	0.270	0.144	0.416	0.061	0.733
Leaf nitrogen	6	$F_{5,28} = 1.101$	0.382	0.192	0.278	0.153	0.388

*Time frame 1 = 4 weeks, 2 = 6 weeks, 3 = 8 weeks, 4 = 12 weeks, 5 = 16 weeks, 6 = 20 weeks.

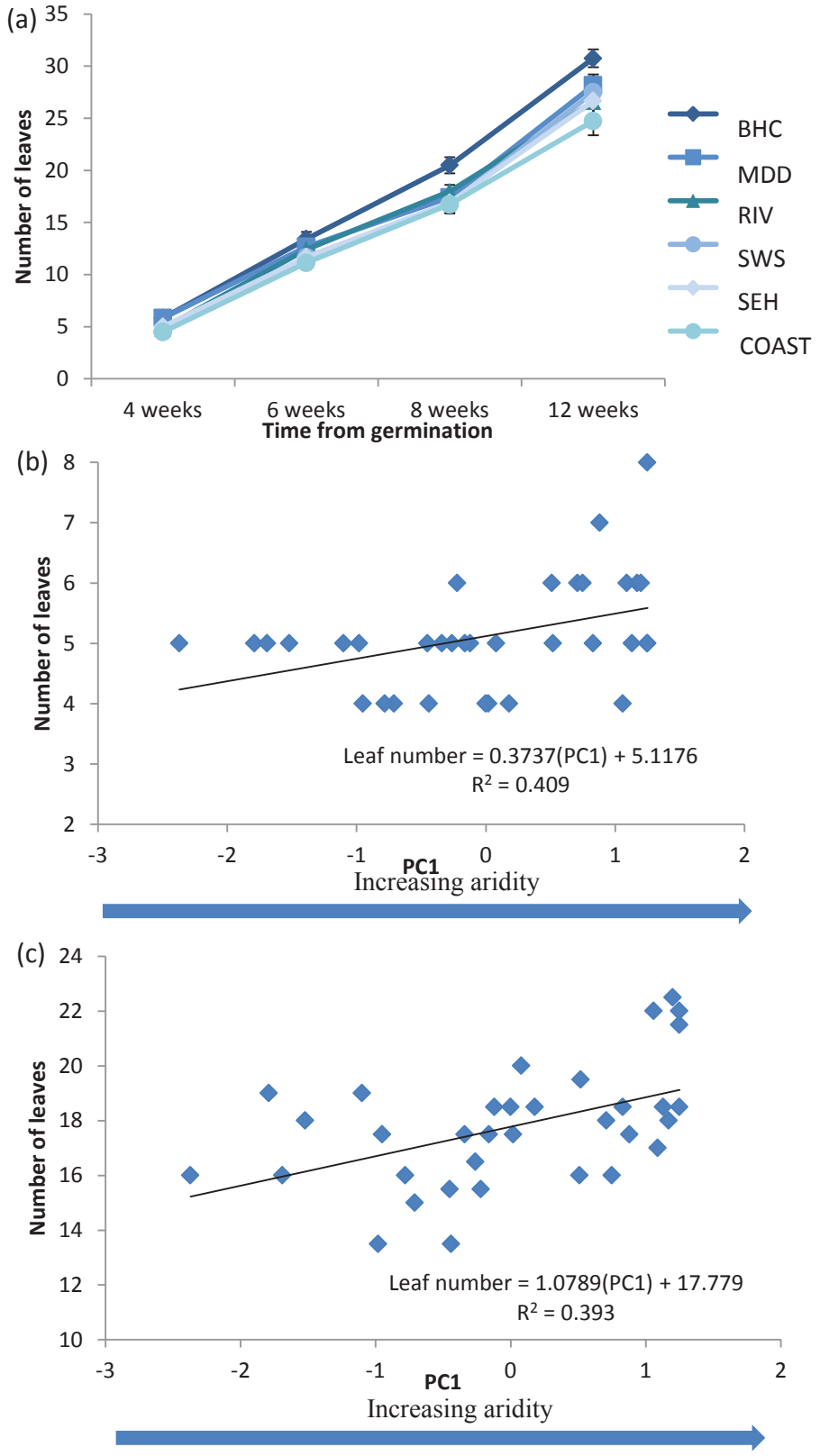

Figure 3.4 Leaf number was measured over four time intervals four, six, eight and 12 weeks from germination during a common garden glasshouse study; (a) actual means ± SE; (b) leaf number first harvest site level scores on PC1 and (c) leaf number third harvest site level scores on PC1. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

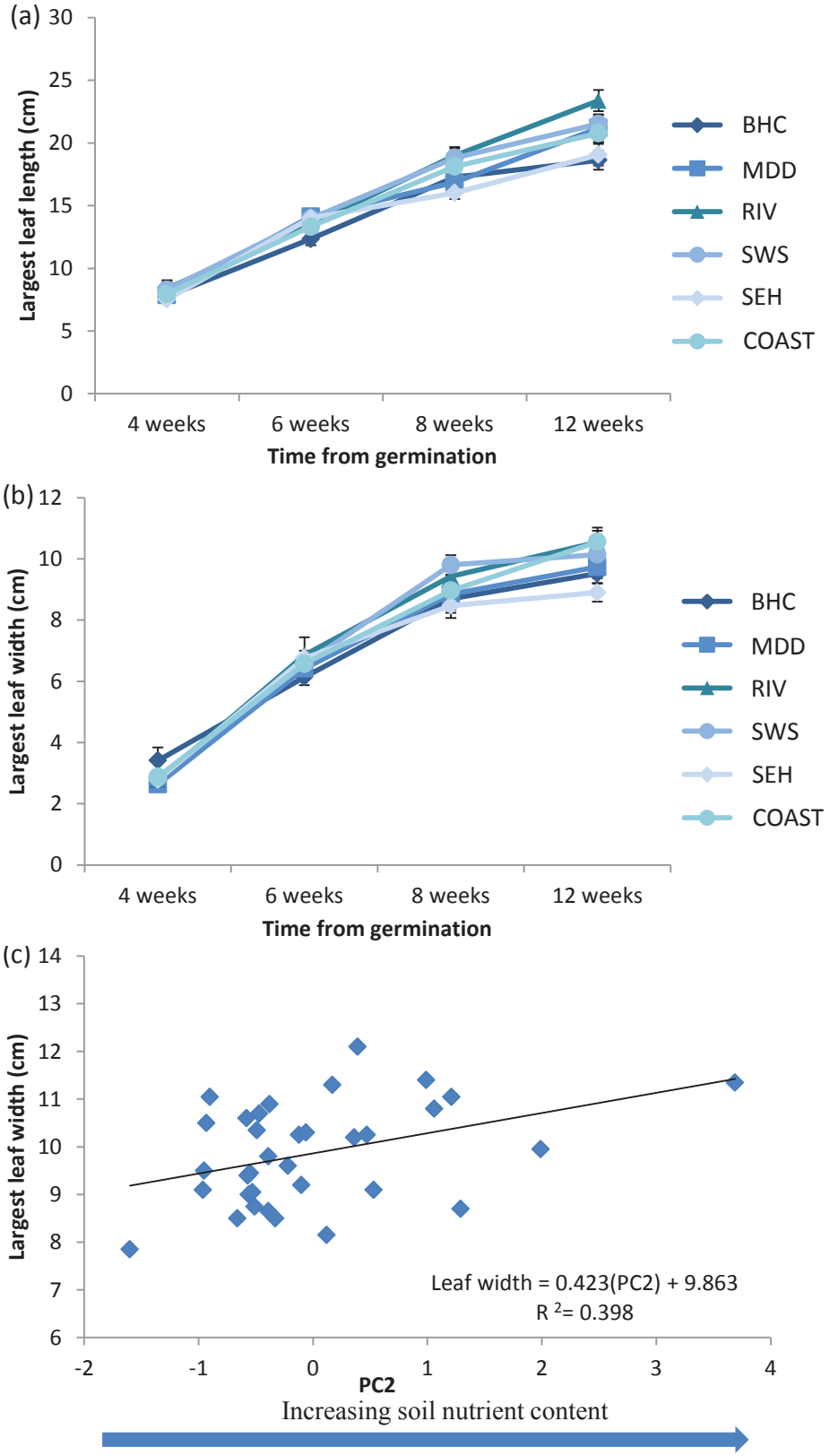

Figure 3.5 Leaf dimensions measured during common garden glasshouse study measured during four time intervals four, six, eight and 12 weeks from germination; (a) largest leaf length actual means ± SE; (b) largest leaf width actual means ± SE; (c) largest leaf width fourth harvest compared to site level scores on PC2. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

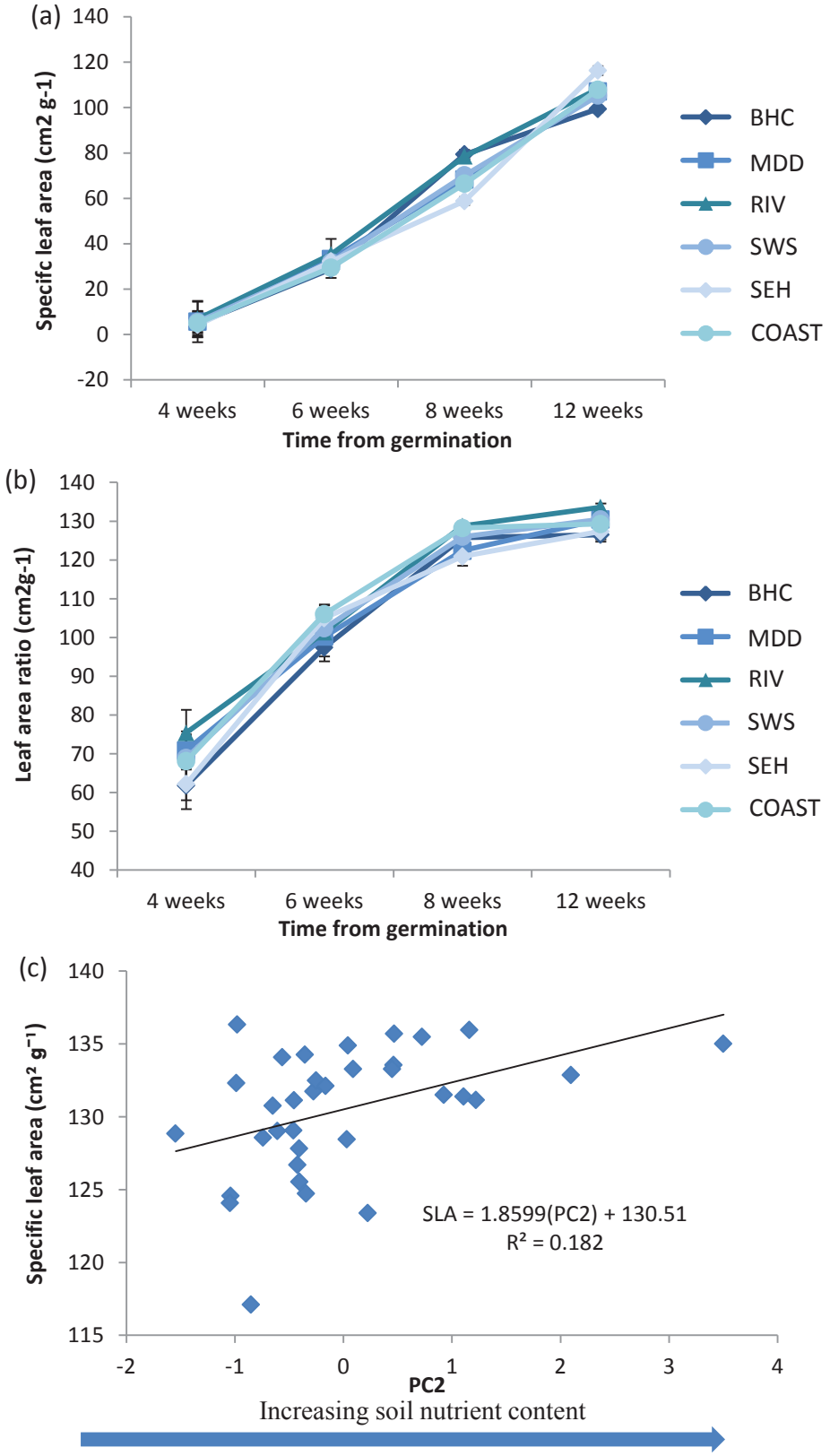

Figure 3.6 Leaf traits measured during common garden glasshouse study measured during four time intervals four, six, eight and 12 weeks from germination; (a) specific leaf area (SLA) actual means ± SE; (b) leaf area ratio (LAR) actual means ± SE; (c) SLA third harvest site level scores on PC2. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

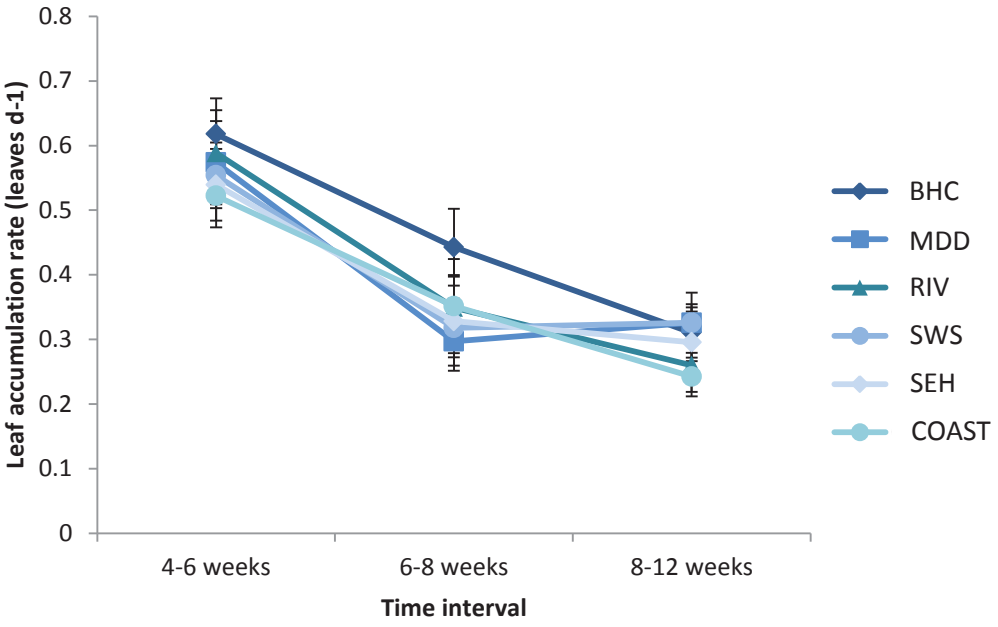

Figure 3.7 Leaf accumulation rate (actual means ± SE) measured during common garden glasshouse study measured during four time intervals four, six, eight and 12 weeks from germination. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

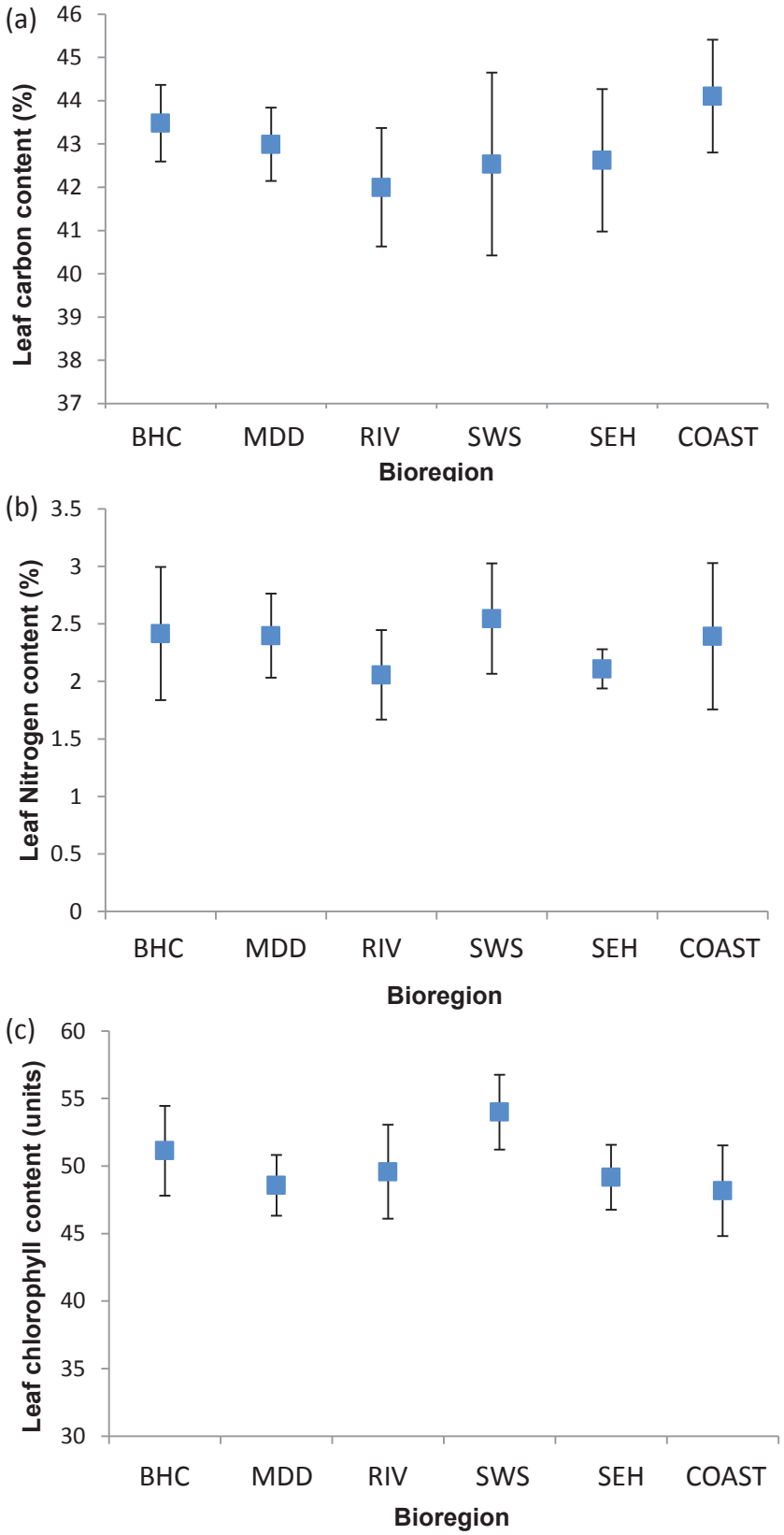

Figure 3.8 Leaf nutrient content measured once during a common garden glasshouse study; (a) leaf nitrogen content (±SD); (b) leaf carbon content (±SD) and (c) leaf chlorophyll content (±SD). Abbreviations denote the individual bioregions, see Fig. 2.1 for full names. Bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).

3.3.2 Stature traits

Height, as a measure of plant fitness, varied among bioregions during the third harvest (Fig. 3.9a) and simple linear regression revealed a positive correlation with climate (Table 3.2). Shorter plants were observed from mesic environments while taller plants were observed from more arid environments (Fig. 3.9b), however, at the fourth harvest there was no difference among bioregions indicating that during the third harvest there may have been a significant change in resource allocation (Table 3.2). Average petiole length did not vary among bioregions at any point during the experiment ($P>>0.05$; Table 3.2; Fig. 3.9c). P_{area} varied among bioregions during the third and fourth harvest (Fig. 3.10a), however, it was not correlated with either climate variables or edaphic factors (Table 3.2), Although during the third harvest, P_{area} was marginally correlated with edaphic factors ($P = 0.06$; Fig. 3.10b), suggesting that plants in high nutrient environments had more photosynthetic area than plants from mesic environments.

Table 3.2 Results of linear mixed models and linear regressions of stature traits.

Plant trait	Time frame*	Linear mixed model		Regression			
		Bioregion		PC1		PC2	
		F	*P*	R^2	*P*	R^2	*P*
Petiole length	3	$F_{5,28} = 1.726$	0.161	0.217	0.218	0.182	0.303
	4	$F_{5,28} = 0.796^{\#}$	$0.562^{\#}$	0.118	0.506	0.111	0.530
Height	3	$F_{5,28} = 3.189$	**0.021**	0.373	**0.030**	0.106	0.550
	4	$F_{5,28} = 1.842^{\#}$	$0.137^{\#}$	0.058	0.746	0.060	0.735
Photosynthetic area (P_{area})	1	$F_{5,28} = 0.540$	0.744	0.001	0.993	0.097	0.586
	2	$F_{5,28} = 1.065$	0.400	0.052	0.770	0.118	0.506
	3	$F_{5,28} = 3.758$	**0.010**	0.212	0.228	0.326	0.060
	4	$F_{5,28} = 2.735$	**0.039**	0.028	0.874	0.219	0.212
Above ground biomass (AGB)	1	$F_{5,28} = 5.637$	**0.001**	0.482	**0.004**	0.114	0.522
	2	$F_{5,28} = 1.341$	0.276	0.322	0.063	0.210	0.232
	3	$F_{5,28} = 2.268$	0.075	0.285	0.102	0.019	0.914
	4	$F_{5,28} = 5.244$	**0.002**	0.569	**<0.001**	0.004	0.984
Below ground biomass (BGB)	1	$F_{5,28} = 5.331$	**0.001**	0.328	0.059	0.403	**0.018**
	2	$F_{5,28} = 0.688^{\#}$	$0.637^{\#}$	0.145	0.414	0.024	0.893
	3	$F_{5,28} = 2.112$	0.094	0.387	**0.024**	0.198	0.261
	4	$F_{5,28} = 1.665^{\#}$	$0.176^{\#}$	0.147	0.407	0.015	0.932
Root : shoot ratio (RSR)	1	$F_{5,28} = 3.426^{\#}$	$\mathbf{0.015}^{\#}$	0.043	0.811	0.404	**0.018**
	2	$F_{5,28} = 2.525$	0.052	0.300	0.085	0.180	0.307
	3	$F_{5,28} = 2.399$	0.062	0.074	0.678	0.150	0.397
	4	$F_{5,28} = 1.579$	0.198	0.204	0.247	0.063	0.724

*Time intervals; 1 = four weeks, 2 = six weeks, 3 = eight weeks, 4 = 12 weeks.
[#] Denotes logarithmic (base 10) transformed

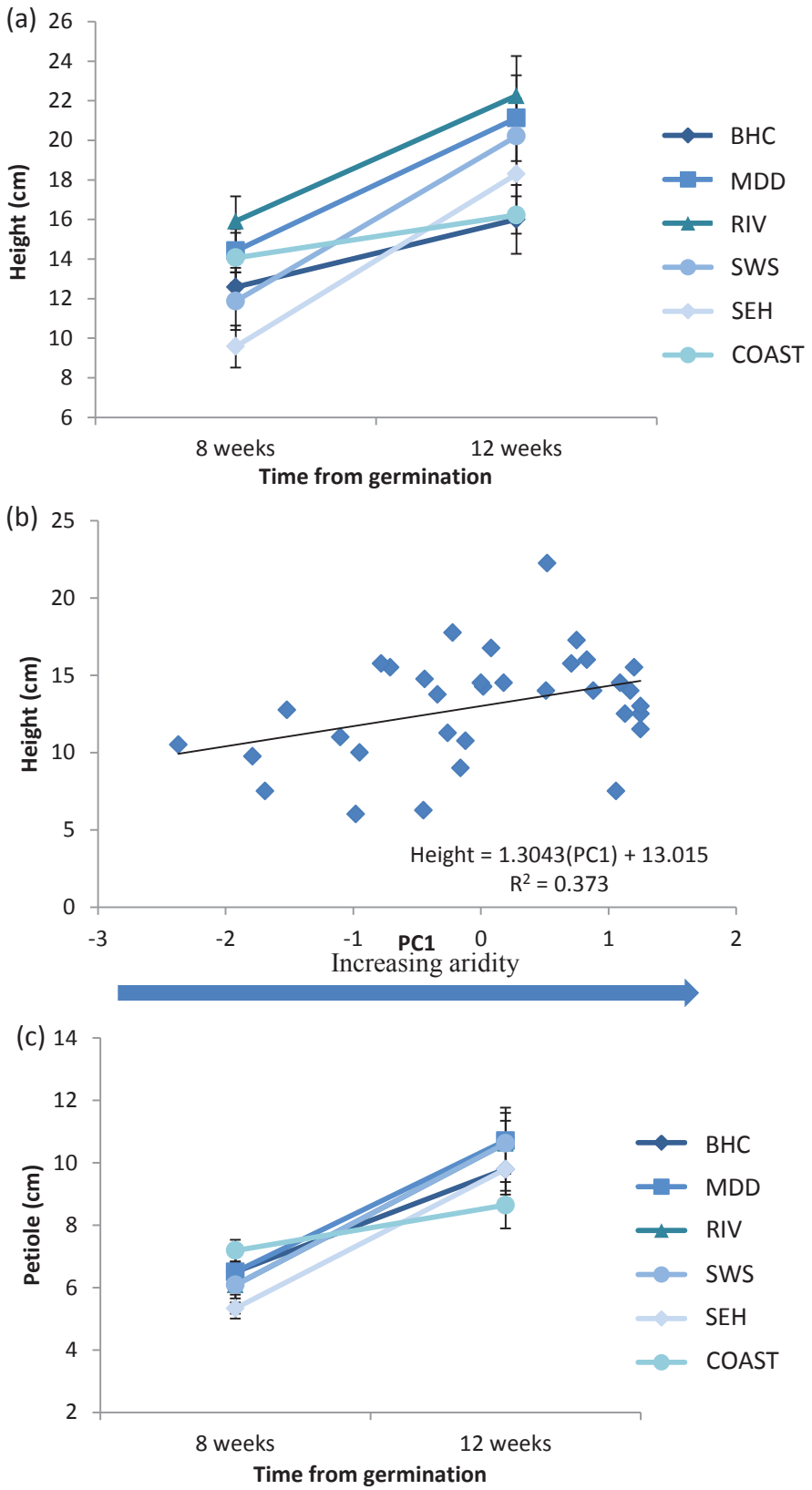

Figure 3.9 Plant height and petiole length measured over two harvest sessions eight and 12 weeks from germination during a common garden glasshouse study; (a) plant height actual means ± SE; (b) height at eight weeks site level scores on PC1; (c) plant petiole length of an average leaf actual means ± SE. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

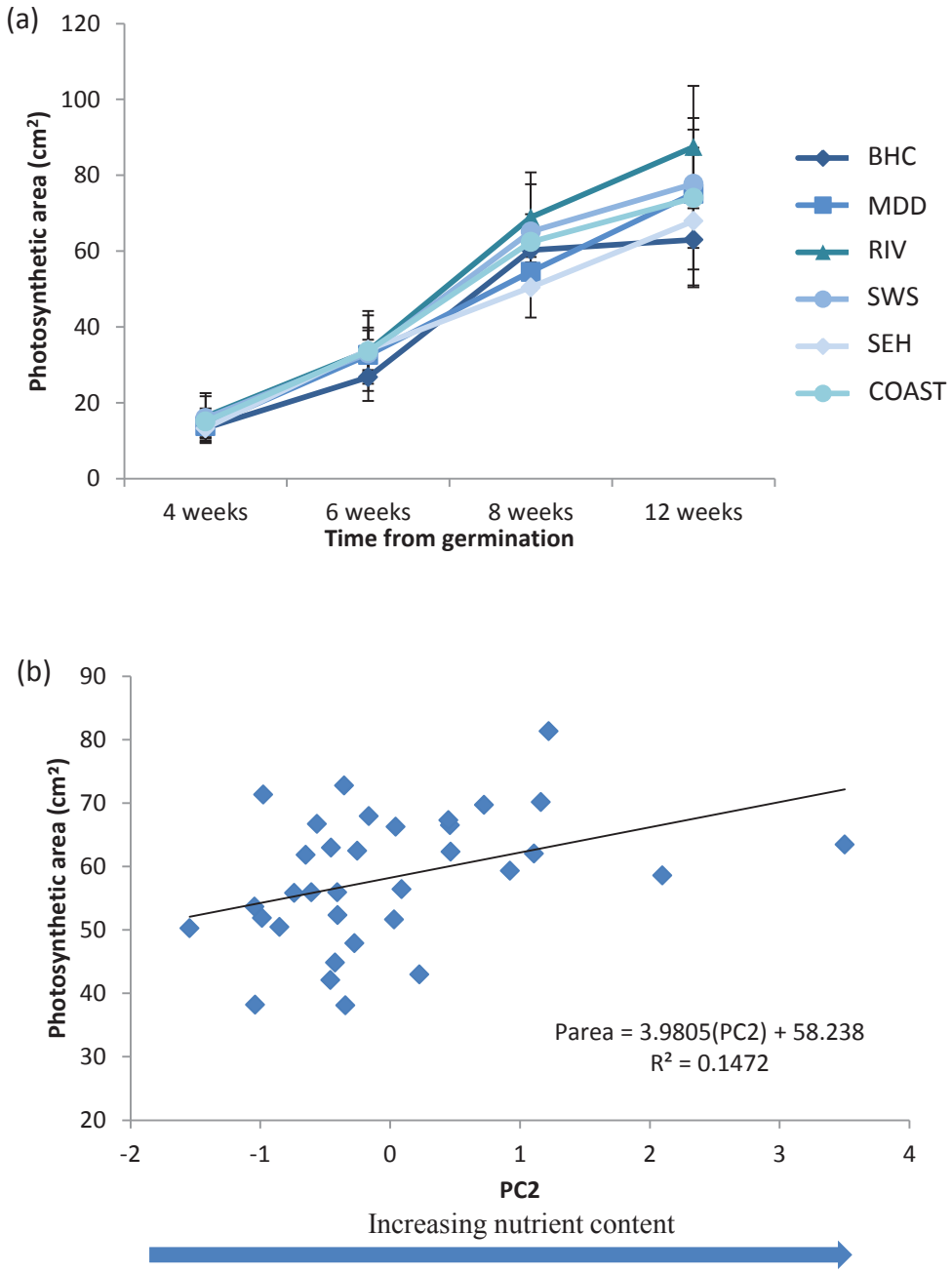

Figure 3.10 Photosynthetic area (P_{area}) was measured over four time intervals four, six, eight and 12 weeks from germination during a common garden glasshouse study; (a) P_{area} actual means ± SE; (b) P_{area} third harvest site level scores on PC1. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

Initially AGB was found to vary among bioregions and negatively correlate with climatic variables (Fig. 3.11a, b; Table 3.2). However, in the final harvest this trend was reversed (Fig. 3.11c; Table 3.2) and plants were positively correlated with climate, with larger plants produced with increasing aridity. BGB and RSR were significantly different among bioregions during the first harvest (Fig. 3.12a; 3.13a) and were highly negatively correlated with edaphic characteristics, exhibiting a significant decrease in below ground traits in response to increasing nutrient availability corresponding with increased foraging for resources in nutrient-poor environments (Fig. 3.12b; 3.13b). However, this relationship was not found for the remaining harvests (Table 3.2) and edaphic characteristics were not related to any other physiological variables at any other time frame ($P >> 0.05$). This indicates that soil characteristics may be important during the early stages of below ground biomass and become less important as the plant matures or are not expressed later in life due to restriction by the pot.

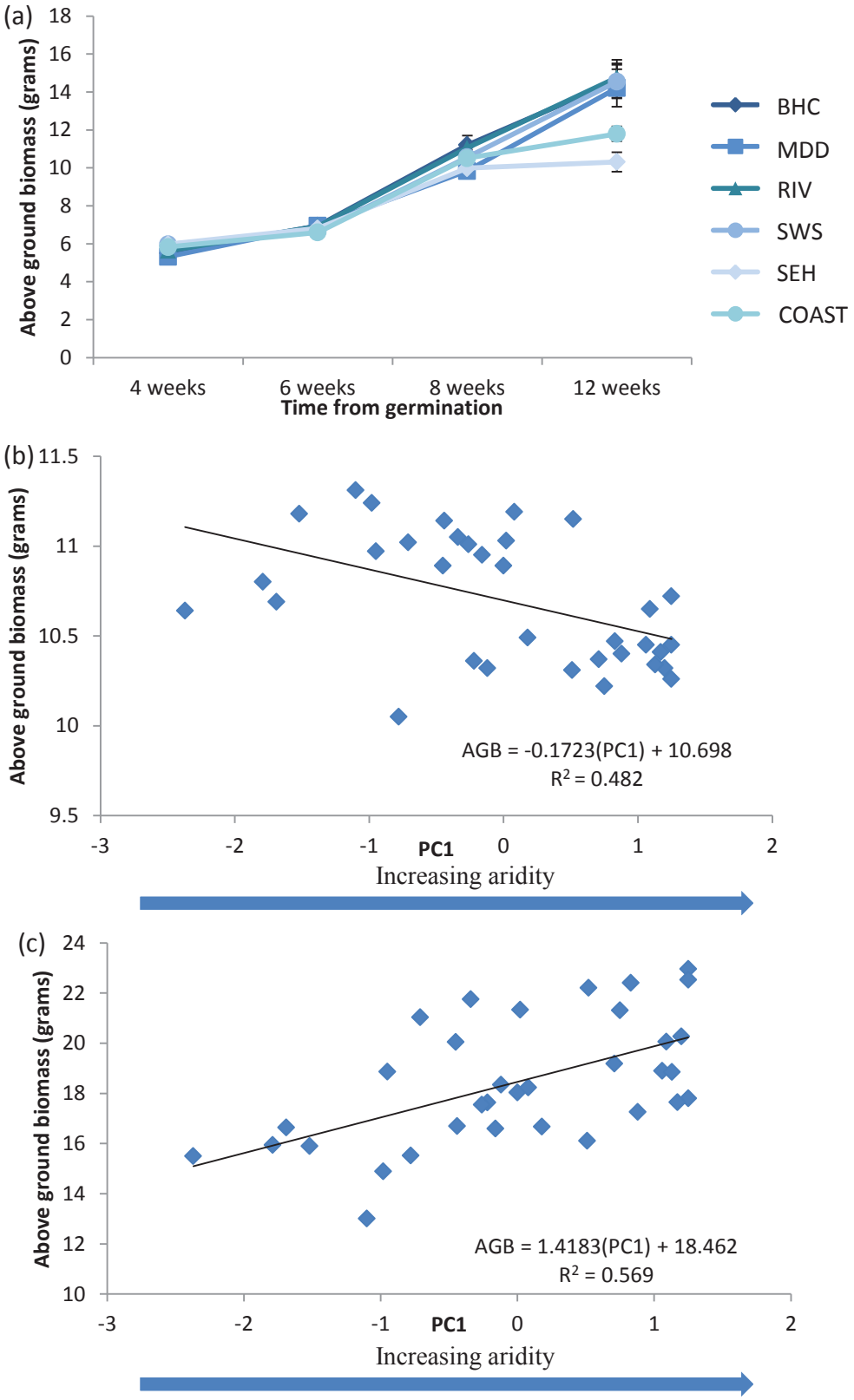

Figure 3.11 Above ground biomass (AGB) was measured over four time intervals four, six, eight and 12 weeks from germination during a common garden glasshouse study; (a) AGB actual means ± SE; (b) AGB first harvest site level scores on PC1 and (c) AGB third harvest site level scores on PC1. Abbreviations denote the individual bioregions; Fig. 2.1 for full names.

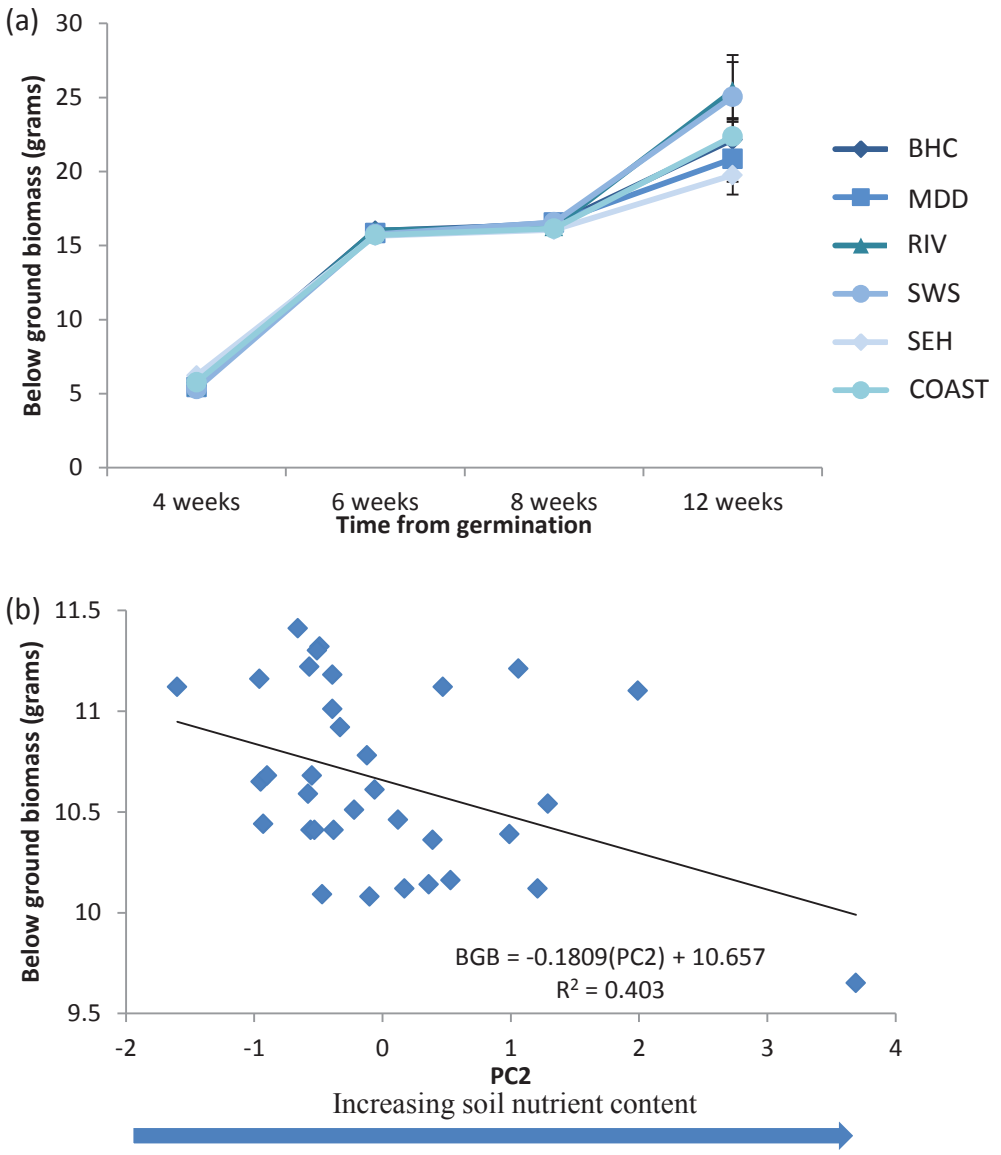

Figure 3.12 Below ground biomass (BGB) was measured over four time intervals four, six, eight and 12 weeks from germination during a common garden glasshouse study; (a) BGB actual means ± SE; (b) BGB first harvest site level scores on edaphic factors (PC2) Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

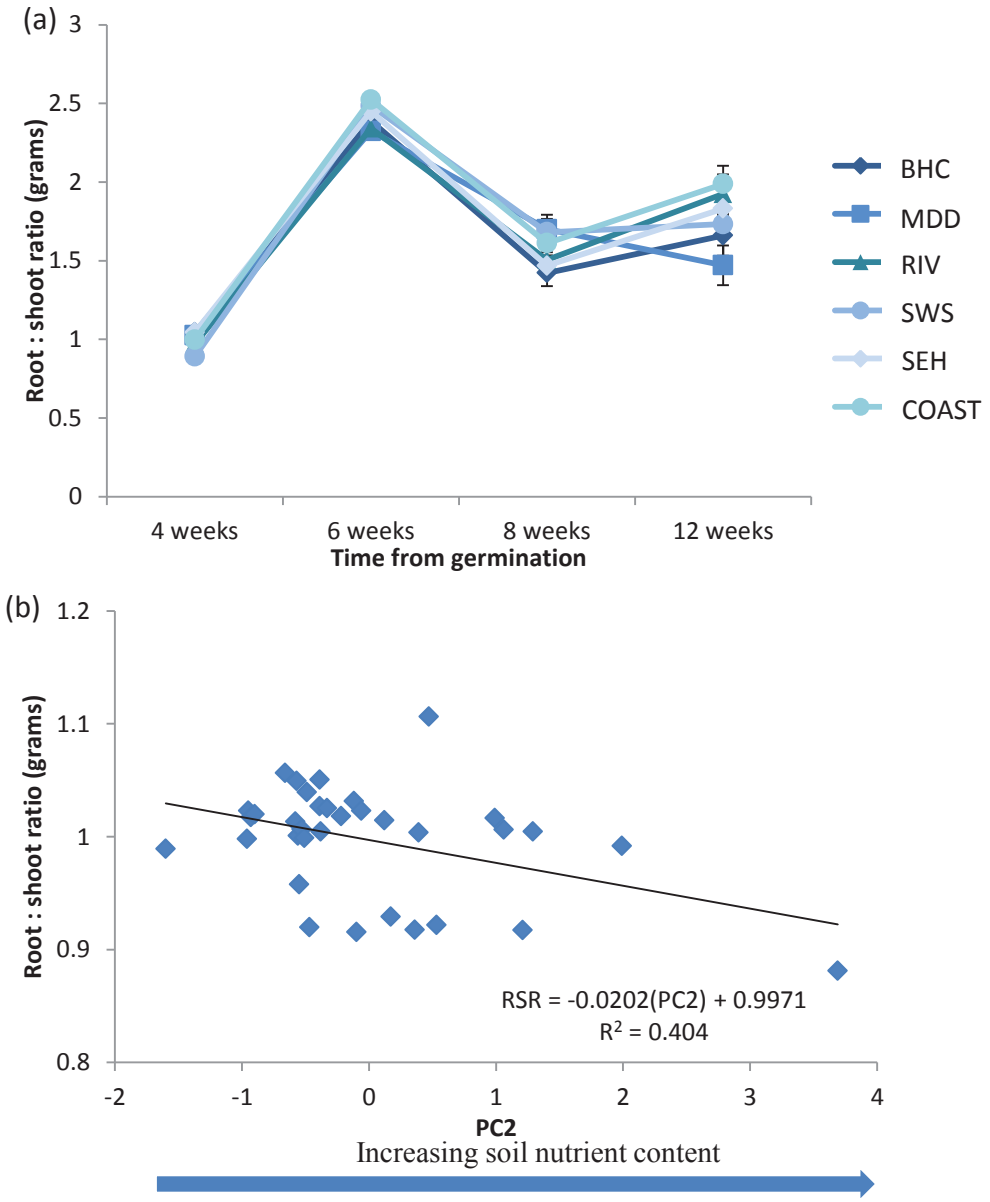

Figure 3.13 Root : shoot ratio (RSR) was measured over four time intervals four, six, eight and 12 weeks from germination during a common garden glasshouse study; (a) RSR actual means ± SE; (b) RSR first harvest site level scores on edaphic factors (PC2). Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

3.3.3 Water use traits

Although daily water use and daily increase in biomass varied among plants (240.7 ml to 304.3 ml and 7.1 g to 30.0 g, respectively) they did not statistically differ among bioregions (P > 0.05). In addition, WUE (daily water use per increase in biomass) also varied from 9.2 g/ml to 31.5 g/ml but was also not statistically different among bioregions ($F_{5,28}$ = 0.43, *P* = 0.82; Table 3.3) or sites (Wald Z = 1.35, *P* = 0.18). Transpiration rate measured with a porometer was also not found to differ among bioregions or sites for the adaxial or abaxial sides of the leaves (*P* > 0.05; Table 3.3). Carbon isotope ratios (Δ) differed by 9% however this was also not significantly different among bioregions ($F_{5,28}$ = 0.80, *P* = 0.56; Table 3.3). Simple linear regression was conducted for all water use variables against climatic variables (PC1) and edaphic (PC2) characteristics, however, none of the dependant variables were explained by the components (*P* >> 0.05; Table 3.3).

Table 3.3 Results of linear mixed model analysis of water use traits with bioregion as a fixed explanatory factor and site within bioregion as a random factor.

Trait	Linear mixed model		Regression			
	Bioregion		PC1		PC2	
	F	*P*	R^2	*P*	R^2	*P*
Physical water use efficiency*	$F_{5,28}$ = 0.432	0.822	0.157	0.375	0.040	0.823
Carbon Isotope ratio Δ	$F_{5,28}$ = 0.799	0.560	0.258	0.140	0.011	0.952
Porometer – Adaxial~	$F_{5,27}$ = 1.052	0.408	0.080	0.659	0.061	0.736
Porometer – Abaxial^	$F_{5,28}$ = 0.352	0.877	0.020	0.908	0.058	0.746

* Physical water use efficiency refers to the daily water use divided by the biomass.
~ Adaxial is the upper surface of the leaf.
^ Abaxial is the lower surface of the leaf.

3.4 Discussion

All bioregions appear to have significant differences at various life stages suggesting that selection pressures for growth traits do exist. Growth traits such as leaf number and SLA followed predictions with plants from arid environments producing many small leaves and reducing investment in leaf structure per unit resource input which is consistent with arid ecology theory (Table 3.4). Conversely, growth traits such as RGR and LAR did not follow predictions resulting in faster growing plants from arid regions. Stature traits such as AGB, BGB and RSR initially followed predictions with smaller AGB and larger below ground traits corresponding with increased foraging for nutrients in arid environments. However, stature traits such as height, petiole length and P_{area} did not follow predictions with plants from arid environments being taller than plants from mesic environments and consistent petiole length and P_{area} across the range (Table 3.4). In addition, final AGB was larger in arid environments indicating that the growth strategy of this species changes throughout its life.

Table 3.4 Predicted and observed trends in plants collected from arid environments compared to mesic environments. The following symbols indicate: + increasing and – decreasing trait values with respect to aridity ([a]) or soil nutrient content ([n]). Abbreviations denote trait names see tables 3.1, 3.2 and 3.3 for full names.

Trait group	Trait	Predicted	Observed
Growth	RGR	–	+ [a]
	Leaf No.	+	+ [a]
	Largest leaf length	–	**0**
	Largest leaf width	–	– (final) [n]
	SLA	–	– [n]
	LAR	–	+ [a]
	Leaf accumulation	+	**0**
	Leaf chlorophyll	+	**0**
	Leaf carbon	+	**0**
	Leaf nitrogen	+	**0**
Stature	Height	–	+ (final) [a]
	Petiole length	–	**0**
	P_{area}	–	**0**
	AGB	–	– (initial), + (final) [a]
	BGB	+	+ (initial) [n]
	RSR	+	+ (initial) [n]
Water use	WUE	+	**0**
	Carbon Isotope ratio	–	**0**
	Porometer *	–	**0**

* Measure of stomatal conductance.
\# initial = harvest 1 and 2 (4-6 weeks), final = harvest 3 and 4 (8-12 weeks).

Water use traits did not behave as predicted and did not vary across the range, suggesting that this species may use a drought avoidance strategy in which plants rapidly reproduce to avoid drought conditions, rather than a drought tolerance strategy, where plants reduce water use and withstand drought until conditions improve. In contrast, water use efficiency traits may form a trade-off with rapid growth traits associated with growth and resource allocation, as observed by Angert *et al.* (2007) where high water use efficiency was observed in populations with low growth rates in a guild of desert annuals. However some traits such as leaf N, C and chlorophyll did not differ among bioregions suggesting either low selection pressure or static nature. While some of these growth patterns are contradictory to the literature on arid environments (e.g. high RGR compared to the predicted low RGR), these adaptations are likely to contribute to the invasiveness of this species (Piggin & Sheppard 1995; Siemann & Rogers 2001; Grotkopp & Rejmánek 2007).

Given that trait responses varied among environments and over time, I discuss reasons below for the observed rapid growth, large stature and commonality in water use strategy in plants from arid environments compared to mesic environments. Previous work has shown that rapid adaptation (< 200 years) in life history traits has been observed in many plants species across a range of growth (e.g. Siemann & Rogers 2001; Buschmann *et al.* 2005) and reproductive traits (e.g. Weber & Schmid 1998; Kollmann & Bañuelos 2004). As a result, it is not surprising that growth traits such as RGR were found to differ among bioregions and positively correlate with climate. Similar population variation in RGRs correlating with environmental gradients has been demonstrated in native (e.g. Clevering *et al*. 2001) and invasive species (e.g. DeWalt *et al*. 2004) indicating that adaptation of this trait is likely to be

important for survival in locations peripheral to the typical climatic envelope (Siemann & Rogers 2001; Buschmann *et al.* 2005).

Leaves are highly plastic, especially during early development and reflect resource availability and environmental conditions (Castro-Díez *et al*. 1997). Consequently, the observed difference in leaf quantity and shape, in response to climate is not surprising as smaller leaves are advantageous in hot, dry, high light and low nutrient environments, such as the BHC and MDD bioregions, as they have a smaller surface area and a higher heat exchange capacity (Fonseca *et al*. 2000; Ackerly *et al*. 2002; Bragg & Westoby 2002; McDonald *et al*. 2003; Kleiman & Aarssen 2007). Similarly, Piggin *et al*. (1973) found a similar trend of leaf number positively correlating with temperature in glasshouse populations of *E. plantagineum*. Smaller leaves have also been linked to reduced leaf expansion resulting in lower herbivory (Moles & Westoby 2000) and efficient vertical distribution in a multilayered crown (Horn 1971). In addition, the leaf trait LAR varies among bioregions and is correlated with climate indicating that leaf resource capture and use varies in response to local climatic factors. High LAR in the fertile coastal bioregion is consistent with large leaves that are being produced and may correlate with increased shade in eastern bioregions allowing larger leaves (Peperkorn *et al*. 2005).

Resource allocation was also found to vary across time, with plants from arid environments initially allocating resources to below ground growth resulting in reduced above ground growth. In contrast, during the final harvest BGB was found to be consistent among bioregions while AGB was found to be higher ensuring reproductive success before conditions become unsuitable. This trade-off between AGB and BGB indicates that resource allocation can be beneficially altered to assist with pressures at different life stages. In arid environments, access to water and

nutrients is essential for survival and rapid development of an extensive root system is vital to secure these resources for future growth (Grotkopp & Rejmánek 2007). Below ground traits such as BGB and RSR exhibited a significant decrease in response in increasing nutrient availability. This trend has also been noted in two other Mediterranean species, *Halimium halimifolium* and *Pinus pinea*, which also displayed an increase in RSR with increasing light availability (Peperkorn *et al*. 2005). This may also be the case with *E. plantagineum* as arid environments had lower plant densities resulting in less shade and less competition (above and below ground) than in mesic environments (T. Konarzewski, personal observation). Thus, rapid establishment through rapid BGB accumulation may assist this species to not only compete with other species but also within species, as *E. plantagineum* germinates in multiple dense cohorts that can produce up to 883 established seedlings per meter (Burdon *et al*. 1983).

3.4.1 Observation of new defence mechanism

Although few changes in growth were detected, a new defence mechanism was observed for this species (L. Weston, *pers. com.*). Discolouration and raised bumps (trichomes; Serrato-Valenti *et al*. 1997; Fig. 3.14a) in a uniform pattern were found on the leaves of plant populations from Mildura and Oberon, ten weeks from germination. These patterns have been observed in the closely related species *Echium vulgare* (Klemow *et al.* 2002); however, (to my knowledge) they have never been recorded on *E. plantagineum*. In addition, this phytotoxin (napthoquinone), a toxic compound produced by a plant, is usually found in the roots where it leaches into the soil and prevents other plants from growing nearby (Weston *et al.* 2011). These spots were found in two distinct patterns (1) spread uniformly on the surface of the leaf (Fig. 3.14b) and (2) on the edge of the leaf (Fig. 3.14c). The location of the trichomes

(Fig. 3.14a) on the leaf may be an indication of the type of leaf eating insect that is attacking each plant. Further research on why these populations display these trichomes on the leaf surface and whether other populations contain these compounds is outside the scope of this project but could be the subject of future research. Appearance of this new protection strategy may explain why I did not see all of the predicted adaptation in growth traits, as defence investment is commonly associated with trade-offs in growth investment (EICA Hypothesis, Blossey & Nötzold 1995; e.g. Messina *et al.* 2002; DeWalt *et al*. 2004; Fine *et al.* 2006), although this is just a hypothesis.

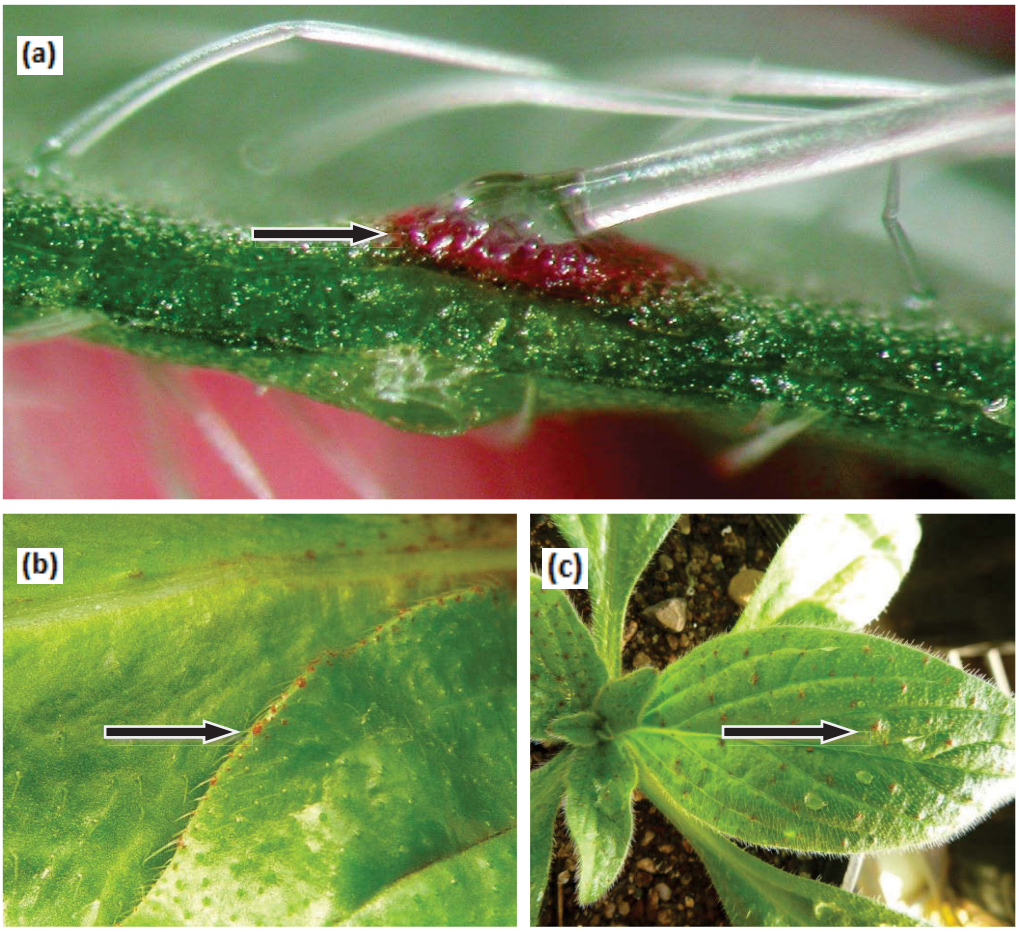

Figure 3.14 *Echium plantagineum* plants with red trichomes, raised bumps on the leaf surface, predicted to be used in defence against insects; (a) leaf cross section with trichome; (b) trichomes on leaf edge and (c) trichomes on leaf surface.

All *E. plantagineum* plants in this study were found to have growth strategies that corresponded to local site conditions for example, traits such as RGR, leaf number, AGB, BGB and RSR displayed evidence of potential selection and adaptation. However, there are many factors that may influence trait expression including species characteristics and resource availability. For example, *E. plantagineum* is not restricted by size during its reproductive phase and can produce flowers at any age and size (Piggin & Sheppard 1995; Buckley *et al*. 2005). Resource limitation can prevent populations from evolving or developing favourable adaptations, as plants are only able to respond until they have reached the limit of available resources (Bone & Farres 2001). Many traits did not differ among bioregions and this may suggest low selection pressure, inability to changes these traits or trade-offs among plant traits. In addition, it is not surprising that some plant variables measured during the first harvest did not significantly differ among bioregions as this experiment was initiated using seeds of the same size however differences become more apparent over time.

The growth strategy of *E. plantagineum* varied among environments and across time with plants in arid environments initially using a combination of growth and stature traits to rapidly grow and establish such as increased RGR, leaf number, $P_{area,}$ initial BGB and initial RSR. In addition, as these plants start to mature, their allocation strategy appears to change to taller plants with increased AGB and LAR. These alterations in life history traits suggest potential adaptation to the unpredictable local conditions of arid environments since their introduction in to Australia in 1850. These adaptations in growth strategy and resource allocation allow this species to accumulate resources, mature, reproduce and expand their range efficiently under comparatively harsh growing conditions. In addition water use traits were not found

to vary among environments, thus, this species is likely to use a drought avoidance strategy rather than a drought tolerance strategy and as a result, in the next chapter I will expect to see early maturation and early flowering in plants from arid bioregions and later flowering in the cooler temperature mesic bioregions. The next chapter of the thesis will look at the reproductive processes of this invasive species and how they contribute to the survival and spread of *E. plantagineum* in arid environments.

Chapter 4: Variation in reproductive traits of *Echium plantagineum* populations along an arid-mesic gradient

4.1 Introduction

Invasive species have considerable potential for rapid adaptation and evolution, enhancing their ability to establish, proliferate and reproduce in novel environments (Barrett *et al.* 2008; Prentis *et al.* 2008). Adaptive evolution in invasive species is driven by genetic diversity, immigration history, range expansion, available resources and local climatic conditions (Barrett *et al.* 2008). Range expansion and heterogeneous environmental factors drive rapid adaptive evolution in invasive species and can increase selection for local adaptation (Maron *et al.* 2004; Barrett *et al.* 2008; Colautti *et al.* 2010). For example, Leger & Rice (2007) observed rapid evolution through local adaptation of life-history and reproductive traits in the invasive poppy *Eschscholzia californica* over the last 110-150 years since its introduction to Chile in the 1890s. Moreover, Maron *et al*. (2004), Etterson *et al*. (2008) and Montague *et al*. (2008) found similar adaptation in life-history and reproductive traits along environmental gradients in less than 200 years among populations of *Hypericum perforatum, Solidago altissima* and *Lythrum salicaria*, respectively. These studies and others (e.g. Weber & Schmid 1998; Bone & Farres 2001; Reznick & Ghalambor 2001) have demonstrated that rapid changes in traits in response to environmental variation can lead to higher fitness levels and greater reproductive success in novel ranges.

Reproductive processes are critical drivers of adaptive evolution as they are responsible for population characteristics such as genetic diversity distribution, genetic recombination, gene flow and population size (Barrett *et al*. 2008). Invasion success is strongly linked to the reproductive traits responsible for the production, dispersal and genetic composition of propagules (Colautti *et al.* 2006; Barrett *et al.* 2008). Hence, understanding the role of reproductive processes during a biological invasions is critical for predicting microevolutionary changes in invading populations and potential changes in ecosystem composition. Climatic adaptation often produces clinal variation in life-history traits associated with variation in temperature, rainfall and seasonality (Barrett *et al.* 2008). Adaptation in reproductive traits has been observed along latitudinal and altitudinal clines in both native (e.g. Del pozo *et al.* 2002; Olsson & Ågren 2002; Santamaria *et al*. 2003; Becker *et al*. 2006) and invasive species (e.g. Weber & Schmid 1998; Kollmann & Bañuelos 2004; Maron *et al.* 2004). For example Montague *et al*. (2008) observed adaptation of flowering time in the invasive species *Lythrum salicaria* to a latitudinal cline with populations from high latitudes flowering earlier then populations from lower latitudes, under both glasshouse and field conditions in North America. Collectively, these studies provide evidence for species-specific responses that are driven by the availability of abiotic resources and edaphic factors.

Arid environments are characterised by sparse, variable rainfall and low nutrient availability (Chesson *et al.* 2004; Venable 2007) that can considerably reduce reproductive success in invading species. Adaptation and trait plasticity in reproductive processes, such as flowering time, are common attributes of annual species in arid or semi-arid environments (Iannucci *et al.* 2008) and have the capacity to significantly increase flower production in invasive species. Many studies have

observed early flowering, shortening of flowering periods and reduced or cessation of flowering due to drought conditions in native and invasive species (e.g. Bernier & Périlleux 2005), however, very few studies have compared arid or xeric environments with mesic environments. Many studies have looked at trait variation in reproductive traits of invasive species in response to environmental clines such as rainfall and nutrient availability (e.g. Weber & Schmid 1998; Kollmann & Bañuelos 2004; Maron *et al.* 2004; Leger & Rice 2007; Montague *et al.* 2008; Ridley & Ellstrand 2010; Colautti *et al.* 2010) and as a result have concluded that invasive species are capable of rapid adaptation in life-history traits. However, very few studies have looked at trait variation in reproductive traits in native or invasive species in arid regions. Given that trait variation is critical to the survival of plant species in novel environments, especially under extreme environmental conditions, such as those found in arid environments, this area requires further research.

Flowering in annuals, such as *Echium plantagineum*, occurs independently of size or age and is generally controlled by genetic and environmental cues such as genotype, photoperiod, temperature, vernalization, irradiance and water availability (Bernier & Périlleux 2005; Elzinga *et al.* 2007; Iannucci *et al.* 2008). Early maturation comes at the cost of plant size as resources are reallocated away from growth traits towards reproductive traits (Montague *et al.* 2008; Colautti *et al.* 2010). Gradual selection for earlier flowering time should result in smaller plant size and lower rates of fecundity, limiting population growth and slower rates of invasion (Colautti *et al.* 2010). In addition, earlier flowering time induced by environmental factors requires earlier activity of other life-history traits such as leaf expansion, root growth and nutrient uptake (Fitter & Fitter 2002) which are important for niche differentiation, resource distribution and adaptation to novel environments. Abiotic selection

pressures can accelerate flowering and shorten flowering periods as part of a drought avoidance strategy in arid or unpredictable climates (Franks *et al.* 2007; Kolář *&* Seňková 2008). This drought avoidance strategy allows plants to shorten their lifespan to ensure viable seed production (Franks *et al.* 2007; Kolář *&* Seňková 2008). Hence, the timing of flower initiation is vital for reproduction, as flower and seed production need to be completed under the most ideal conditions to maximise reproductive success (Amasino 1996; Simpson *&* Dean 2002). As a result reproductive traits are crucial to the survival of invasive species in novel environments.

4.1.1 Chapter aim and approach

In this chapter, I compare reproductive trait variation among populations of the invasive forb *Echium plantagineum*. *Echium plantagineum* seeds were collected from field populations along an arid-mesic gradient, germinated in the laboratory and transferred to the glasshouse as seedlings until reaching the reproductive phase where they were measured for reproductive traits. The aim of this study was to quantify variation in reproductive traits among populations and gather evidence for climatic adaptation or population differentiation along the arid-mesic gradient. First, I tested the hypothesis that populations from arid environments produce stems and flowers faster than populations from mesic environments. Second, I tested the hypothesis that plants from arid regions produce fewer stems and flowers than plants from mesic environments. Third, I tested the hypothesis that recurrent selection for earlier flowering, in response to low water availability, has resulted in a reduction in flowering time variation in populations from arid environments compared with mesic populations. These responses would indicate that populations are capable of differential adaptation in reproductive traits in response to reduced water availability and low nutrient availability in arid environments. Reproductive traits determine the

quality and quantities of progeny produced and are therefore vital to the survival of invasive species in novel environments.

4.2 Methods

Seeds were collected from 34 populations along an east to west arid-mesic rainfall gradient across southern New South Wales from six IBRA bioregions based on environmental and climatic factors (See 2.2.1). Each bioregion contained six study sites with the exception of the coastal bioregion (COAST) which had four sites due to limited availability of appropriate sites. Maternal effects on the experiment were avoided through the use of seeds of a similar size and weight, and experiments were conducted in a non-competitive glasshouse environment. Seed germination was completed under the same conditions as presented in Chapter 2 (see 2.2.3) with ten seeds from each of ten plants from each of the 34 populations (n = 3400 total) germinated in petri dishes on moist filter paper in dark conditions. After five days, two germinants from each of 340 plants (680 germinants in total), which were similar in size and germinated on the same day, were transported to the glasshouse wrapped in foil and transplanted into biodegradable pots (Jiffypot®). The experiment was run from April to December 2010 under a controlled day/night temperature regime (25/15 + 5ºC) with a photoperiod governed by natural sunlight as detailed in Chapter 3 (See 3.2.1). After two weeks seedlings were transplanted to 10cm pots with standard compost soil and then to 20cm pots after six weeks. Plants were grown to reproductive maturity using the same method described in Chapter 3 (see 3.2.1).

Plants were grown in the glasshouse in a randomised block design with five blocks consisting of four benches to a block. Light intensity, a potential covariate in

the study, was measured using a LI-COR photometer (Model LI-185B). I divided each block into 12 groups and averaged five measurements for each group collected at 8am, 10am, 12pm, 2pm and 4pm on a single day during the middle of the experiment (23/08/2010). Plant position was recorded as edge of block or core of block to ensure that location did not influence the results of the study.

Stem initiation started ten weeks after the beginning of the trial, after which time all plants were inspected daily for stem initiation. Here, the time to stem initiation (SI_{time}) is defined as the first observation of the first leaf of the first stem expressed as the number of days from germination, and represents the transition from the rosette to non-reproductive adult. Floral initiation started approximately ten weeks later and was determined as the time taken for the first flower (FI_{time}) of each plant to fully open. I also calculated the time from first stem to first flower (SF_{time}) as an indication of the time required to produce the first flower after the plant had reached its adult phase. The photoperiod, defined as day length in hours, was recorded on the date of first flower for each plant. Total stem and total flower production were measured at the conclusion of the trial 40 weeks after germination.

Environmental and climate data for each collection site was collected from the SILO enhanced meteorological dataset and data drill procedure (Jeffrey *et al* 2001; see Chapter 2, section 2.2.5). Soil characteristics were collected using the methods of Rayment & Higginson (1992; electrical conductivity and pH) and Dumas (1981; nitrogen content, carbon content; see Chapter 2, section 2.2.4). These data were used in a principal component analysis to determine the amount of variation in reproductive traits that can be accounted for by environmental and site characteristics. Of the 340 plants used in the experiment, 303 produced flowers within the experimental time frame. Plants that did not flower in this time were removed from

analysis. One plant flowered much earlier than all other plants (32 days earlier than the next plant to flower) and was removed from the analysis as an anomaly.

4.2.1 Statistical analysis

Linear mixed model analyses (LMM) were used to assess relationships between six dependent variables including (1) time to stem initiation (SI_{time}), (2) time to flower initiation (FI_{time}), (3) time from first stem to first flower (SF_{time}), (4) photoperiod at first flower, (5) total number of flowering stems at the end of the experiment and (6) estimated total flowers produced at the end of the experiment and the predictor variables including (1) bioregion (fixed), (2) block (fixed), (3) site within bioregion (random), (4) light intensity (fixed) and (5) plant position (edge or core; fixed). The fixed variables were tested using a standard F-test and the random variable was tested using a Wald Z test (Dytham 2011). LMMs were used to test the impact of predictor variables on time to stem initiation variance (SIV) and time to flower initiation variance (FIV) as representatives of the variation in reproductive traits at the site level within bioregion. SIV and FIV were calculated as the variance among plants within the same site. The same analyses were carried out using the coefficient of variation as a representative of variance but they generated the same result and are not reported. SI_{time} was square root transformed and all other variables were left untransformed to be consistent with the assumptions of LMMs.

The above analyses showed that all reproductive traits except total flower production and total stem production varied considerably at the site level (see results below). I then determined whether linear relationships existed between site characteristics and the dependent variables (i.e. the reproductive traits) using linear regression. Six site characteristics and four edaphic factors were reduced using

principal component analysis (PCA) to two principal components that explained 82% of the total variance (Chapter 2, see section 2.2.6). The first component (PC1) was strongly associated with climatic variables while the second component (PC2) primarily reflected site-level soil characteristics. These components were then used as independent variables in linear regressions with the reproductive traits as dependent variables (Sokal *&* Rohlf 1995; Dytham 2011). As FI_{time} significantly varied among bioregions, latitude and longitude were used as independent variables in individual linear regressions, as these variables have been used in earlier work. Data transformations were not required for these variance variables as all data were consistent with model assumptions. Statistics were performed using SPSS (SPSS Inc, version 19, IBM).

4.3 Results

SI_{time} varied among plants from 59 to 164 days (from germination), however it did not vary significantly among bioregions or sites ($P > 0.05$; Fig. 4.1a, b; Table 4.1). Linear regression analysis revealed that SI_{time} was not correlated with climatic variables (PC1; $P > 0.05$), however, it was negatively related to edaphic characteristics (PC2; $P < 0.05$; Table 4.2; Fig. 4.1c), indicating that bioregions with lower nutrient levels, for example southern highlands (SEH) and Broken Hill Complex bioregions (BHC), produced stems later than bioregions with higher nutrient availability, such as the coastal bioregion (COAST; Fig. 4.2).

Table 4.1 Results of linear mixed model analysis with reproductive traits as response variables in separate models. Bioregion and block were fixed explanatory variables and site nested within bioregion was a random explanatory variable. Light intensity and plant position were included in the model as covariates. SI_{time} represents time to first stem; FI_{time} represents time to first flower; SF_{time} represents time from first stem to first flower; SIV represents stem initiation variance and FIV represents flower initiation variance. Significant *P*-values are in bold with a value of $P < 0.05$.

Reproductive characteristic	Bioregion		Site (Bioregion)		Block		Light intensity		Position	
	***F*-value**	***P*-value**	**Wald Z**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
SI_{time} (days)	$F_{5,26} = 1.94$	0.12	0.92	0.36	$F_{4,266} = 1.12$	0.35	$F_{1,286} = 5.93$	**0.02**	$F_{1,289} = 0.62$	0.43
FI_{time} (days)	$F_{5,29} = 5.09$	**< 0.01**	0.37	0.72	$F_{4,270} = 5.31$	**< 0.01**	$F_{1,288} = 0.33$	0.57	$F_{1,290} = 8.01$	**< 0.01**
SF_{time} (days)	$F_{5,27} = 4.06$	**< 0.01**	0.47	0.64	$F_{4,269} = 1.84$	0.12	$F_{1,290} = 3.68$	0.06	$F_{1,288} = 6.52$	**0.01**
Photoperiod (h)	$F_{5,29} = 4.64$	**< 0.01**	0.14	0.89	$F_{4,271} = 4.00$	**< 0.01**	$F_{1,289} = 0.84$	0.36	$F_{1,290} = 5.10$	**0.03**
Total stems	$F_{5,28} = 1.13$	0.37	0.63	0.53	$F_{4,268} = 10.70$	**< 0.01**	$F_{1,287} = 2.74$	0.10	$F_{1,290} = 1.10$	0.30
Total flowers	$F_{5,28} = 1.35$	0.27	-	-	$F_{4,290} = 12.24$	**< 0.01**	$F_{1,290} = 7.28$	**< 0.01**	$F_{1,290} = 0.14$	0.71
SIV	$F_{5,28} = 0.41$	0.84	-	-	-	-	-	-	-	-
FIV	$F_{5,28} = 1.37$	0.27	-	-	-	-	-	-	-	-

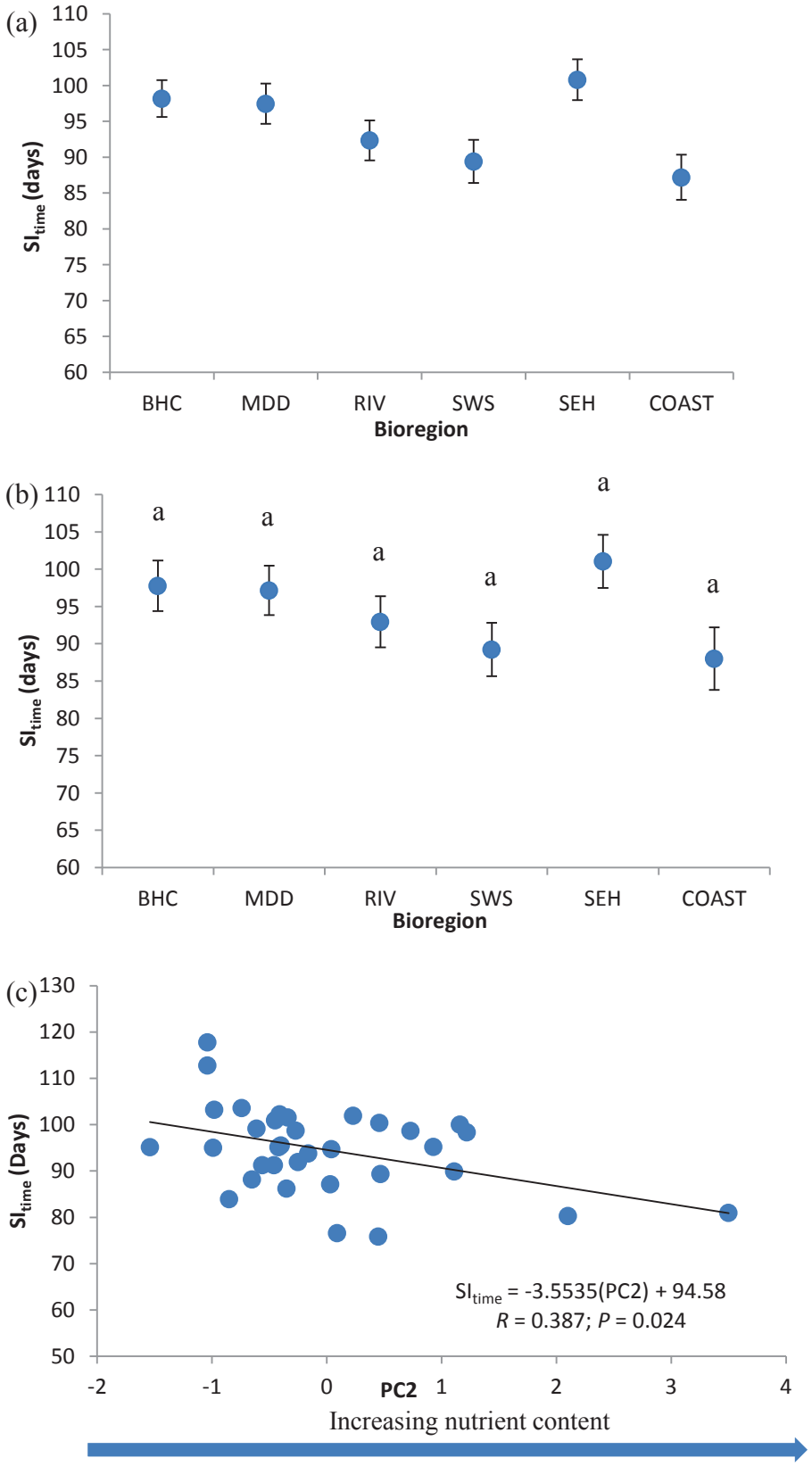

Figure 4.1 Time to first stem initiation (SI_{time}) across the six bioregions; (a) actual means ± SE; (b) adjusted Means ± SE and least square mean post-hoc test. Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names. Bioregions are arranged from most westerly (BHC) to most easterly (COAST).

Table 4.2 Results of simple linear regression analysis with reproductive traits as response variables in separate models. The explanatory variables are components from a principal component analysis with site level environmental variables; PC1 primarily represents climate factors; PC2 primarily represents edaphic factors. Significant *P*-values are in bold with a value of $P < 0.05$. SI_{time} represents time to first stem; FI_{time} represents time to first flower; SF_{time} represents time from first stem to first flower; SIV represents stem initiation variance and FIV represents flower initiation variance.

Trait	PC1		PC2	
	R	***P***	***R***	***P***
SI_{time} (days)	0.047	0.793	0.387	**0.024**
FI_{time} (days)	0.548	**0.001**	0.150	0.398
SF_{time} (days)	0.468	**0.006**	0.450	**0.009**
Photoperiod (h)	0.563	**0.001**	0.147	0.406
Total stems	0.111	0.532	0.387	**0.024**
Total flowers	0.277	0.113	0.258	0.141
SIV	0.027	0.881	0.072	0.686
FIV	0.008	0.963	0.392	**0.022**

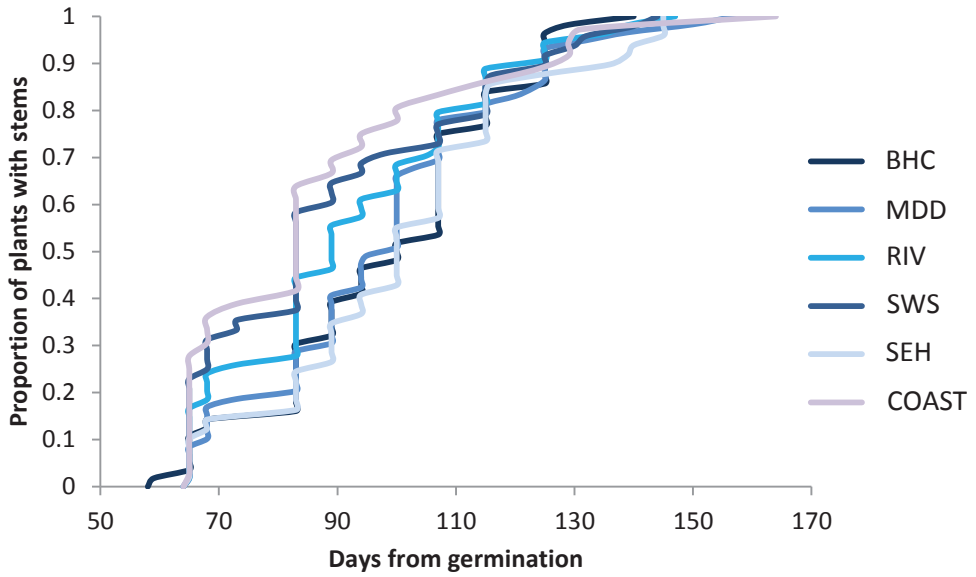

Figure 4.2 Time to first stem initiation (SI_{time}) across the six bioregions; cumulative curves of proportion of plants with stems over time (days from germination). Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

FI_{time} varied among plants from 132 to 220 days (from germination) and differed significantly among bioregions ($P < 0.05$) but not sites ($P > 0.05$; Fig. 4.3a, b; Table 4.1). FI_{time} was highly negatively correlated with PC1 ($P < 0.05$; Fig. 4.4a) but not PC2 ($P > 0.05$; Table 4.2) indicating that plants from arid bioregions, such as BHC and Murray Darling Depression (MDD), flowered earlier than plants from mesic bioregions, such as SEH and COAST (Fig. 4.5). Latitude and longitude were also used in individual linear regressions to determine their individual contribution to variation in FI_{time}. Latitude and longitude were both significant predictors of FI_{time} but the relationship with longitude was much stronger (latitude: $r = 0.36$, $P = 0.04$, longitude: $r = 0.58$, $P < 0.01$; Fig. 4.4b).

SF_{time} varied significantly among bioregions ($P < 0.05$; Fig. 4.6a, b; Table 4.1) and was related significantly to both principal components, with lower SF_{time} in arid environments (PC1; $P < 0.05$; Fig. 4.7a) and nutrient poor environments (PC2; $P < 0.05$; Fig. 4.7b; Table 4.2). Photoperiod at first flower varied significantly across plants from different bioregions ($P < 0.05$; Fig. 4.8a, b) with plants in arid regions flowering at a shorter photoperiod than plants from mesic environments. Linear regression analyses showed that variation in photoperiod at first flower was strongly related to climatic variables (PC1; $P < 0.05$; Table 4.1; Fig. 4.8c) but not to soil characteristics (PC2; $P > 0.05$; Table 4.2). Total stem production and total flower production were not found to vary significantly among bioregions ($P > 0.05$; Fig. 4.9a, b & 4.10a, b), and total flower production did not vary significantly with either PC1 or PC2 ($P > 0.05$; Table 4.2). However total stem production was positively and significantly correlated with soil characteristics (PC2; $P < 0.05$) but not with climatic variables ($P > 0.05$; Table 4.2).

LMM analysis of SIV revealed neither significant differences among bioregions ($P > 0.05$; Table 4.1) nor relationships between either principal component in linear regression analysis ($P > 0.05$; Table 4.2). FIV was also not explained by climatic variables ($P > 0.05$) but was positively correlated with local soil variables ($P < 0.05$; Table 4.2). These results indicate no shift with climate towards reduced variation in flower initiation in arid or mesic populations.

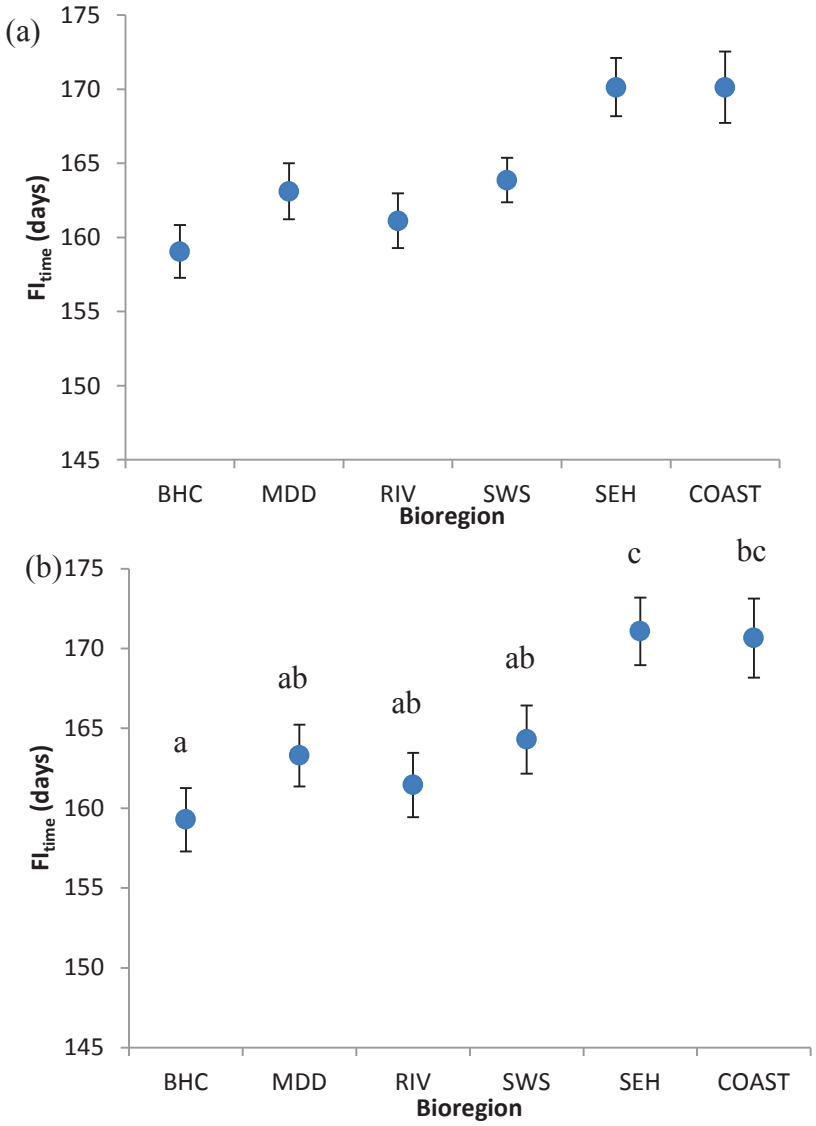

Figure 4.3 Time to first flower (FI_{time}) during a common garden glasshouse trial with *Echium plantagineum* averaged across the six bioregions; (a) actual means ± SE; (b) adjusted means ± SE. Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

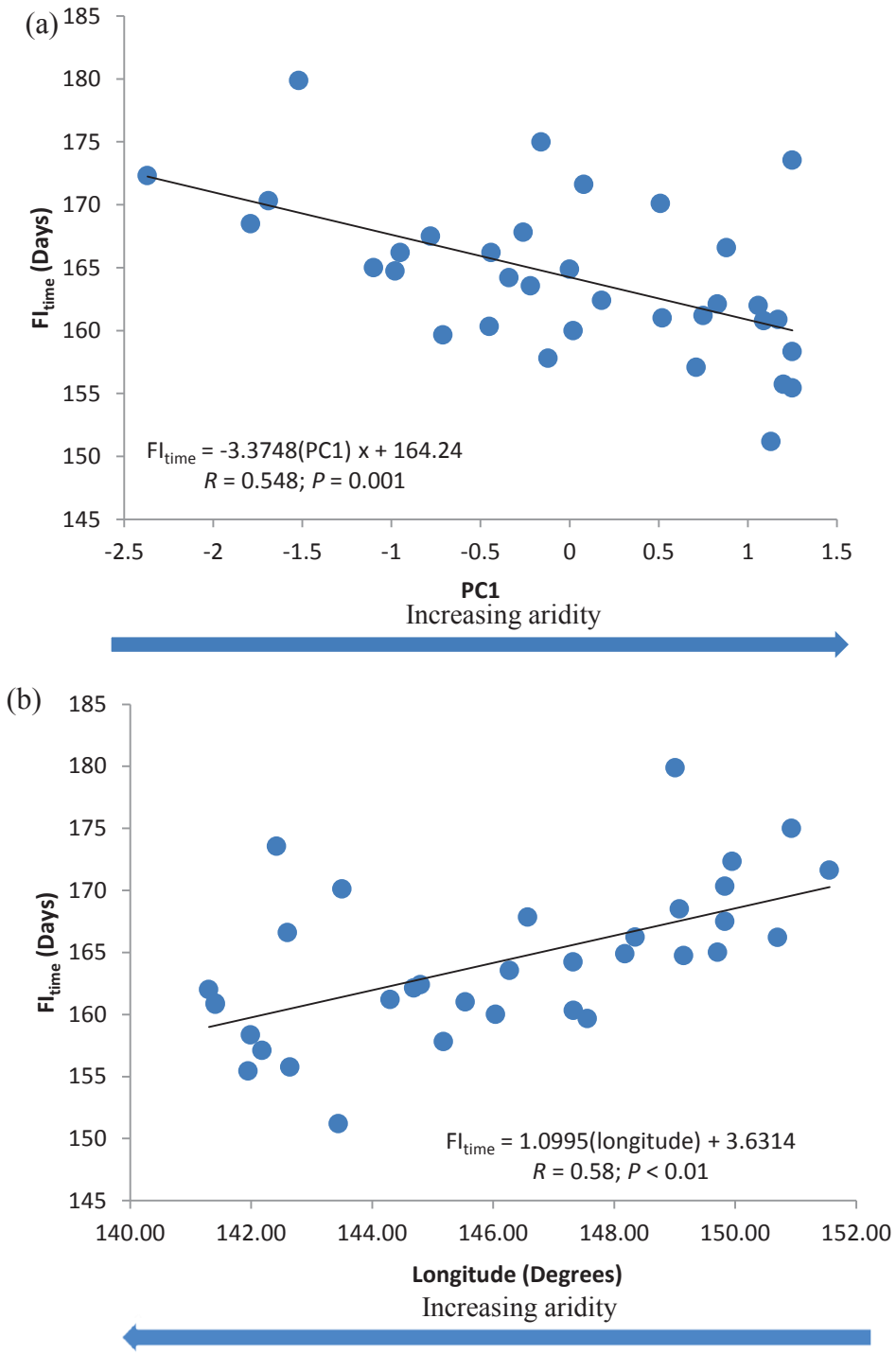

Figure 4.4 Relationships between flower initiation (FI_{time}) and two key predictor variables; (a) site-level scores on PC1 and (b) site longitude.

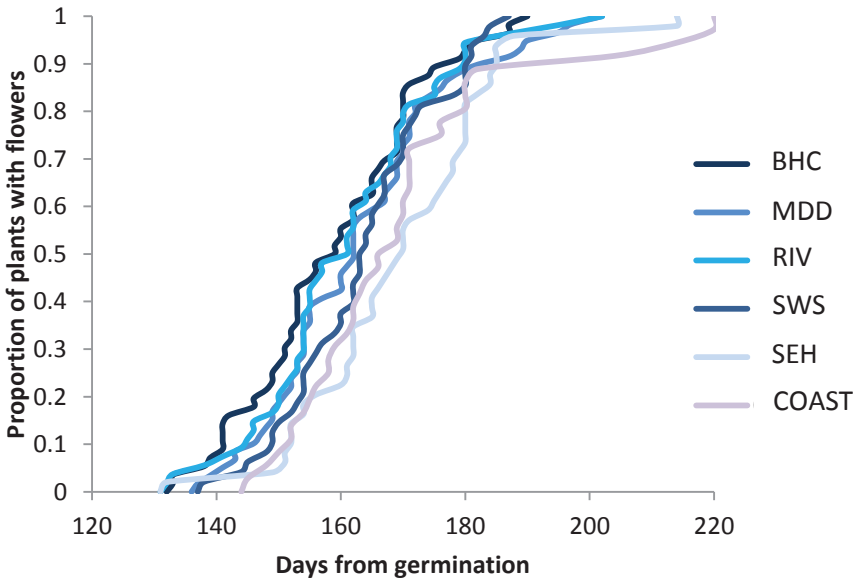

Figure 4.5 Time to first flower (FI_{time}) during a common garden glasshouse trial with *Echium plantagineum* averaged across the six bioregions; cumulative curves of proportion of plants with flowers over time (days from germination). Abbreviations denote the individual bioregions, see Fig. 2.1 for full names.

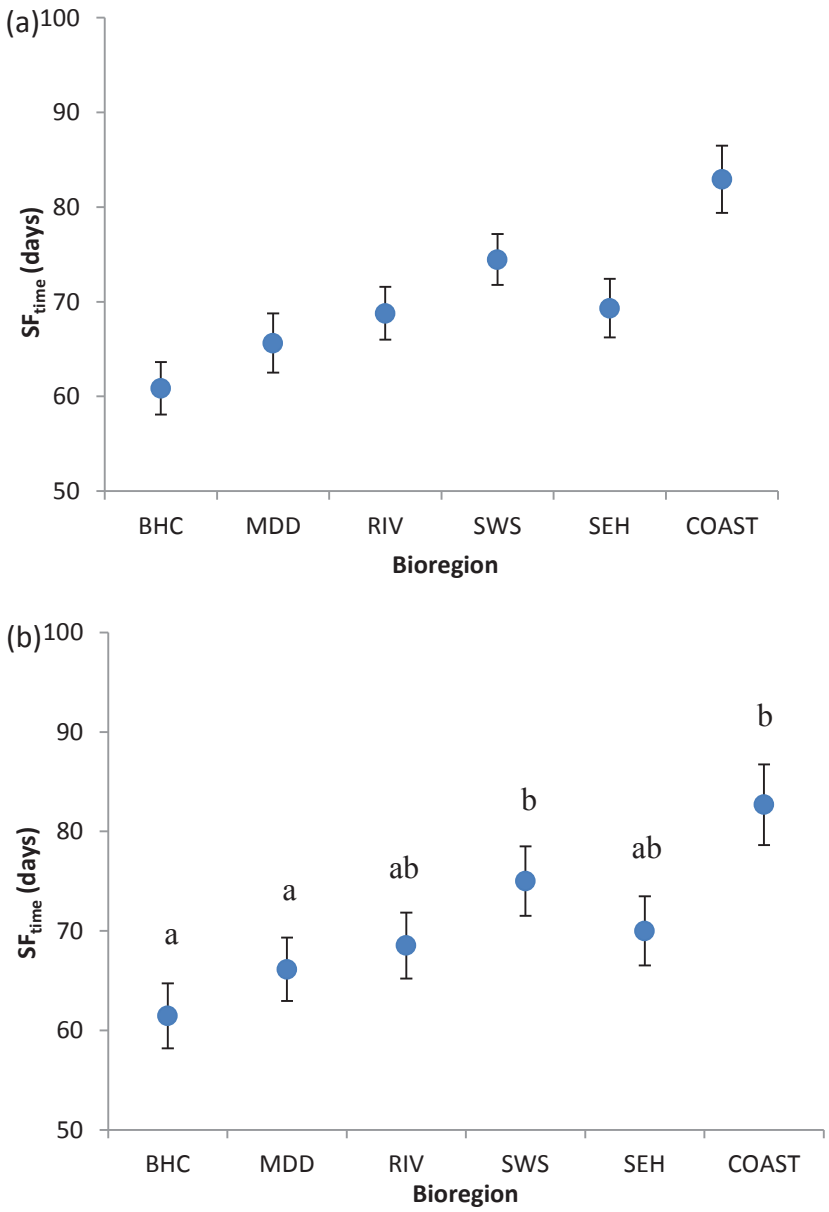

Figure 4.6 Time from first stem to first flower (SF_{time}) averaged per bioregion during a common garden glasshouse trial; (a) actual means ± SE; (b) adjusted means ± SE. Means sharing the same letter did not differ significantly at the 0.05 level PC2. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names. Bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).

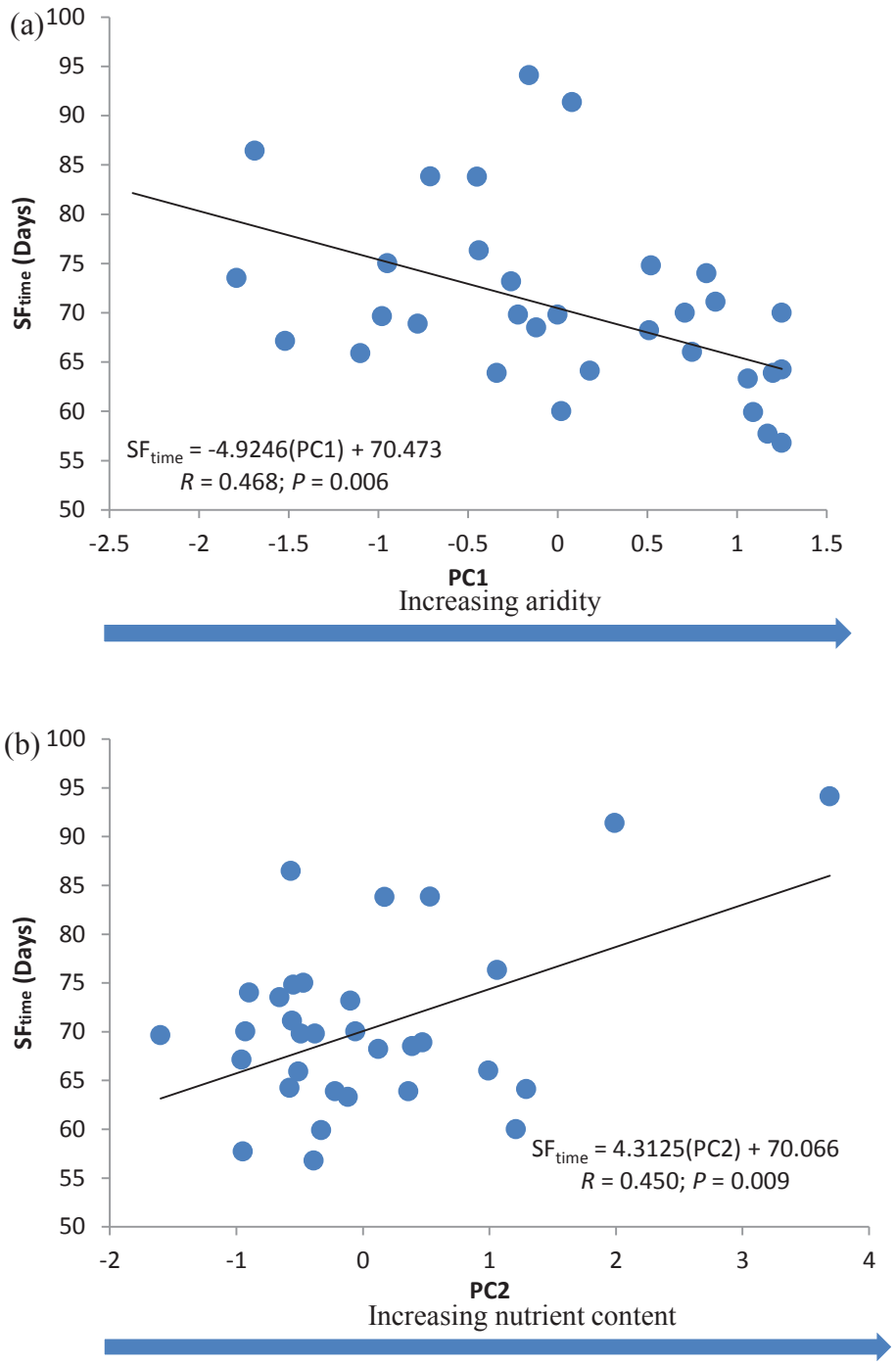

Figure 4.7 Time from first stem to first flower (SF_{time}) averaged per bioregion during a common garden glasshouse trial; (a) Relationship between SF_{time} and site-level scores on PC1 and (b) Relationship between SF_{time} and site-level scores on PC2

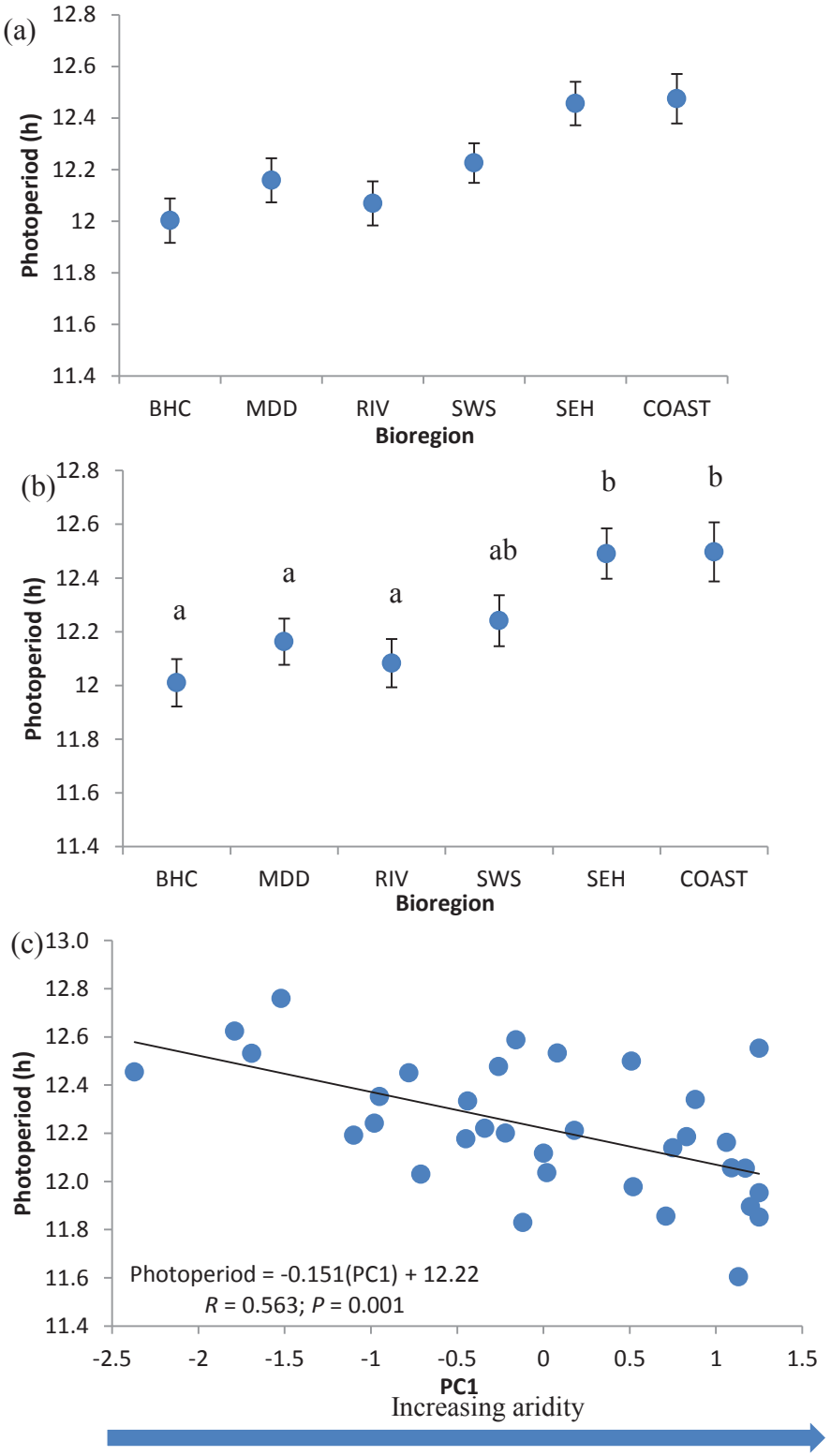

Figure 4.8 Average photoperiod at first flower averaged per bioregion during a common garden glasshouse trial; (a) actual means ± SE; (b) adjusted means ± SE. Means sharing the same letter did not differ significantly at the 0.05 level Abbreviations denote the individual bioregions, see Fig. 2.1 for full names, bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST); and (c) Relationship between photoperiod and site-level scores on PC1.

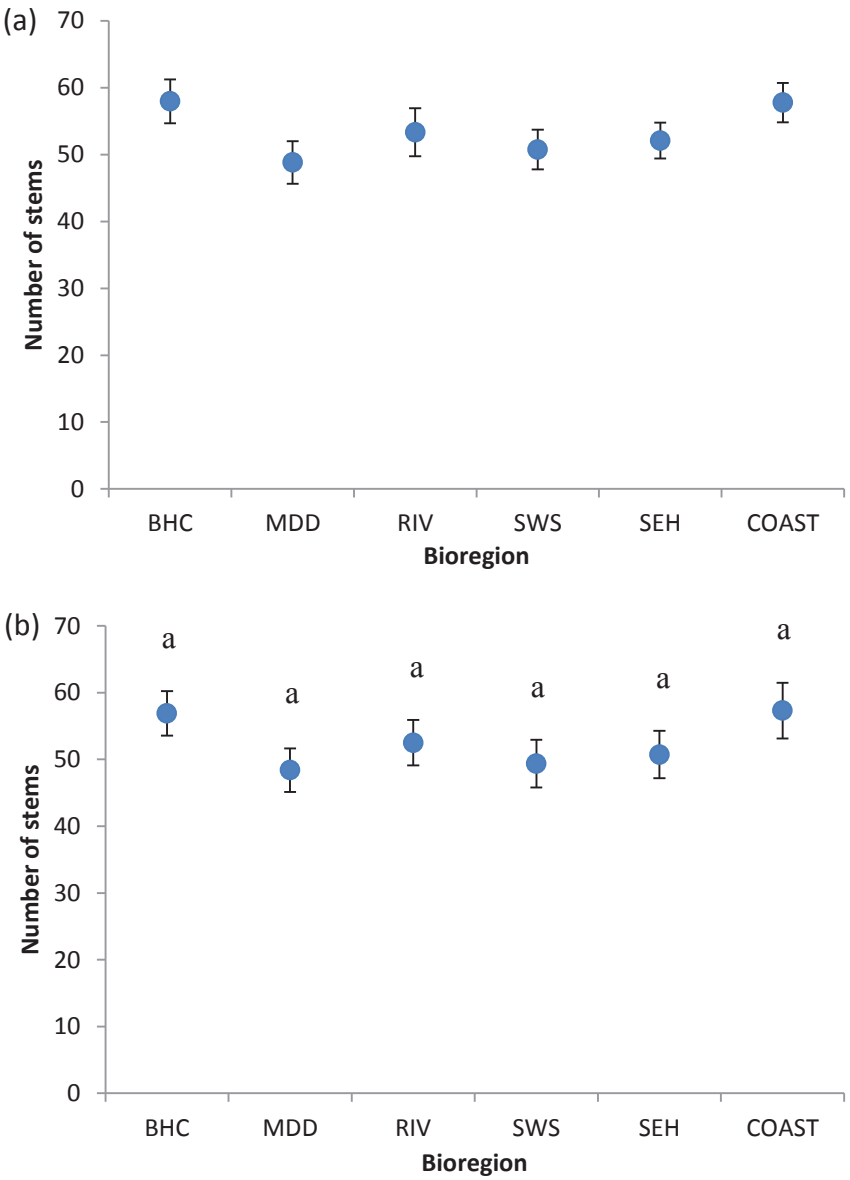

Figure 4.9 Number of stems per plant averaged per bioregion during common garden glasshouse trial; (a) actual means ± SE and (b) adjusted means ± SE. Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names, bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).

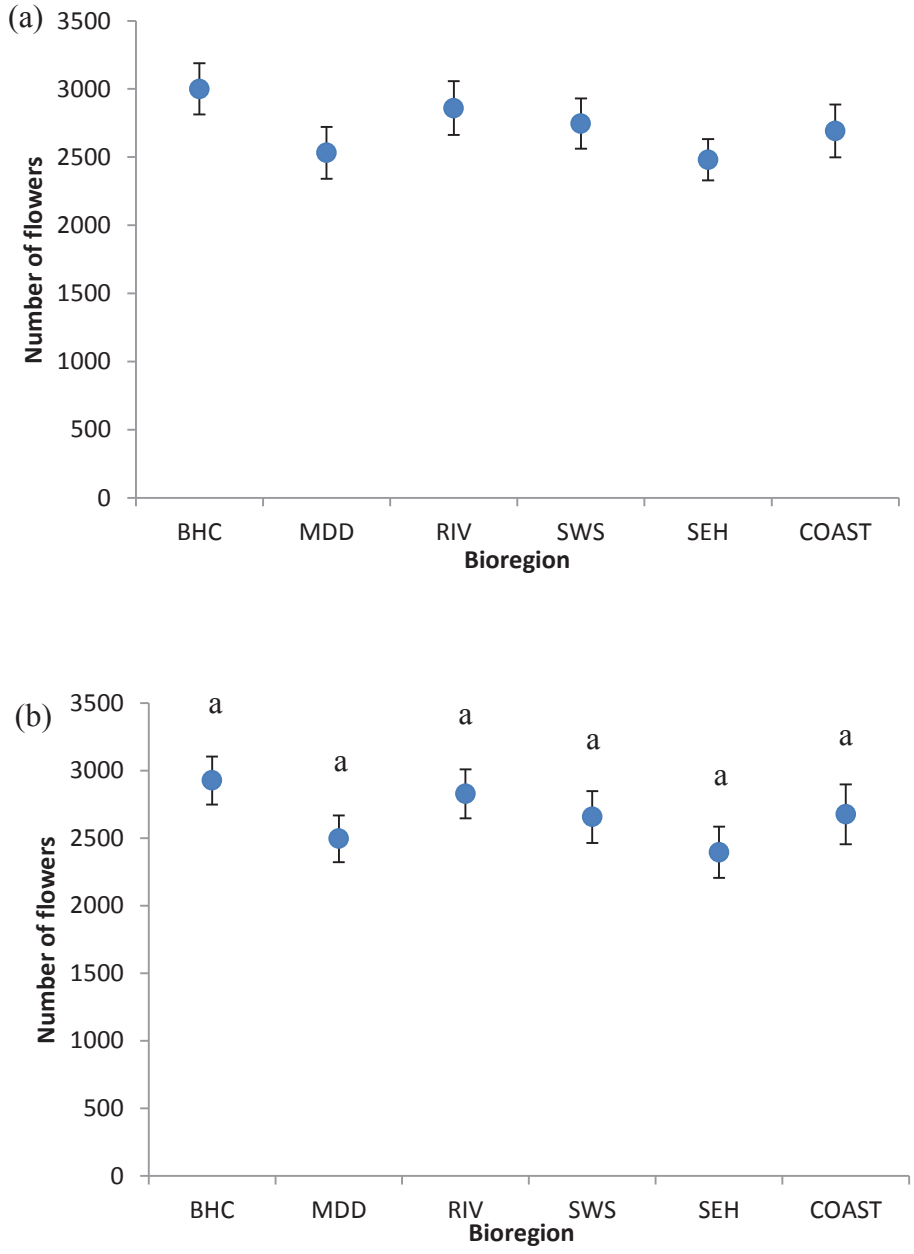

Figure 4.10 Average estimated total flower production per plant averaged per bioregion during a common garden glasshouse trial; (a) actual means ± SE and (b) adjusted means ± SE. Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, see Fig. 2.1 for full names, bioregions are arranged along the x-axis from most westerly (BHC) to most easterly (COAST).

4.4 Discussion

The results of this study indicate that *Echium plantagineum* has undergone clinal shifts in reproductive traits during its invasion of Australian ecosystems, providing evidence for possible climatic adaptation. This adaptation has occurred rapidly (within 100-150 years since introduction to Australia) and is likely to play a vital role in the spread and establishment of *E. plantagineum* in novel environments (Maron *et al*. 2004). There are many examples of species that have experienced similar rates of adaptation (<150 years) since invasion, in various life-history traits (e.g. Jain & Martins 1979; Blair & Wolfe 2004; Leger & Rice 2003; 2007), in response to the new climatic factors present in novel environments. Climate is one of the most important abiotic factors governing species distribution and rapid adaptation to climatic factors in a novel environment is likely to assist with range expansion and increased population survival (Monty & Mahy 2009). Consequently, the success of an invasive species is linked to its ability to efficiently adapt to climatic factors and effectively reproduce when introduced in to novel environments (Piggin *&* Sheppard 1995). *Echium plantagineum* is an example of an invasive species that has been able to adapt its reproductive traits to local climatic factors and these adaptations are likely to have contributed to its invasive potential (Piggin *&* Sheppard 1995; Siemann & Rogers 2001; Grotkopp & Rejmánek 2007).

Given that key reproductive traits of *Echium plantagineum* varied significantly along an arid-mesic cline, I discuss reasons below for the observed rapid stem and flower production in arid environments. The development of clinal trends in reproductive traits may allow newly introduced species to successfully invade and establish in habitats that they were not originally climatically matched with, in their

native range (Siemann & Rogers 2001; Broennimann *et al*. 2007; Beaumont *et al*. 2009). Examples of clinal trends of decreasing flower initiation with increasing latitude include: the annual invasive *Impatiens glandulifera* throughout Europe (Kollmann & Bañuelos 2004), the invasive *Lythrum salicaria* in North American wetlands (Montague *et al*. 2008) and the native annual species *Clarkia unguiculata* in North America (Jonas & Geber 1999; see Table 1.1 for more examples). Consistent with these previous examples, my study has demonstrated a clinal reduction in time to first flower of 1.1 days per degree increase of longitude (Fig. 4.3b) resulting in plants in arid environments initiating flower production on average 12 days earlier than mesic populations. Similar trends of reduced flowering time have been reported in the literature. For example, Fitter & Fitter (2002) reported an average increase in flowering time of 4.5 days for 385 species of native British plants in as little as 50 years. Furthermore, Montague *et al*. (2008) found a difference of 37 days among 25 populations of the invasive species *Lythrum salicaria* and in this case the differences arose in < 200 years. Early flower production is a common strategy of annual arid species, such as *E. plantagineum*, in unpredictable environments, since it provides reproductive assurance (Iannucci *et al.* 2008). Thus, shifts in reproductive traits are likely to play a part in the survival of native and invasive species in response to climatic factors. Collectively, however, there are very few studies that report changes in phenology in response to arid conditions in native or invasive species and as reproductive processes are vital to plant survival, further work is required to better understand the role of phenotypic adaptation.

The results of this study suggest that the reproductive strategy of *E. plantagineum* utilises the alteration of flower initiation in conjunction with several reproductive traits to ensure reproductive success and avoid unfavourable conditions

such as drought. SF_{time} increased from the most arid bioregion (BHC) to the most mesic bioregion (COAST); and plants in arid bioregions were able to produce flowers, on average, 22 days faster than the most mesic bioregion (Fig. 4.6a; b). In addition, this response was significantly explained by both environmental factors (PC1; Fig. 4.7a) and edaphic factors (PC2; Fig. 4.7b) highlighting the influence of site characteristics on this reproductive response. This trend of rapid flower production after stem production in arid environments is likely to be a valuable drought avoidance technique driven by reduced water availability as drought conditions are known to reduce vegetative growth and reproductive output (e.g. Bernier & Périlleux 2005; Mahajan & Tuteja 2005). Thus, it is essential for plants to produce flowers when climatic conditions are most suitable (Elzinga *et al.* 2007) and this is particularly important for annual species (Iannucci *et al.* 2008) as they only have one season to produce viable seed. In contrast, SI_{time} did not significantly vary between bioregions but there is a general trend towards earlier SI_{time} in the mesic bioregions and later SI_{time} in arid bioregions with the exception of one notable outlier (SEH bioregion; Fig. 4.1b). In addition, there was evidence that SI_{time} is influenced by edaphic factors as there is a general trend for SI_{time} to decrease with increasing nutrient content (Fig. 4.1c). Collectively, it is likely that natural selection has favoured *E. plantagineum* populations with flower production that responds to local climatic conditions, thus increasing reproductive success and population survival.

Echium plantagineum has been reported to be a 'long day' plant that starts to produce flowers as the photoperiod increases in late spring/early summer, to allow seed set under favourable seasonal conditions (Piggin & Sheppard 1995), which is consistent with the findings of my study. This response to photoperiod is likely to be selected for to ensure that plants produce flowers during the optimal time of the

season and avoid poor conditions later in the season (Elzinga *et al.* 2007). Previous studies have observed variation in growth (e.g. Bastlová & Květ 2002 and examples therein) and reproductive (e.g. Weber & Schmid 1998) responses of invasive species to different photoperiod regimes. Populations in arid areas are likely to use this response as a technique to avoid drought conditions preventing flower production.

The restricted supply of resources, such as water and nutrients, in arid environments is predicted to reduce stem and flower production, however, *E. plantagineum* plants in the glasshouse were found to produce the same amount of flowers and stem regardless of their original location. Thus, given enough time and resources these plants are capable of having the same reproductive output. During favourable years this could result in prolific seed production (Burdon *et al*. 1983; Piggin & Sheppard 1995) and may be a valuable part of the species' invasion strategy, which has resulted in vast stretches of land contaminated with thousands of plants. On the other hand, in poor years or arid locations, this is not likely to be the case and adaptations are required to cope with a reduced reproductive season. Collectively, given the same amount of time all plants are able to produce similar amounts of stems and flowers, which suggests a role for plasticity under field conditions. Although beyond the scope of this project, such an exploration of the role of phenotypic plasticity in the invasion process is warranted.

Shifts in flowering time towards earlier flowering in arid environments may have evolved as the result of a number of species specific, population specific and environmental factors. For example, self-compatibility in its introduced range (Petanidou *et al*. 2011) and high genetic diversity (Brown & Burdon 1983; Burdon & Brown 1986) may have allowed this species to spread to inhabit new environments with different climate envelopes that may not exist in its native range (e.g.

Broennimann *et al*. 2007; Beaumont *et al*. 2009). In contrast, there is the possibility that this species may have established in Australia under pre-adapted conditions, however, this is not likely to be the case as the introduction history of this species indicates that it has spread large distances away from its initial introduction zones (>800 km; Piggin & Sheppard 1995) and has maintained high levels of genetic diversity (Burdon & Brown 1983) including ongoing production of novel genotypes. Likewise, maternal effects may potentially influence progeny responses, however, maternal effects are unlikely to contribute to the large scale differentiation of the *E. plantagineum* populations observed in my study (Montague *et al*. 2008), since maternal effects were mitigated through the selection seeds of a similar mass, similar sized seeds and germinants of similar size and germination date. Therefore, this study provides evidence for rapid adaptation of this species to local conditions however, this does not confirm that this rapid local adaptation increases the invasiveness of this species nor the specific mechanism (Ridley & Ellstrand 2010) and further work is required to determine the role of this adaptation in the invasion history of *E. plantagineum*.

This study provides evidence for rapid shifts in reproductive traits that correlated with reduced water availability and intermediate to low nutrient availability in arid environments. As reproductive traits determine the quality and quantity of progeny produced, they are vital to the survival of invasive species in novel environments. Moreover, rapid adaptation must have occurred since introduction (<150 years) for such large shift to be observed under common garden conditions. Despite the short timeframe, examples of rapid adaptation in life-history traits can be found over similar timeframes in the literature. While reproductive traits potentially play a part in the success of this species in novel environments, seed characteristics of

the progeny are predicted to be just as important. The next chapter of this thesis investigates geographic patterns in seed size variation of *E. plantagineum* along the arid-mesic gradient.

Chapter 5: Rapid development of adaptive, climate-driven clinal variation in seed mass in the invasive annual forb *Echium plantagineum* L.

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5.1 Introduction

Successful invasion of novel environments by exotic plant species requires that species maintain positive population growth and spread in the face of environmental heterogeneity and new selection pressures (Barrett *et al*. 2008; Monty *et al*. 2009). While many factors determine the demographic characteristics and spatial spread of invading plant populations (Arim *et al.* 2006), rapid evolutionary changes in fitness-related traits increase the reproductive output of local populations and often play a fundamental role in the invasion process (Kawecki & Ebert 2004; Barrett *et al*. 2008; Ridley & Ellstrand 2010). Indeed, significant evolutionary capacity has been identified in many invasive plant species (Weber & Schmid 1998; Reznick & Ghalambor 2001; Lee 2002; Maron *et al.* 2004; Leger & Rice 2007; Etterson *et al.* 2008; Monty & Mahy 2009). This is perhaps not surprising, since considerable theoretical and empirical evidence supports the notion that the capacity for rapid evolutionary change exists widely in plant populations (e.g. Snaydon & Davies 1982; Linhart & Grant 1996; Mealor & Hild 2007; Barrett *et al*. 2008).

Recently it has been suggested by Montague *et al.* (2008) that, at large spatial scales, the spread of invasive populations is mainly determined by evolutionary adaptation and population-level genetic differentiation, while phenotypic plasticity becomes more important where small-scale variation in abiotic conditions impact on population fitness. While the adaptive importance of phenotypic plasticity is well understood (Bradshaw 1965; Schlichting 1986; DeWitt *et al.* 1998; Parker *et al.* 2003; Davidson *et al.* 2011), the capacity for invasive species to undergo adaptive differentiation along broad-scale climatic gradients has been more poorly documented. Adaptive clinal variation in life-history traits has been observed in some invasive species (e.g. Weber & Schmid 1998; Parker *et al*. 2003; Kollmann & Bañuelos 2004; Maron *et al.* 2004; Leger & Rice 2007; Etterson *et al.* 2008; Monty & Mahy 2009), and their ability to occupy new climatically distinct envelopes in their introduced range is likely to be a valuable strategy in general (Broennimann *et al.* 2007).

However, not all invasive species display clinal differentiation (Ebeling *et al.* 2011; Alexander *et al*. 2012), perhaps due to the wide range of genetic, demographic, developmental and environmental factors that influence evolutionary divergence of plant populations in new habitats (Barrett *et al.* 2008; Fenner & Lee 2001; Mitchell & Power 2003; Hinz & Schwarzländer 2004; Colautti *et al.* 2010). Peripheral populations located in marginal habitats, for example, suffer numerous evolutionary constraints related to population size, gene flow and migration rates (Kirkpatrick & Barton 1997; Lenormand 2002; Bridle & Vines 2007). Levels of phenotypic plasticity (DeWitt *et al.* 1998), seed dormancy (Rees 1996), co-variation among fitness traits (Colautti *et al.* 2010), and pathogen load (Bossdorf *et al.* 2005) are also known (among other factors) to limit evolutionary adaptation, and many are especially

relevant for invasive plant populations. Given this conflicting evidence, there is a clear need for a more comprehensive understanding of the species, circumstances, and traits in which adaptive clines are likely to develop.

Seed mass is a key fitness-related trait that might be expected to show strong clinal adaptation when the ability of a species to produce seed of a particular size underpins reproductive success and survival in new environments (Leishman & Westoby 1994; Mason *et al.* 2008). Seed mass influences many life-history traits including dispersal ability, seed bank viability and persistence, progeny fitness, flower size and plant longevity (Vaughton & Ramsey 1998; Guo *et al*. 2000). Large seed size appears to be especially important in arid zone species probably due to the increased temperature-related metabolic costs and the requirements for seedling establishment in arid environments (Leishman & Westoby 1994; Leishman *et al.* 2000; Murray *et al*. 2004). Evolution of seed mass in response to environmental gradients is indeed well documented on a local (Aizen & Woodcock 1992; Winn & Gross 1993; Boulli *et al.* 2001; Murray *et al*. 2004; Moles *et al.* 2005; Daws *et al.* 2007) and global scale (Buckley *et al.* 2003; Moles *et al.* 2007) although few studies have considered whether such patterns exist among invasive species (but see Hurka & Benneweg 1979; Telenius & Torstensson 1999; Buckley *et al.* 2003).

The aim of this study was to test whether, over the past ~150 years since introduction, invasive populations of the annual plant species *Echium plantagineum* L. (Paterson's curse) have developed adaptive, population-level differentiation in seed mass in response to broad climatic gradients in south-eastern Australia. *Echium plantagineum* is a genetically diverse (Brown & Burdon 1983), globally significant weed (Piggin & Sheppard 1995). In Australia, it has invaded arid, temperate and coastal environments, costing the meat and wool industry upwards of $125 million

annually (Carter 2009). We hypothesized that invasive populations of *E. plantagineum* have developed a cline in seed mass in response to aridity, with larger seeds prevailing in populations sourced from warmer, drier habitats than in those sourced from cooler, wetter temperate and coastal habitats. We also hypothesized that ongoing selection for seed size will have resulted in a narrowing of seed size variation among populations within bioregions and among individual plants within populations in the most arid and unfavourable environments relative to populations from a more favourable core habitat (Kirkpatrick & Barton 1997; Colautti *et al.* 2012). To test these hypotheses, we compared the weights of glasshouse-produced seed from invasive *E. plantagineum* populations sourced from 34 sites across a very large (1,000 km) temperature and rainfall gradient in south-eastern Australia.

5.2 Methods

Originally native to Europe and the Mediterranean region, *Echium plantagineum* (Boraginaceae) is an annual forb that was introduced to Australia in around 1850 (Piggin & Sheppard 1995). It is a globally invasive species that has become successfully established in 30 million hectares of agricultural land in Australia (Piggin 1982; Grigulis *et al.* 2001; See Chapter 1, Fig. 1.2). *Echium plantagineum* is insect pollinated and can produce up to 10,000 seeds with seed production of up to 30,000 per m^2. Seeds are dispersed via water, contaminated fodder, garden waste, animal fur and the alimentary tracts of birds or grazing animals (Grigulis *et al.* 2001; Blood 2001), and while some seed can remain dormant in the soil for up to ten years (Blood 2001; Shea *et al.* 2000), most germinate more rapidly (Piggin & Sheppard 1995). Seedlings most effectively colonise bare ground (Muyt 2001) and can have

recruitment rates of >1,000 m^{-2} (Shea *et al.* 2000). In annual species like *E. plantagineum* frequent seed production is a critical driver of population fitness, making it an ideal model species for studies of adaptive capacity and evolutionary responses of seed-related traits in response to broad-scale climatic variation.

5.2.1 Seed collection and field sites

Echium plantagineum seed was collected during the 2009 reproductive season (October to December) from a total of 34 sites across seven IBRA (Interim Biogeographic Regionalisation for Australia scientific framework; Thackway & Cresswell 1995), bioregions in New South Wales, south-eastern Australia (See Chapter 2; Fig. 2.1). These bioregions are large, geographically distinct areas of land with similar climate, land systems, vegetation and animal communities (Thackway & Cresswell 1995). The study sites followed a 1000 km long climatic cline which varies from arid (Broken Hill Complex bioregion) to coastal (Sydney Basin and South-East Coast bioregions) and cool temperate (South-East Highlands bioregion). These bioregions capture a large majority of *E. plantagineum* habitats in SE Australia. Due to similarities between the two coastal bioregions (Sydney Basin and South-East Coast) and the limited number of sites containing *E. plantagineum*, these bioregions have been combined to form a single coastal bioregion (COAST) for our analysis. Sites were randomly selected from across each bioregion; all had at least 50 seed-producing plants. Seed was collected from ten randomly selected individual plants at each site between October 2009 (Broken Hill Complex bioregion) and December 2009 (COAST bioregion), when plants were producing mature seed. No permits were required for the field collections since *E. plantagineum* is an introduced, invasive species. No collections were made on private land. Seeds were transported to the laboratory (CSIRO Black Mountain Laboratories, Canberra, ACT; S 35.27°, E

149.12°), extracted from the mature fruit using a rubbing board (consisting of two flat rubber pads), and stored in paper bags at room temperature until used in the following glasshouse experiment.

5.2.2 Common garden glasshouse experiment

The objective of the glasshouse experiment was to compare the mass of seeds produced by different plants under common growing conditions, thus allowing for a more controlled assessment of the genetic basis of existing variation. Experimental maternal effects were minimised by using seed from different populations that were equivalent in mass, size, germination time and level of dormancy. Seed choice was facilitated by the fact that field-collected seed from different bioregions did not differ in mass (see Chapter 2), unlike glasshouse produced seed (see below). This is likely to reflect the dry conditions experienced during the collection year (2009), especially in the most westerly bioregions, since drought is well known to cause reduced seed mass (Stamp 1990) and general divergence of plant traits under field and glasshouse conditions (Winn & Gross 1993; Kollmann & Bañuelos 2004). Nonetheless, all populations produced large numbers of viable, fully mature seed which were adequate for experimental use.

Ten seeds from each of ten plants from each site (3400 seeds in total) were germinated in the laboratory on moist filter paper in petri dishes under dark conditions at room temperature. After the radicle had emerged, one similar sized embryo (based on radicle length) from each plant (340 in total) was transplanted into small biodegradable pots (Jiffypot®) in a temperature controlled glasshouse at the CSIRO Black Mountain site. After ten days the pots were planted into 10 cm pots of standard potting mix, and then four weeks prior to the commencement of the experiment,

plants were again transplanted into 20 cm pots containing standard, high nutrient compost potting soil (consisting of a mix of calcium carbonate lime, dolomite lime, blood and bone, and NPK fertiliser; pH = 6.5). Pots were arranged in a randomised block design with five blocks each consisting of three benches; two plants from each of the 34 study sites were randomly placed in each block. Plants were grown from April to December 2010 under a photoperiod governed by natural sunlight and a targeted day/night temperature regime of 25/15ºC with an average of 20°C. Temperatures were logged from August to October and followed the targeted regime reasonably closely, with daily averages of 16-20ºC, although spot temperatures as high as 27ºC and as low as 12ºC were observed. Of the 340 plants used in the experiment, two died and 34 plants did not flower within the duration of the experiment (250 days); these were removed from further consideration. After 27 weeks sufficient flowers were produced to allow pollination. Pots were fertilised with Aquasol® Soluble Fertiliser (Yates, Australia) fortnightly or as required.

Suitable plants for open pollination were defined as plants with ten or more open receptive healthy flowers which were identified by the shape and size of the flower and the length and maturity of the stigma. Between October and November 2010 plants from each site were transported together but separately from plants from other sites to a pollination chamber (a small naturally-lit glasshouse) to ensure that cross pollination occurred among plants that originated from the same site. Between six and ten plants with suitable numbers of flowers were available for each site. Plants were stored in a separate, insect-free glasshouse for 24 hours prior to placement in the pollination chamber and previously mature flowers were removed to ensure that only newly developed flowers were pollinated. Pollination was performed by European honey bees (*Apis mellifera*) with an exposure period of 24 hours. Plants were changed

at night when the bees returned to their hive to reduce the risk of cross pollination between sites. All plants were then moved back to the glasshouse (which was also insect free) to complete their development. Seeds were collected from all plants after five weeks following seed maturation and placed into paper bags for storage at room temperature. Seed production was only observed in flowers that were exposed to bee pollination.

5.2.3 Seed measurements

Seed mass per individual plant was determined as the weight of ten viable seeds dried for one week at a temperature of 80ºC, expressed as seed weight (g) per 100 seeds. Seed viability was determined by visual inspection and lightly pressing on either side of the seed with forceps (see 2.2.2). Experience with germination of field seed indicated that seeds were viable if the seed coat did not crack or deform under light pressure (i.e. the seed was filled).

5.2.4 Site characterisation

One representative soil core, 10 cm^2 and 10 cm deep, was collected at each field site, sealed in a plastic bag, and stored at room temperature in the laboratory. A representative subsample (five grams) was ground to a fine powder using a tissuelyser at a frequency of 30.1 rpm for ten minutes, and analysed for percentage carbon (C) and percentage nitrogen (N) using a Europa 20-20 isotope ratio mass spectrometer with an automated nitrogen carbon analysis preparation system (Dumas 1981). Soil electrical conductivity and soil pH ($CaCl_2$) were measured as described in Rayment & Higginson (1992).

Climate data for the period 1910-2010 were obtained for all study sites using the SILO enhanced meteorological dataset and datadrill procedure hosted by the State

of Queensland Department of Environment and Resource Management 2012 (Jeffrey *et al.* 2001; See 2.2.5). We then derived five key climatological variables for each site, focusing on the time frame of May to October during which *E. plantagineum* growth and effective precipitation are highest (Piggin 1976a): total precipitation (P), mean maximum temperature, mean minimum temperature, mean temperature, and total potential evapotranspiration (PET; see Table 2.2 for summary). We also derived two measures of aridity for each site: 1) the annual atmospheric water balance (AWB) (Rasmusson 1968), defined as AWB = P- PET and 2) the aridity index (AI) (Lioubimtseva & Adams 2004), defined as AI = P/PET. Total precipitation increases from around 100 mm in the arid Broken Hill complex to > 300 mm in COAST and South-East Highland bioregions (Fig. 5.1a). Higher bioregions in the east (e.g. NSW South-West Slopes and South-East Highlands) experience cooler temperatures than coastal or far inland locations (Fig. 5.1b). The combination of increasingly drier and warmer conditions towards the west of the study region results in a sharp increase in aridity from the South-East Highland to Broken Hill Complex bioregions (Fig. 5.1c); aridity is intermediate in coastal habitats due to the high overall rainfall.

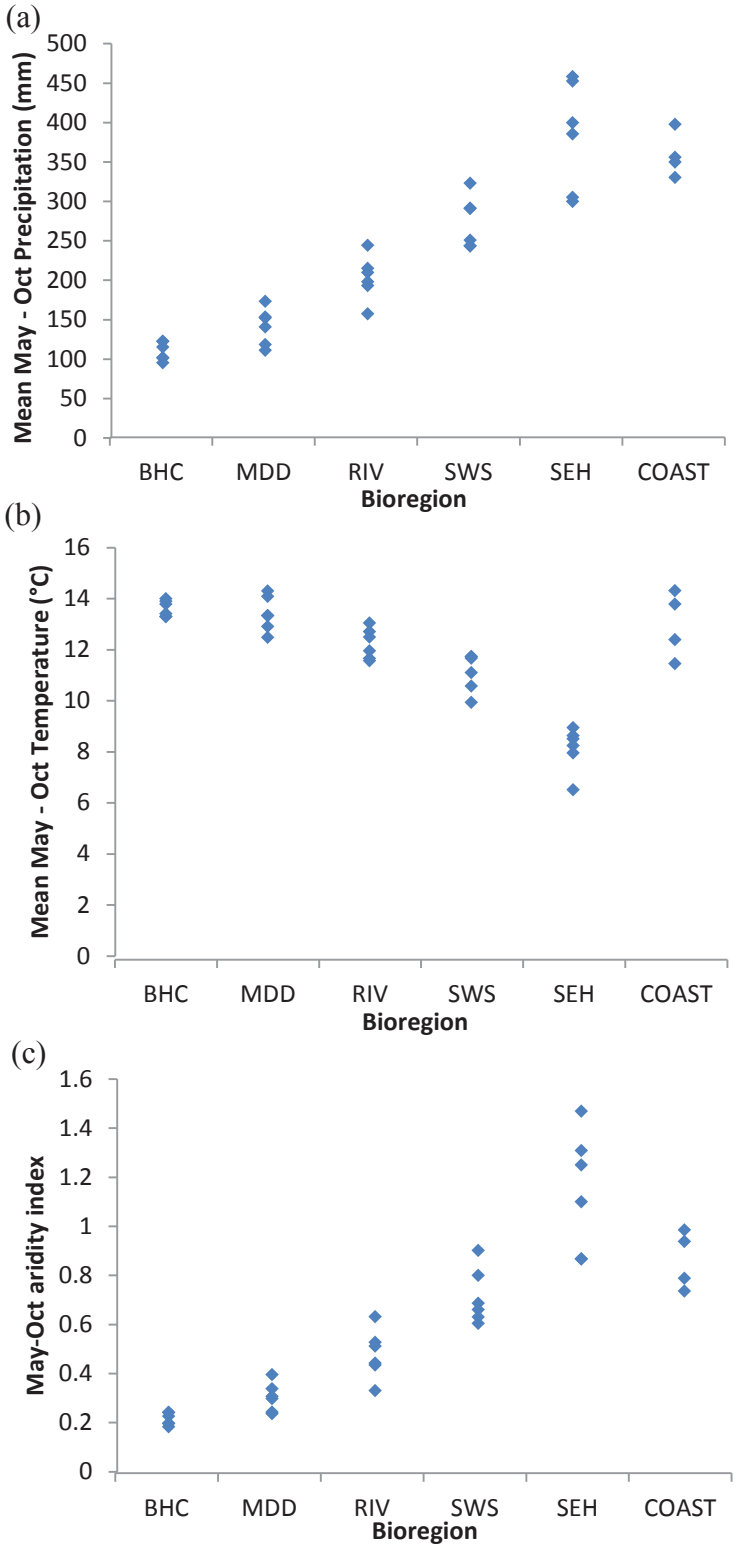

Figure 5.1 Climatological data for the May-October growing season for bioregions in the study area. (a) Total precipitation (mm). (b) Mean temperature (°C). (c) Aridity Index. Abbreviations denote the individual bioregions, Abbreviations denote the individual bioregions, See Fig. 2.1 for full names. Bioregions are arranged along the *x* axis from most westerly (BHC) to most easterly (COAST).

5.2.5 Statistical analyses

The primary data set consisted of mean seed mass for 189 seed-producing plants across 34 study sites (the remainder either died, produced no flowers, or produced no viable seeds). We constructed the final data set for analysis by removing data for the parental plants (n = 45) that produced fewer than three seeds since in most cases the seeds produced were very small due to early abortion or senescence of the fertilised flowers. For one site we retained data from a single plant that produced two healthy, viable seeds because it was the only datum available for that site. The final data set thus consisted of mean seed mass for 144 seed-producing plants. While we report the results of analyses conducted on the final data set, because the presence of small or aborted seed may reflect varying levels of self incompatibility or inbreeding depression (Petanidou *et al.* 2011), we conducted all analyses on both data sets. This decision had no impact on interpretation of the results, although the exclusion of smaller seed did slightly reduce among-site variation in seed mass.

Linear mixed model analysis was used to relate seed mass to bioregion (fixed predictor variable), site within bioregion (random) and block (fixed). Effects of fixed variables were tested using standard F-tests and the effect of the random variable was tested using the Wald Z test (Dytham 2011). The final seed mass data set was square-root transformed according to $y = \text{sqrt}(x)$ to meet model assumptions. Post-hoc tests were performed on bioregion means using the Tukey-Kramer adjustment for multiple testing (Tukey 1953).

We also quantified the direct relationships between seed mass and specific environmental characteristics of maternal field site using linear regression. We first used principal component analysis (PCA) to reduce the ten correlated environmental

variables describing the sites (six climatic, four soil variables; see Chapter 2, Table 2.3) to two components that, combined, accounted for 82% of the total variance. The first component (PC1) accounted for 58% of the variation in the data (Chapter 2, Table 2.3). Scores on PC1 decrease from the arid (BHC) to mesic (SEH) bioregions, with coastal, slopes and Riverina regions having intermediate scores (see Chapter 2, Fig. 2.3). The second component (PC2) accounted for 24% of the variation and primarily reflected site-level soil characteristics (see Chapter 2, Table 2.3). PC2 primarily distinguished between coastal and highland bioregions (see Chapter 2, Fig. 2.3), with low-elevation coastal areas having higher soil fertility (see Chapter 2, Table 2.3). The relationship between soil pH and PC1 (see Chapter 2, Fig. 2.3; Table 2.3) is indicative of the general tendency for soil acidity to increase from the western to eastern parts of the study area (Scott *et al.* 2000), although soil pH also loaded on PC2.

Both PC1 and PC2 were related to mean site-level seed mass using linear regression analysis. Finally, we used individual linear regression analyses (Dytham 2011; Sokal & Rohlf 1995) to directly assess the impact of latitude and longitude on mean site-level seed mass since these relationships have been previously assessed for a number of species in Australia (e.g. Murray *et al.* 2003). Mean growing season (May-October) precipitation, temperature, and aridity index were also used in individual regression analyses on seed mass. No data transformations were required for the regression analyses.

We next tested whether site-level seed mass variance differed across bioregions. First, we performed Levene's test of homogeneity on non-transformed data to determine whether variance among populations differed among bioregions. We then performed a one-way analysis of variance to determine whether mean among

population seed mass variance differed across bioregions. Data from two sites were excluded because plants produced insufficient seed to determine variance. Finally, we used simple linear regression to determine whether among population seed mass variance was related to longitude, latitude and scores on PC1 and PC2. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Carey, NC, USA).

5.3 Results

Seed mass varied significantly among bioregions ($F_{(5,28)}$ = 3.42, P = 0.02, Fig. 5.2a, b), but not among sites within bioregion (WALD Z = 1.20, P = 0.12). Seed mass did not vary significantly across blocks ($F_{(4,106)}$ = 0.77, P = 0.54). There was an overall pattern for seeds sourced from populations found in drier bioregions (especially the Broken Hill Complex and Murray Darling Depression) to be heavier than those sourced from more mesic coastal and south-eastern highland habitats (Fig. 5.2a, b). Mean seed weight in Broken Hill Complex populations was 23% higher than that of COAST populations (Fig. 5.2a), with seeds produced by plants from semi-arid (Riverina) and temperate (NSW South-Western Slopes, South-Eastern Highlands) bioregions being intermediate in weight (Fig. 5.2a).

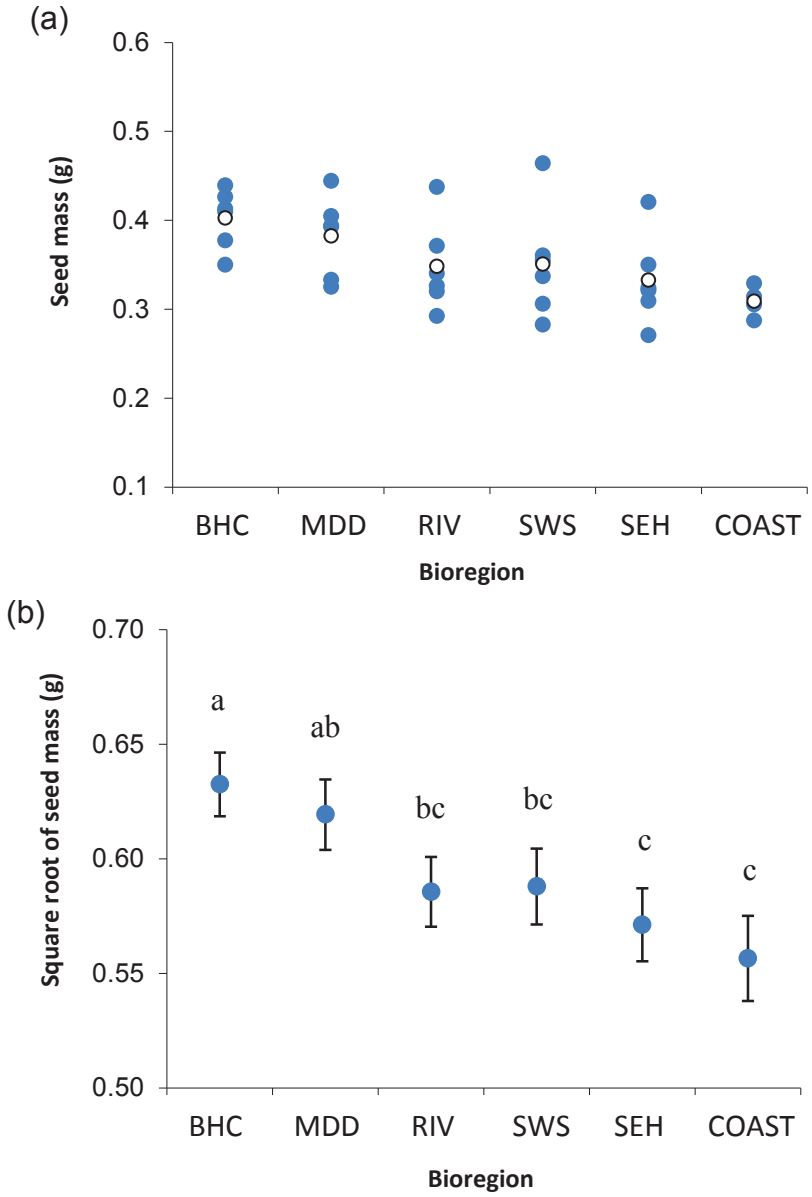

Figure 5.2 Seed mass derived from plants collected from six bioregions in the study area. (a) Mean site-level seed mass (g; 100 seeds) across all six bioregions based on the final data set (with small seeds removed). Site means are shown as filled circles while bioregion means (average of all sites within a bioregion) are shown as unfilled circles. (b) Estimated mean seed mass (± 1 SE) for each bioregion based on linear mixed model analysis (see methods) of final data set (square root transformed). Means sharing the same letter did not differ significantly at the 0.05 level. Abbreviations denote the individual bioregions, See Fig. 2.1 for full names.

At the population level, seed mass was significantly related to PC1 (*B* (unstandardised regression coefficient) = 0.021, R^2 = 0.15, P = 0.02; Fig. 5.3) but not PC2 (*B* = -0.012, R^2 = 0.05, P = 0.19), indicating that climatic factors were more important than soil-related factors, in determining variation in seed mass. Seed mass was strongly related to longitude (*B* = -0.009, R^2 = 0.32, P < 0.001, Fig. 5.4a). Each degree of longitude reduced predicted 100-seed weight by around 0.01 g (from a maximum of ~0.40 g in the Broken Hill Complex to a minimum of ~0.30 g in the COAST bioregion; Fig. 5.4a). This represents a decline of ~25% over around 10° of longitude, or, on average, ~2.5% per degree of longitude. Seed mass also declined with May-October rainfall (1910-2010) (*B* = -0.0002, R^2 = 0.18, P = 0.01), aridity index (*B* = -0.058, R^2 = 0.15, P = 0.02) but only marginally with mean temperature (*B* = 0.007, R^2 = 0.09, P = 0.09). Finally, seed mass was also significantly related to latitude (*B* = 0.016, R^2 = 0.14, P = 0.03, Fig. 5.4b), with each degree of latitude reducing predicted mean seed mass by 0.016 g, or around 4% (on average over the entire study region).

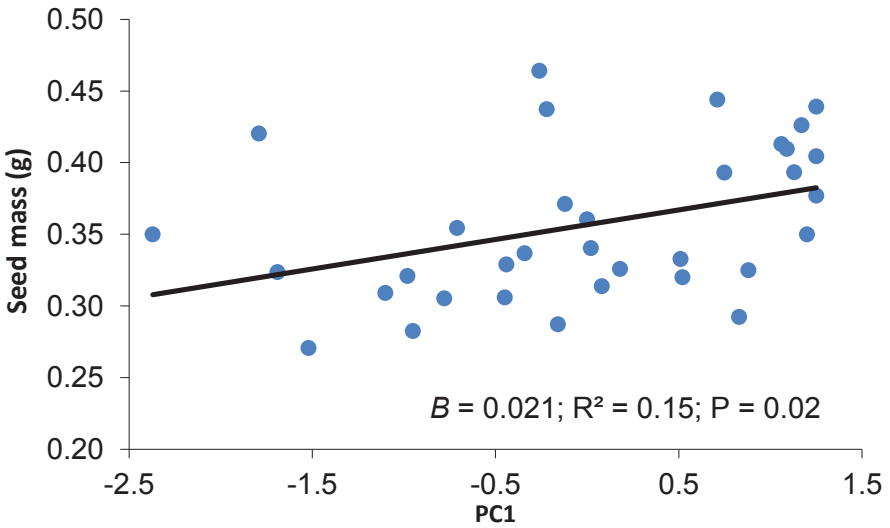

Figure 5.3 Scatterplot depicting linear relationship between seed mass and site-level scores on PC1 (primarily related to climate). *B* = unstandardised regression coefficient.

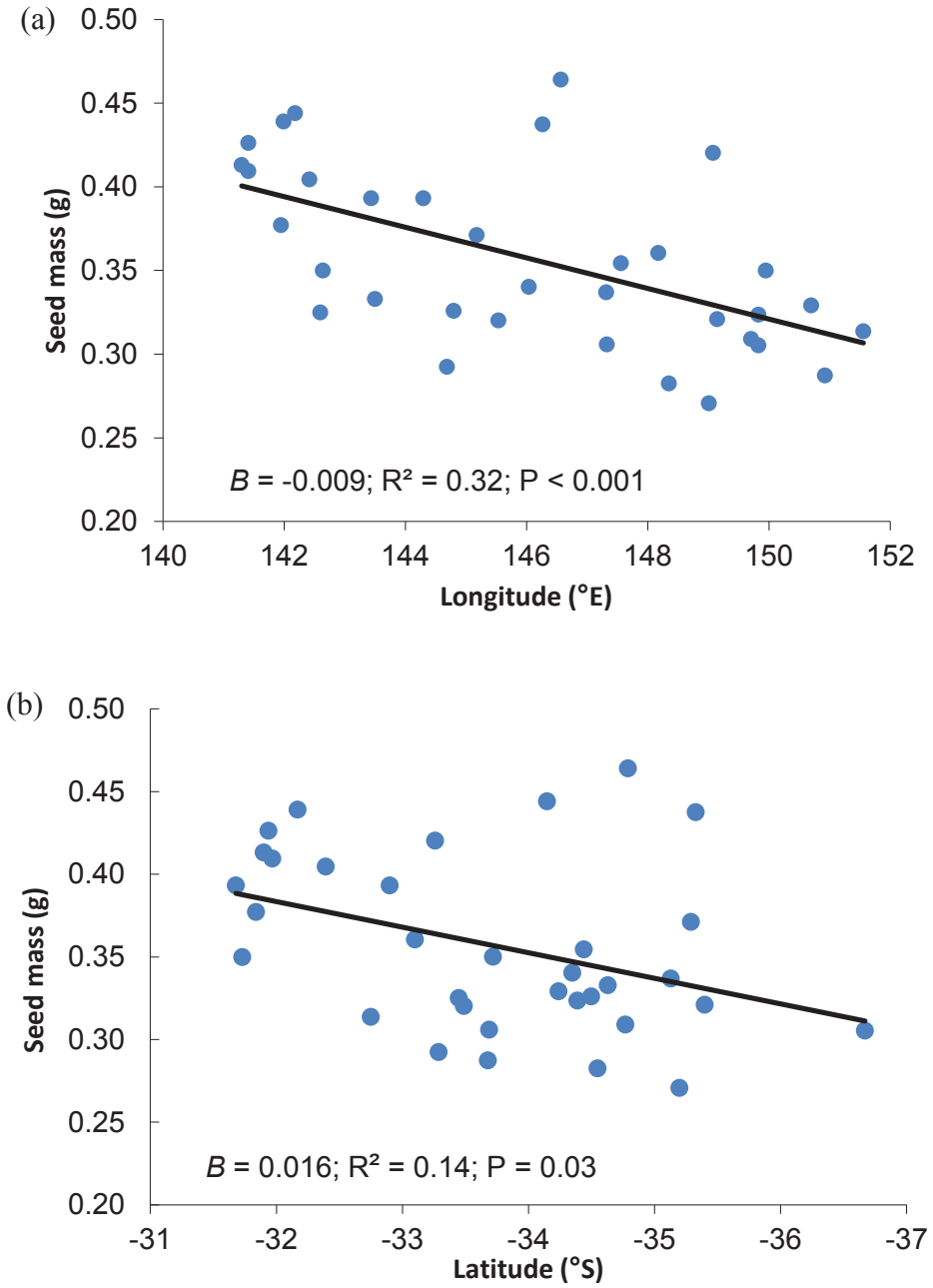

Figure 5.4 Scatterplots depicting linear relationships between seed mass and two key predictor variables: (b) site longitude, and (c) site latitude. *B* = unstandardised regression coefficient.

Variance among populations within bioregion did not differ significantly (Levene's test of homogeneity on untransformed data $F_{(5,26)} = 1.36$, $P = 0.27$; Fig. 5.5), and mean site-level variation in seed mass (calculated as the variance in seed mass among plants within each site) did not differ significantly across bioregions ($F_{5,26} = 1.63$, $P = 0.19$; Fig. 5.5). There was a general trend for between-site variance in seed mass (Fig. 5.5) to be greatest towards the core of the species distribution (e.g. NSW South-Western Slopes and South-Eastern Highland bioregions) and least towards the edge (e.g. Broken Hill Complex and COAST bioregions). This result was not observed in among-plant variation at the site level (Fig. 5.5). Site-level variation in seed mass was also not related to longitude, latitude, or scores on PC1 or PC2 ($P >> 0.05$ for all).

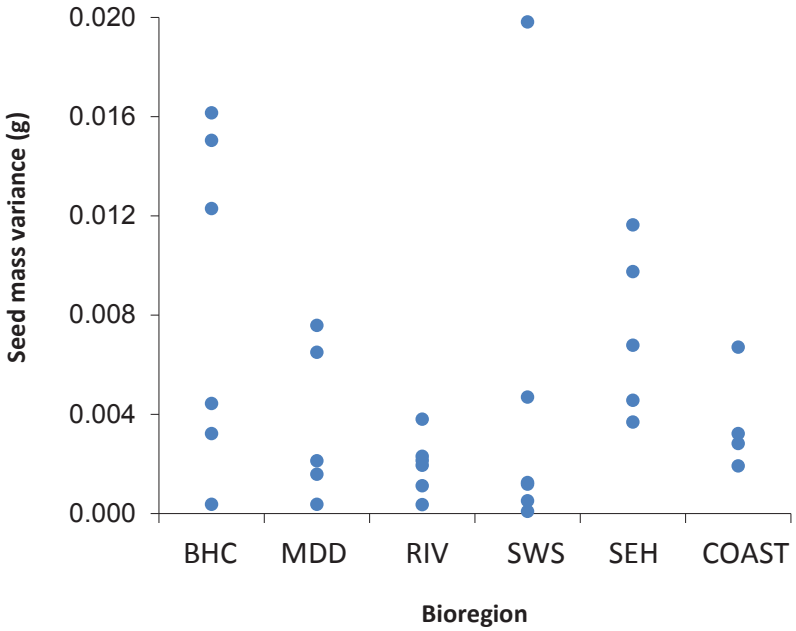

Figure 5.5 Variance in seed mass for all study sites in each bioregion, determined as the variance in seed mass among seed-producing plants using the final data set. Abbreviations denote the individual bioregions, See Fig. 2.1 for full names.

5.4 Discussion

The results of this study support the hypothesis that relatively recent climatic adaptation has resulted in the development of a cline in *Echium plantagineum* seed mass with aridity in south-eastern Australia. Our data indicate that *E. plantagineum* seed mass declines by around 25% across the 1,000 km west-east gradient spanning the study area, with smaller seed being produced by populations from cooler, wetter environments (e.g. COAST bioregion) than warmer, drier environments (e.g. BHC bioregion). Seed mass correspondingly declines by close to 2.5% (on average) per degree of longitude, and broadly increases with site aridity, which is highest in the west of the study region. We also found that seed mass declines with increasing latitude, although the strength of the association is lower than for longitude (Fig. 5.4a).

These data suggest that selection pressure, associated with aridity, has acted on invasive populations of *E. plantagineum* to increase seed size in arid relative to mesic habitats. This supports the general argument that seed mass plays an important role in maintaining population fitness in arid-adapted species (Leishman & Westoby 1994; Leishman *et al.* 2000), and that seed-related traits have the capacity to undergo evolutionary shifts that increase the invasive potential of newly introduced plant populations (Daws *et al.* 2007). Adaptive variation in seed mass in response to broad environmental gradients has been well documented (Aizen & Woodcock 1992; Winn & Gross 1993; Boulli *et al.* 2001; Murray *et al*. 2004; Moles *et al.* 2005; 2007; Daws *et al.* 2007); but this study is one of the first to document such a change in an invasive species (see Hurka & Benneweg 1979; Telenius & Torstensson 1999; Buckley *et al.* 2003 for other examples).

Interestingly, the decline in seed mass of 2.5% per degree of longitude and 4% per degree of latitude in *E. plantagineum* is remarkably similar to that reported by (Murray *et al.* 2003) for native perennial *Glycine* species in Australia. They attributed the presence of larger seed in inland areas and at low latitudes to the increased metabolic costs of high temperature and increase in availability of photosynthate in these environments (Murray *et al.* 2004), which is a plausible explanation for existence of the same pattern in *E. plantagineum*. The high degree of spatial convergence of *Echium* and *Glycine* strongly supports the view that, in Australia, both native and exotic species experience similar climatic selection pressures and have the capacity to develop adaptive clinal variation in seed mass in response. However, while seed mass evolution is likely to have increased the overall distribution, abundance and fitness of *E. plantagineum*, other factors, such as overall seed size (Daws *et al.* 2007), broad differences in other life history traits, or the effects of disease (Bossdorf *et al.* 2005) are also likely to play a key role in determining the performance of this and other invasive species relative to sympatric native species.

We also hypothesized that variation in seed mass should decrease in populations subjected to the strongest directional selection pressure (see Colautti *et al.* 2010), which in this case is more arid areas where seed mass is known to be a key determinant of fitness (Leishman & Westoby 1994; Leishman *et al.* 2000). However, in contrast to mean seed mass, we did not find a clear pattern in seed mass variation across the study region. Variation in seed mass among plants at the individual site scale was unrelated to longitude, latitude, or variation in climate and soil (i.e. PC1), and did not differ across bioregions (Fig. 5.5). This is consistent with other studies that have shown *E. plantagineum* populations in Australia to be extremely genetically diverse, with geographically isolated peripheral populations as diverse as those

located in core habitat (Brown & Burdon 1983; Burdon & Brown 1986). It is worth noting, however, that the magnitude of variation across different plants within each study site was relatively low (coefficients of variation mainly in the 10-30% range; data not shown), and could reflect experimental error. Another possibility is that other processes, including gene flow and resulting migration-selection balance (Kirkpatrick & Barton 1997; Lenormand 2002; Phillips 1996), genetic drift in peripheral populations with small effective population sizes (Vucetich & Waite 2003), increased local genetic diversity arising from genotypic admixture (Kirkpatrick & Barton 1997) or "genetic rescue" (Lenormand 2002) could have resulted in the lack of clinal development in variation for seed mass.

Variation among sites in seed mass also did not differ among bioregions, but there was a suggestion that sites had greater similarity in mean seed size (i.e. lower variance) at either ends of the cline than in the centre (Fig. 5.2a, b). The overall relationship between seed mass variance and clinal location (e.g. longitude) appears to be curvilinear, with a peak in the SWS bioregion. If true, this could reflect the effects of directional selection operating more strongly on heritable genetic variation in marginal than in core populations. Indeed, the observed pattern differs from that predicted under simple genetic drift, i.e. greater differentiation among small, isolated (peripheral), populations than larger, more interconnected (core) populations (Lee 2002). However, the reduced variance observed in marginal populations could also reflect the generally lower levels of climatic variation across survey sites in the most westerly and easterly bioregions (BHC and COAST bioregions respectively) compared with those towards the centre of *E. plantagineum'*s distribution. Clearly, further data are required to resolve whether convergence in seed mass among range-

edge populations has occurred as a result of selection, other demographic or evolutionary processes, or simply as a sampling artefact.

From an evolutionary standpoint, the development of clinal population-level variation in fitness-related traits observed in invasive species both here and globally (e.g; Etterson 2004; Kollmann & Bañuelos 2004; Maron *et al*. 2007; Mason *et al.* 2008; Montague *et al*. 2008; Etterson *et al*. 2008; Monty & Mahy 2009 although see Alexander *et al.* 2012 for a contrasting example) can arise via several different mechanisms. Clinal differentiation can occur via adaptive radiation, the evolution of diversity within a rapidly expanding lineage or during range expansion as a restricted number of founder genotypes incrementally diverge over time (Maron *et al.* 2004; Montague *et al.* 2008). This process can be facilitated by an admixture of populations sourced from different parts of the native range and generations of novel genotypes for selection to act upon (Bossdorf *et al.* 2005; Etterson 2008; Montague *et al.* 2008). Alternatively, a broad base of genotypes may be introduced across the species range, mean trait shifts occurring as a result of selective filtering of pre-adapted or climatically matched genotypes (Maron *et al.* 2004; Montague *et al.* 2008). While the latter process is sometimes not seen as adaptive evolution (Maron *et al.* 2004), it does still involve incremental improvement in population fitness via natural selection of phenotypes with heritable traits, which is a condition necessary for evolutionary change (Hoffman & Merilä 1999).

In *E. plantagineum*, both processes have probably taken place, as is the case in invasions more generally (Montague *et al.* 2008). Early introductions of *E. plantagineum* are thought to have come from a variety of areas in the native range, including England, Morocco and France, with multiple introductions occurring across eastern Australia in the mid- to late 1800's (Piggin & Sheppard 1995). Between 1910

and 2010 populations expanded and merged (Chapter 1, Fig. 1.2), and during this time significant mixing of genotypes has been likely. Populations in Australia are extremely genetically diverse, recombinants are ubiquitous (Brown & Burdon 1983), and overall levels of genetic diversity are similar to that observed in the native range (Burdon & Brown 1986). The breeding system of *E. plantagineum* has also diverged in Australian and native range populations (Petanidou *et al.* 2011) with Australian populations being self compatible and able to outcross (Brown & Burdon 1983). These lines of evidence suggest that the clinal development observed in *E. plantagineum* in this study can be at least in part be explained by evolutionary adaptive radiation and not simply by fitness optimisation of populations via filtering of pre-adapted genotypes.

The rate at which adaptive variation in seed mass exhibited by *E. plantagineum* in Australia has developed is noteworthy. Mean differences in seed mass of around 25% have occurred in, at the very most, 150 generations, which is towards the lower end of the evolutionary rates observed, in traits related to invasiveness, elsewhere (Thompson 1998; Weber & Schmid 1998; Maron *et al.* 2004; Franks *et al.* 2007; Leger & Rice 2007; Friedman *et al*. 2008; Montague *et al.* 2008; Whitney & Gabler 2008; Ridley & Ellstrand 2010). Despite this short timeframe, geographic patterns in seed mass observed in *E. plantagineum* have apparently converged with those observed in other native Australian forbs.

The results of this study have significant long-term management implications for *E. plantagineum* and other invasive species globally. Predicted global climate change is expected to favour species that have the capacity to rapidly adapt to new conditions (Mason *et al.* 2008), and these are the same characteristics that facilitate the invasion of new environments (Bone & Farres 2001). Our study supports the view

that the fitness and range potential of invasive species can rapidly increase as a result of genetic divergence of populations along broad climatic and geographic gradients, and that selection for seed mass can play in important role in this process.

Chapter 6: Discussion and synthesis

6.1 Overview of important findings

The central aim of this thesis was to use the invasive species, *E. plantagineum* as a model system, to investigate the potential for population differentiation in key life-history traits. This required the collection of seed material from 34 sites across a 1 000 km arid-mesic gradient in south-eastern NSW. With this seed I conducted a germination trial in the laboratory to determine the germination strategy of *E. plantagineum* immediately following water exposure (Chapter 2). This was followed by glasshouse studies of plant growth, water use (Chapter 3) and reproductive strategies (Chapter 4). Finally I used open pollination with European honey bees (*Apis Mellifera*) to assess the impact of the parental environment on progeny seed mass and size (Chapter 5).

The goal of this design was to observe genetically based trait variation and to determine which traits and life stages correlate with environmental factors in Australian ecosystems. The information gathered in this thesis provides a whole-plant perspective on the potential evolution of plant traits in response to site-specific environmental selection pressures. Thus, this thesis provides an insight into the ability of this species to overcome the introduction-to-invasion pathway and has identified several traits that have undergone rapid adaptation to local climatic conditions since introduction to Australia, therefore, the aim of this thesis has been met. This work has covered a significant gap in the literature as very few empirical studies have been able to identify rapid population divergence in life-history traits across the entire lifecycle of an invasive species along an arid-mesic gradient. The research conducted in this study has provided valuable information about the evolutionary potential of *E. plantagineum* and an indication of evolutionary potential of other genetically diverse, broad leaf invasive species.

6.1.1 Summary of major results and conclusions

The major results of this study are summarised in Table 6.1 and I discuss below four specific predictions that were addressed in this thesis.

1. **Populations from arid environments will germinate faster and reserve a greater portion of seeds for later germination (bet-hedging) compared to populations from mesic environments**

   The germination rate and time of *E. plantagineum* populations (Chapter 2) did not differ among bioregions suggesting that this species has a similar germination strategy across its introduced range, however when compared to other native and invasive species it germinates very rapidly (within 48 hours). This indicates that this species is able to rapidly germinate and take advantage of space and resources before surrounding species. As rapid germination is linked to invasion success in many species (Baker 1974; Radford & Cousens 2000; Kudoh *et al.* 2007) this trait is likely to have played a key role in the invasion strategy of *E. plantagineum*.

2. **Plants from arid environments have slower growth, smaller stature and higher water use efficiency compared to plants from mesic populations.**

   Arid region populations had a faster growth rate, more leaves, faster below-ground growth and larger above-ground growth than mesic populations (Chapter 3). This suggests that these traits have been selected for and may be favourable for resource security in arid environments, where plants must rapidly acquire resources when available. In contrast, water use strategies did not vary across the range indicating that this species is likely to use a drought avoidance strategy rather than tolerating drought

conditions. This strategy is supported by the early flower production strategy of plants from arid environments.

3. **Plants from arid environments will produce stems and flowers earlier and in higher quantities than plants from mesic populations.**

Plants from arid environments produced flowers on average 12 days earlier than plants from mesic environments resulting in the development of a cline in flowering time decreasing with aridity (Chapter 4). Resource constraints in arid environments are likely to have selected for earlier flowering varieties. Rapid flower production comes at a cost of resource collection capacity, however, in arid environments rainfall variability and low rainfall can significantly reduce resource availability, especially late in the growing season, favouring rapid flower production. Rapid flower production and dehydration avoidance reduces the requirement for water use efficiency as plants that produce flowers earlier in the season do not need to tolerate drought conditions if they have already produced viable seeds. In mesic environments slower growth and reproduction allows this species to collect large amounts of resources before producing flowers. This might be an advantage if biomass is high and there is greater competition for resources. Interestingly, however, given enough time and resources plants from arid and mesic environments were able to produce the same amount of flowers and flowering stems, suggesting that accumulating biomass during earlier stages of development does not provide a reproductive advantage.

Table 6.1 Summary of major results of the thesis with predicted and observed trends in *E. plantagineum* plants collected from arid environments compared to mesic environments.

Trait group	Traits measured	Predicted	Observed	Significant finding
Germination	Germination rate and germination speed	Rapid germination	Uniform very rapid germination	Very rapid germination (<2 days) especially when compared to native species
		Bet-hedging[#]	No evidence for bet-hedging	
Growth	*Growth:* Relative growth rate (RGR), leaf number, leaf dimensions, specific leaf area (SLA), leaf area ratio (LAR), leaf accumulation rate and leaf chlorophyll, carbon and nitrogen content.	Slower growth	Faster growth	Rapid growth, reduced leaf size and increased leaf number in arid regions
	Stature: Height, petiole length, photosynthetic area (P_{area}), above ground biomass (AGB), below ground biomass (BGB) and root : shoot ratio (RSR).	Smaller stature	Larger stature	Rapid early BGB and RSR with increased final height and AGB in arid areas
	Water use: Water use efficiency (WUE), carbon isotope ratio and stomatal conductance	Efficient water use	No change in water use	Evidence for drought avoidance
Reproduction	Time to stem initiation (SI_{time}), time to flower initiation (FI_{time}), time from first stem to first flower (SF_{time}), photoperiod, total stems and total flowers	Rapid stem production	No change in stem production	Development of flower initiation cline with plants in arid areas producing flowers much faster than mesic populations
		Earlier flowering	Earlier flowering	
	Variation in stem initiation (SIV) and flower initiation (FIV)	Fewer stems and flowers	No change in stem and flower quantity	
Progeny seed	Seed mass and dimensions	Larger seed mass and size	Larger seed mass and size	Development of a cline is seed mass with larger seed being produced in arid environments
	Variation in seed mass	Reduced variation in seed mass at range margins	No change in seed mass variation	

[#]For the purpose of this study bet-hedging is defined as the spread of germination over time after a single water exposure event.

4. **Larger seeds will be produced in arid environments compared to mesic environments.**

   Variation in seed mass developed a cline along the arid-mesic gradient as larger seeds were produced by plant populations from arid environments and smaller seeds were produced by plant populations from mesic environments. This cline suggests that larger seeds provide an advantage in arid environments which corresponds with seed mass theory and other published examples suggesting that larger seeds are required in arid environments due to the added metabolic costs and increased photosynthate in these environments (e.g. Murray *et al.* 2004).

6.2 Implications for the ecology of *Echium plantagineum* in Australia?

6.2.1 Evidence for evolution since introduction

Several traits throughout the life-history of *E. plantagineum* display convincing evidence for evolutionary change with the most persuading evidence coming from the development of clines in flowering time and progeny seed size along an arid-mesic gradient. The presence of clinal variation, in flowering time and progeny seed size in introduced ranges, suggests that adaptive processes have selected for faster reproduction and larger seed size in arid environments. When combined with rapid germination and fast initial growth this species is able to rapidly establish, take advantage of available resources and reproduce before conditions become unsuitable. The high level of genetic diversity in this species (Brown & Burdon 1983) is likely to play a large role in the ability of this species to rapidly modify its traits to match its environment. Although rapid evolution has been observed in many invasive species

across similar timeframes (e.g. Weber & Schmid 1998; Maron *et al.* 2004; Leger & Rice 2007) this study is one of the first to observe rapid development of clines in flowering time and seed size across an arid-mesic gradient in an invaded range.

While evolutionary adaptation is the most likely driver of this cline, there are several other possible factors that may influence the development of clines in flowering time and seed mass. For example, colonization history through the introduction of pre-adapted genotypes may also result in a similar cline (Montague *et al.* 2008), however, due to the high level of genetic diversity throughout the invaded range (Brown & Burdon 1983) and the four isolated points of successful introduction in to Australia (Forcella *et al*. 1986), it is unlikely that this cline resulted from the distribution of pre-adapted genotypes transported from climatically similar environments in the native range (climate matching; Montague *et al.* 2008). Definitive proof of the role of natural selection in the development of these clines will require reciprocal transplant experiments (e.g. Lacey 1988; Rice & Mack 1991) to demonstrate local adaptation, combined with phenotypic selection analysis to determine the strength of the selection pressures (Montague *et al.* 2008), and this would be a fruitful area for further research.

6.2.2 Response to arid environments

One of the most interesting aspects of the life-history of this species is its ability to modify its traits to suit the surrounding environment. For example, in response to arid environments *E. plantagineum* has increased its growth rate, leaf number (Chapter 3), flowering time (Chapter 4) and seed size (Chapter 5). In addition, theses adaptations are likely to increase population reproductive success and ultimately population survival. These adaptations allow this species to cope with the added metabolic costs (Murray *et al.* 2004), reduced precipitation (Fischer & Turner 1978; Armas &

Pugnaire 2005) and low nutrient availability (Maestre *et al*. 2005) typical of arid environments. These adaptations are likely to have occurred within the last 150 years since introduction to Australia and are likely to have contributed to ability of this species to overcome the naturalisation-to-invasion pathway and its invasive success in Australia.

Echium plantagineum has the capacity to persist in extremely harsh environments, for example *E. plantagineum* populations have been recorded in semi-arid and arid environments located in places such as Nyngan (annual rainfall 445 mm) and Broken Hill (annual rainfall 248 mm) since 1900 and 1946 respectively (AVH). However, the robust nature of *E. plantagineum* is best displayed through its ability to use the same germination, growth and water use strategies regardless of the surrounding environmental conditions. There are two main possibilities for this response; (1) these traits may be static in nature and unable to be altered, perhaps due to: trade-offs or associations with other traits, low trait genetic diversity or lack of heritable variation, or (2) they are not influenced by selection pressures and do not need to adapt to surrounding environmental conditions. Regardless of the reason this species is able to establish and reproduce under difficult climatic conditions.

6.3 Implications of climate on the distribution and management of *E. plantagineum*

Future changes in climate throughout Australia are predicted to increase the average temperature and reduce precipitation throughout the main distribution of *E. plantagineum*'s introduced range (Whetton *et al.* 1993; Hughes 2003; Anwar *et al*. 2007). Thus, adaptation to such changes will depend on how species are able to cope

with environmental heterogeneity. The distribution of *E. plantagineum* is not likely to be reduced under these changes, as pre-adapted genotypes already exist across a wide range of climate regimes and environments which increases the chance of pre-existing genotypes matching future climates. However, it is unclear whether changes in future climate in range margins (e.g. hotter and drier conditions in arid environments), will reduce population sizes, remove existing populations or induce rapid adaptation. While this would be a worthwhile area for future research, current management programs must focus on the control of existing populations and how selection may cause adaptation in the near future.

6.3.1 Management implications for *E. plantagineum*

Research on the biological control of *E. plantagineum* started in 1971 at the CSIRO (Nordbloom 2003) and many control efforts have relied on unsuccessful biological control methods. Biological control methods have been successful through the centre of its invaded range, however, due to the large dormant seed bank (> six years), populations of *E. plantagineum* are able to recover the following year. The effectiveness of biological control agents in arid areas is significantly reduced, as during periods of drought, this species is able to senesce without producing seed and recover from the seed bank the following year. Biological control species require re-distribution each year to allow effective control resulting in significant cost for land managers. Furthermore, increased temperature and reduced rainfall are expected to reduce the effectiveness of these biological controls (Knepp *et al* 2005).

In the past, control methods such as competitive crop growing, grazing management, herbicide treatment, biological control, slashing, burning and hand weeding have been used to reduce populations size (Naughton *et al.* 2006), however, many of these techniques can reduce the productivity of the land. Reducing the spread

of *E. plantagineum* is likely to require a combination of these techniques in conjunction with the correct timing to reduce population size. Invasive spread is most often limited by dispersal, however *E. plantagineum* is capable of producing prolific numbers of small, easily dispersed seeds allow populations to be transported large distances. Therefore, preventing flower and seed production through grazing techniques (Grigulis *et al*. 2001), physical removal and herbicides can significantly reduce population size, however, population flowering time varies based on population location (see Chapter 4). In addition, as *E. plantagineum* uses rapid germination strategy (see Chapter 2), germinating seeds could be treated with a fast acting herbicide immediately after rain events to reduce population size with minimal risk to later germinating species.

6.3.2 Adjusting management programs for potential evolution

Historically evolution has been considered on a scale of millennia however research over the last decade has shown that evolution can be extremely rapid and many species are capable of rapid evolution within 10-100 years from establishment (e.g. Jain & Martins 1979; Rice & Mack 1991; Siemann & Rogers 2001; Leiss & Mueller-Scharer 2001; Franks et al. 2007). This rapid evolution has become increasingly important for land managers as the time frame for management decisions is usually a 100 year period (Thompson 1998) and does not generally account for possible evolution and range expansion beyond native climatic envelopes. For example, species distribution models, such as ecological niche models (ENM), assess the potential spread of invasive species through the assumption that the best indicator of climatic conditions is the distribution in their native range (Beaumont *et al*. 2009). However, there is increasing evidence that invasive species do not conserve their climatic niche during invasion of novel environments and they are able to establish,

grow and reproduce under novel climatic regimes (e.g. Siemann & Rogers 2001; Broennimann *et al*. 2007; Fitzpatrick *et al*. 2007; Beaumont *et al*. 2009). In their native range species may be subject to biotic constraints (such as competitors or pathogens), dispersal limitation or geographic barriers that reduce potential distribution (Beaumont *et al*. 2009) which may not be present in invaded environments. As a result, potential distribution, range expansion and future impacts of introduced species may be under estimated. Therefore, future management programs that depend on species distribution data must consider life-history trait divergence and evolutionary capacity to determine whether an exotic species will occupy a climatic niche beyond their predicted climatic envelope. This can be done through the inclusion of population distribution and flowering time surveys to ensure that evolution is accounted for and that control methods are conducted at the most optimal time to ensure maximum control effort for minimum cost.

6.4 Key areas for future research

From the conclusions of this thesis there are seven key areas that require further research:

1. **Have these changes in life-history traits increased the invasiveness of *E. plantagineum* arid or mesic environments?**

   My study provides evidence for the divergence of life-history traits throughout the lifecycle of *E. plantagineum*, however, it is unable to determine how these changes have influenced the invasiveness of this species. Further research is required to determine whether these adaptations may lead to range expansion in the future. This may have broader implications for invasions of *E.*

plantagineum around the world but may also provide an insight into the capability of other invasive species to adapt to local conditions.

2. Does the competitive ability of *E. plantagineum* vary across the range

This study identifies several traits that vary among arid and mesic populations, however, it was unable to determine the competitive ability of each population. The evolution of increased competitive ability hypothesis (EICA) suggests that invasive species that are released from natural enemies, such as herbivores or pathogens, will switch their resources from defence to growth (Blossey & Nötzold 1995; e.g. Messina *et al.* 2002; DeWalt *et al*. 2004; Fine *et al.* 2006). However, there is conflicting evidence for this hypothesis (e.g. Colautti *et al.* 2004; Joshi & Vrieling 2005; Handley *et al.* 2008) and *E. plantagineum* may provide another example where this hypothesis may not apply as a novel defence technique was observed during the trial (discussed in section 3.4.1 of Chapter 3) in populations across the arid-mesic gradient. Red trichomes were observed on the surface of the leaves on individual plants at multiple locations throughout the introduced range (e.g. Oberon, SEH bioregion and Mildura, MDD bioregion), suggesting that resources are not being allocated away from defence mechanisms in these locations however further work is required.

3. How will *E. plantagineum* populations cope with future changes in climate?

This study has shown that *E. plantagineum* is capable of population divergence in life-history traits over a relatively short period of time, however

does *E. plantagineum* have the capacity to rapidly adapt further changes in climate in the future? Due to the changes in climate predicted for Australia, range margin populations are going to be under pressure to either rapidly adapt, disperse to more favourable locations or perish. Populations in range margins are often limited from further range expansion by barriers such as geography, climate, genetic availability, resource availability or reduced population fitness (Bridle & Vines 2007). While *E. plantagineum* populations in range margins have adapted to local conditions it is unclear as to whether they are able to adapt further to escape changes in climate.

4. **How does the growth and reproductive strategy of *E. plantagineum* compare to its native range?**

Various studies have identified differences in native and introduced ranges in germination (e.g. Kudoh *et al.* 2007; Hierro *et al.* 2009), growth (e.g. DeWalt *et al*. 2004; Etterson *et al*. 2008) and reproductive (e.g. Leger & Rice 2007; Alexander *et al*. 2009) life-history traits. These studies have found that populations in the introduced range are often larger and more fecund than populations within their native range (Maron *et al*. 2004). While my study has found that *E. plantagineum* is capable of rapid adaptation to climatic conditions in its novel range, it would be interesting to determine the adaptive capacity of this species in its native range or other introduced populations. This would provide an insight in to the amount of adaptation that has occurred since introduction to Australia, the adaptive capacity of this species and potential future range distribution of native and invasive *E. plantagineum* populations.

5. What are the community impacts of *E. plantagineum* in Australian ecosystems?

How does *E. plantagineum* influence the population dynamics of communities that it invades? *E. plantagineum* is known to produce large leaves that smother surrounding species (Parsons & Cuthbertson 1992) and release toxins in to the soil to reduce competition (Weston *et al.* 2011). Do the adaptations identified in my study, such as smaller leaves in arid environments, allow it to develop faster and use resources before other species? Will *E. plantagineum* prevent adaptation or influence the survival of native species? This will have broad implications for communities that *E. plantagineum* has invaded and may invade in the future.

6. Implications for the adaptive capacity of native species

When adapting to local climatic conditions there are several ecological and genetic barriers that populations must overcome when adapting to local conditions. Ecological barriers may include environmental heterogeneity, habitat type, competition with surrounding species or human disturbance, while, genetic barriers may include low genetic diversity due to small population size, genetic bottlenecks or hybridization with surrounding species (Beaumont *et al*. 2009). My study has shown that plant populations of the introduced species, *E. plantagineum*, are able to develop clines in life-history traits throughout various life stages in response to climate suggesting that other native and invasive species will be able to adapt similar traits in comparable timeframes. This research will have broad implications for

conservation efforts in the future as the adaptive capability of species may determine their ability to survive climatic changes in the future.

7. Proof of natural selection

This study has been able to provide convincing evidence of rapid adaptation in flowering time and progeny seed mass. The length of the gradient, introduction history and high genetic diversity of *E. plantagineum*, suggests that it is unlikely that the development of these clines are the result of climate matching, however the only way to be definitely certain of the size and magnitude of selection pressures is to conduct a reciprocal transplant experiment (Montague *et al.* 2008). This would indicate the role of natural selection, local adaptation and phenotypic plasticity in the success of an invasive species in its novel range.

6.5 Final conclusion

The invasion of *E. plantagineum* in Australia has provided a unique opportunity to observe the adaptation of an invasive species to multiple novel environments along an arid-mesic gradient. This study has demonstrated the ability of an invasive species, *E. plantagineum*, to rapidly develop clinal patterns in life-history traits in response to key climatic drivers. The robust nature of *E. plantagineum* has enabled it to invade a wide range of climatically variable environments and adjust its life-history traits accordingly. This study has used a whole lifecycle approach to determine population differentiation of an invasive species over large arid-mesic gradient. This technique is a powerful tool to determine the ability of invasive species to adapt to local environmental conditions throughout its life, despite the challenges that accompany

the invasion of novel environments. This study highlights a requirement for the integration of evolutionary information into the assessment and prediction of invasive species.

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