Responding to change: phenotypic plasticity and local adaptation, case studies using native and invasive *Erodium* species

A thesis submitted for the degree of Doctor of Philosophy of The Australian National University

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Declaration

I declare that with the exception of technical advice from statistical and other technical experiments, occasional field and glasshouse assistance, advice from my supervisors on experiment design and key questions as well as assistance by supervisors and co-authors (where indicated) with editing chapters; the main chapters presented in this thesis represent my own work (i.e. approximately 90% of concepts for chapters, methodology, data analysis, and writing are attributable to me).

The three appendix chapters are all published papers of which I am a co-author and as such my contribution to these appendices is less than that for the main thesis chapters. My contribution to Appendix A: "*Plant phenotypic plasticity in a changing climate*" (a manuscript with eleven authors) was approximately 10 per cent contribution to ideas and six per cent contribution to writing of the manuscript. My contribution to Appendix B: "*Adaptive phenotypic plasticity and plant water use*" was approximately 40% contribution to ideas and writing, I also conducted the small meta-analysis in the paper. My contribution to Appendix C: "*Beware: alien invasion. Where to next for an understanding of weed ecology?*" was approximately 60% contribution to ideas and writing.

Signed: .... Date: 3/4/14

Amy Davidson.

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Abstract

Phenotypic plasticity is often postulated to play a role in plant invasions. This thesis explores the role of plasticity in plant invasions with particular emphasis on responses to water availability. The thesis employed two main approaches: a meta-analysis of published literature and the use of greenhouse experiments on populations of a congeneric native (*Erodium crinitum*) – invasive (*E. cictuarium*) species pair using seed collected along a natural rainfall gradient. The meta-analysis found that phenotypic plasticity is generally greater in invasive compared to non-invasive species. However, this higher plasticity did not always translate to a fitness advantage. In particular, the fitness was partially dependent on whether conditions changed from stressful to average or from average to favourable.

The first of three greenhouse studies assessed whether plasticity is highest in the invasive *Erodium* species and also whether it is highest in populations from the more heterogeneous site (dry site). While some support for the latter was found there was no evidence that the invasive species was more plastic. The study investigated how patterns of adaptive plasticity in the different populations and species change at different points along the water gradient.

Patterns of plasticity in the native and invasive *Erodium* species were also investigated with respect to responses to water dose applications. Halving the dose at which water was applied had a greater effect on the fitness proxies: seed number and total biomass, than did halving the total amount of water supplied. Consistent with the first greenhouse study, dry site populations displayed higher average seed number across all treatments than did wet site populations. However, the wet site populations grew faster. While higher plasticity in key traits resulted in higher fitness for seed number, homeostasis in total biomass was associated with lower plasticity.

The third greenhouse study investigated constraints on plasticity in key traits in response to water availability and competition. I expected that constraints would be greater when multiple stresses were present. However, my hypothesis was rejected. Although several constraints to plasticity were detected, such constraints were as common when only one stress was present as when both stresses were imposed.

The thesis discusses the findings of these studies in the context of our current knowledge on plant invasions and species adaptation to climate change. Differences between the findings of the meta-analysis in which invasive species were more plastic than co-occurring native species, and findings of the greenhouse study, in which little difference was detected between an invasive and native species pair are also discussed. The results suggest that when one controls for range size, invasive species are not inherently more plastic. Both the meta-analysis and the greenhouses studies also suggest that the shorter residency time of invasive species increases the probability that many plastic responses will be maladaptive. Indeed, the greenhouse studies found that species tended to respond adaptively to situations that were more relevant to their ecological context. For example, dry site populations displayed greater adaptive plasticity than wet site populations with respect to changes in water availability.

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Chapter 1 Introduction

1.1 Background

Invasive plants and climate change are two of the most significant threats to Australia's native and agricultural flora (Pittock, 2003). These threats will interact and together impact upon the plant communities of tomorrow, so plant characteristics shift to reflect the selective pressure of new, changed climatic regimes and competitive interactions (Dukes, 2007). Despite considerable scientific interest in understanding the mechanisms surrounding successful invasion of alien plants (Erfmeier & Bruelheide, 2004), there are currently few widely accepted theories regarding why invasive species are so competitive in their non-native ranges (Dietz & Edwards, 2006). Empirical tests of common theories (such as increased competitive ability due to enemy release and/or traits which enable higher resource capture) yield conflicting results (Dietz & Edwards, 2006, see *Table 1.1* for a list of common invasion theories). Plasticity so far, has received less empirical attention than other theories in invasion ecology, but is often proposed as a characteristic that enhances the ability of weed species to adapt to new climates in their invading regions. Improved knowledge about the mechanisms behind ecological invasions is critical to their successful management (Hill *et al*., 2005).

Phenotypic plasticity refers to the effect of the environment on an organism's morphological, anatomical and developmental expression (Scheiner, 1993; Schlitching, 1986). Studies have shown plasticity to be both heritable and in some cases adaptive (Bradshaw, 2006). Results from the literature tend to support the concept that invasive plants display higher plasticity (Richards *et al*., 2006) and certainly many invasive plants come from variable environments where plasticity may

Table 1.1 Common biological invasion theories.

Theory	Synopsis	Key reference/s
Fluctuating resources	Exotic species are more likely to invade when there is a change in the supply of resources in the site to be invaded	Davis *et al.*, 2000
Enemy release	Species which escape their natural enemies in the invaded region will have a competitive advantage over the native organisms.	Maron & Vilà, 2001; Keane & Crawley, 2002
Rapid evolution	Invasive species are able to evolve at super fast rates to adapt to changing conditions thus giving them an advantage over native species.	Bossdorf *et al.*, 2005
Botic resistance hypothesis	Biodiverse communities are more resistant to invasion because the use the available resources and niches more effectively than communities with low biodiversity.	Moulton & Pimm, 1983; Case, 1990; Kennedy *et al.*, 2002
Empty niche hypotheses	Invasive species have traits that are unique to the native community which enables the invaders to exploit 'empty niches'.	Darwin, 1859; Elton, 1958; Levine & D'Antonio, 1999; Fridley *et al.*, 2007
Invasion meltdown hypotheses	As more and more invasive species enter a system the native community reaches a threshold at which there is too much change and it can no longer resist the invaders.	Simberloff & Von Holle, 1999
Evolution of increased competitive ability	Species which have escaped their native enemies will evolve to increase their competitive performance.	Blossey & Notzold, 1995
Reproductive traits:	Reproductive characteristics of invasive species	Baker, 1965
Propagule pressure:	A major determinant of whether a pant will be invasive is number of propagules that are introduced at in anyone introduction event.	Lockwood *et al.*, 2005; Simberloff, 2009

be an advantage, however, there is limited empirical evidence linking plasticity with competitive or colonising ability (Alpert & Simms, 2002, Sultan, 2001).

Because phenotypic plasticity may enable organisms to modify their phenotype in response to changes in environmental conditions it may also play an important role in enabling sessile organisms such as plants to tolerate rapid climate change (Ghalambor *et al.*, 2007, Jump & Peñuelas, 2005). Indeed, plasticity may facilitate tolerance to changing climate conditions both in the short-term through plastic changes in the phenotype leading to tolerance of new environments as well as by facilitating rapid evolution of genotypes through selection on novel phenotypes expressed, at least initially, through plasticity (Nicotra *et al.*, 2010, Pfennig *et al.*, 2010). Similarly, it is often proposed that organisms from more heterogeneous environments will develop higher phenotypic plasticity in key traits than those from more stable environments (Sultan & Spencer, 2002, van Tienderen, 1991). Evidence for this hypothesis, however, is mixed, for example Sultan (2001) and Baythavong (2011) found higher plasticity in populations from the more heterogeneous site whereas Pohlman *et al.* (2005) did not. It is suggested that plasticity may not always be favoured, even in heterogeneous environments, if the environmental changes do not occur at a scale relevant to the organisms' lifecycle (Alpert & Simms, 2002) or if responding plastically incurs costs (DeWitt *et al.*, 1998).

Costs of plasticity may include information acquisition costs (producing the sub-optimal phenotype), developmental instability (large phenotypic variance resulting in lower average fitness, Scheiner *et al.*, 1991), production costs associated with the phenotypic change (sometimes argued to include the former two costs), maintenance costs and intrinsic genetic costs (epistasis and pleitropic effects), (DeWitt *et al.*, 1998, reviewed in van Kleunen & Fischer, 2005 and Auld *et al.*, 2010). Plasticity may also be constrained by phenotypic integration of traits (the phenomenon of functionally related traits to co-vary, Pigliucci, 2003, Schlichting & Pigliucci, 1998). Auld *et al.* (2010) suggest that costs of plasticity may be exacerbated under stressful conditions. Furthermore Valladares *et al.* (2007) suggest resource constraints may further limit plasticity, for example through greater phenotypic integration. However Funk (2008) and Baythavong (2011) found high levels of plasticity even in very resource limited conditions.

While significant progress has been made in understanding the role of adaptive phenotypic plasticity and its constraints, we are still a long way from being able to make general statements about the value of plasticity under different conditions. Or example, we know little regarding when plasticity is likely to be adaptive and in what conditions, when plasticity is likely to be limited nor how prevalent plasticity may be in different species / populations and traits (Nicotra *et al.*, 2010). Such information will be critical in helping to inform predictions of species responses to climate change, including the relative responses of invasive compared to non-invasive species. Comparisons of phenotypic plasticity in populations, species and traits require not only quantification of the plastic responses but more importantly assessments of its adaptive value under different conditions (Bradshaw, 1965). Unfortunately many studies do not provide such information. For example, although phenotypic plasticity in water-use-efficiency (WUE) (a presumably important trait for adapting to variable rainfall) has been measured in several studies, few assessed the adaptive value of this plasticity, impeding the ability to make ecologically useful predictions about the importance of plasticity in this trait (Nicotra & Davidson, 2010).

This PhD investigates the role of phenotypic plasticity in both plant invasions and adaptation to a range of conditions from stressful to highly favourable. In doing so, the work in this thesis further develops various methods for comparing trait plasticity, the adaptive value of this plasticity between populations and species as well as methods for understanding constraints (i.e. costs and limits) of this plasticity. The methods are applied to data from the published literature as well as my own empirical greenhouse experiments on a sympatric native and invasive *Erodium* species pair from south-eastern Australian. The experiments focus on responses to water availability as a decrease in annual rainfall and increase in the variability of rainfall is predicted for many Mediterranean and temperate regions (IPCC, 2007) including south-eastern Australia (Watterson *et al.*, 2007). I found general support in the literature for greater plasticity of invasive compared to co occurring native species. However, in my own experiments when range size was controlled I found little difference between the species. I did, however, find greater evidence of adaptive plasticity in plants sourced from the more heterogeneous dry site compared to those soured from the wet site.

1.2 Species selection for greenhouse studies

There are over 70 species of *Erodium* (Geraniaceae) worldwide, over 60 of these occur across the Mediterranean basin (Fiz *et al.*, 2006), where the species often occupy disturbed sites (Fiz *et al.,* 2008). Consistent with a pioneering role, many *Erodium* species are autogamic annuals and the majority can self pollinate (Fiz *et al.*, 2008). The characteristics of *Erodium* species make them successful pioneers, and the genera is found in temperate through to arid regions (e.g. see http://www.anthos.es/ for information on distributions in Spain) and in every continent except Antarctica. There are 34 species listed on the global compendium of weeds (http://www.hear.org/gcw/) of which 17 are declared “weeds” (as opposed to naturalized or occasional aliens). The short life cycles, ability to self and broad environmental ranges make the genera an ideal choice for studies of phenotypic plasticity.

The *Erodium* species chosen for my study were *Erodium crinitum* Carolin, which is native to Australia and *E. cicutarium* (L.) L'Herit. ex Aiton, which is native to the Mediterranean region (images of species at Figure 1.1). Both species are relatively common herbaceous annuals in temperate, semi-arid and arid southern Australia (Cox & Conran, 1996). The species co-occur across a broad rainfall gradient ranging from approximately 650 mm yr^{-1} in temperate regions down to less than 200 mm yr^{-1} in arid areas (see http://www.chah.gov.au/avh/ for distribution maps of both species, Figure 1.2 a, b).

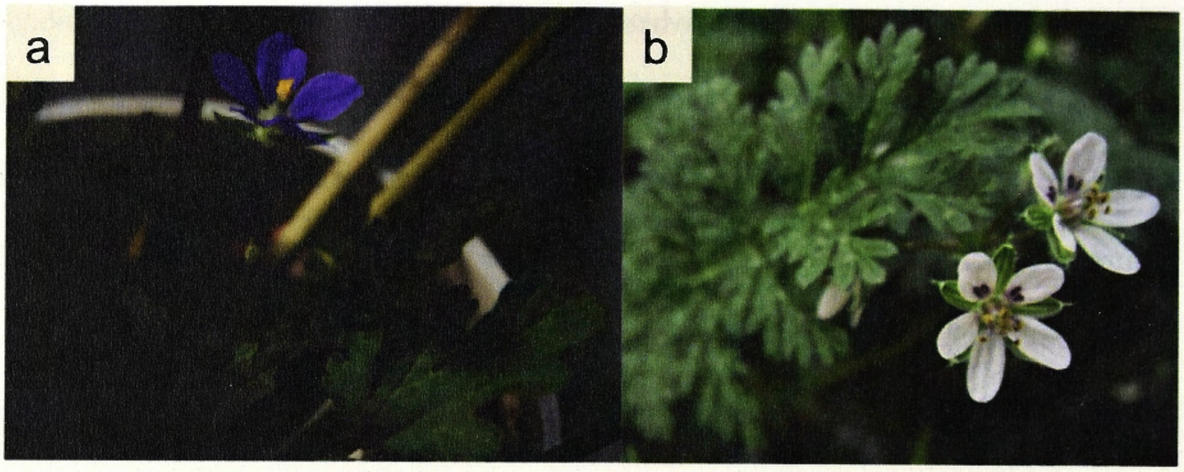

Figure 1.1 a) *Erodium crinitum* b) *E. cicutarium*.

Despite having several characteristics of a pioneering species and occupying disturbed sites in Australia, *E. crinitum* is not known to be invasive in any other country although it is reported to have naturalized in Japan (http://www.hear.org/gcw/species/erodium_crinitum/). The failure of *E. crinitum* to become invasive in Japan suggests the species can be labeled as non-invasive, noting that such a title can never really be provided with certainty given the well-documented cases of sleeper weeds. Comparisons of invasive species with native species whose invasiveness is unknown may result in erroneously comparing two invasive spices and thus impede the ability to capture information relevant to the invasive potential of a species (Burns & Winn, 2006).

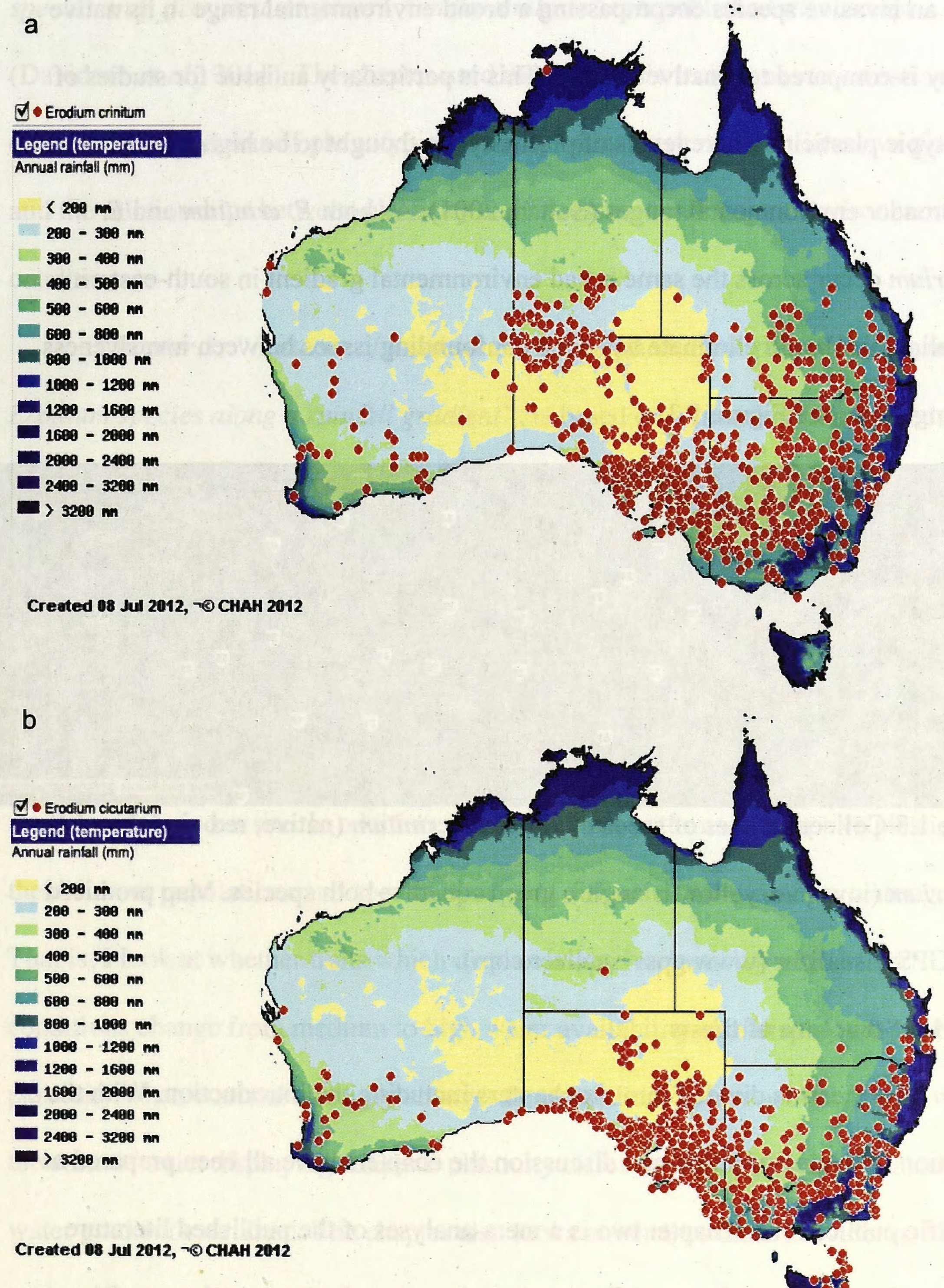

Figure 1.2 a) Distribution of *Erodium crinitum* (native) and *b) E. cicutarium* (invasive) in Australia. Maps created using Australia's Virtual Herbarium tool (www.cha.org.au).

Likewise, environmental range may also be confounded with invasiveness where an invasive species encompassing a broad environmental range in its native country is compared to a native species. This is particularly an issue for studies of phenotypic plasticity where levels of plasticity are thought to be higher in species with broader environmental ranges (Sultan, 2001). As both *E. crinitum* and *E. cicutarium* occur across the same broad environmental gradient in south-eastern Australia, this should eliminate any such confounding issues between invasiveness and range size, see Figure 1.3.

Figure 1.3 Collection sites of seeds of *Erodium crinitum* (native, red circle) and *E. cicutarium* (invasive, yellow triangle), green square = both species. Map produced from GPS visualizer (www.gpsvisualizer.com).

1.3 Structure of thesis

This thesis is divided into six chapters including the introduction. With the exception of the introduction and discussion the chapters have all been prepared as scientific publications. Chapter two is a meta-analyses of the published literature whereas chapters three, four and five are based on greenhouse experiments. In addition, three other manuscripts are provided as appendices. These appendices are literature reviews of which I am a co-author and are included as they provide relevant background information to the thesis topic. The appendices were written during my PhD candidature.

Chapter 2: *"Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis"* is a published manuscript (Davidson *et al.*, 2011). The chapter used meta-analysis techniques to investigate differences in patterns of phenotypic plasticity in invasive and non-invasive plants and the relationships between plasticity and fitness under different environmental conditions.

Chapter 3: *"Patterns of local adaption and phenotypic plasticity in sympatric Erodium species along a rainfall gradient"*, is based on a manuscript prepared for submission to Oikos. The chapter investigates patterns of phenotypic plasticity in response to changes in water availability between a native and invasive *Erodium* species as well as between populations of both species from the wet and dry ends of their range in south-eastern Australia. The chapter investigates whether plasticity is highest in the invasive species and also whether it is highest in populations from the more heterogeneous site. In addition, I describe how patterns of adaptive plasticity in the different populations and species change at different points along a water gradient. That is, I look at whether traits which display adaptive plasticity when water conditions change from medium to high water availability also display adaptive plasticity when water availability changes from medium to low availability or whether the suite of traits displaying adaptive plasticity differ at different segments along the water gradient examined. The chapter also looks at whether there is evidence for a trade-off between local adaptation and phenotypic plasticity.

Chapter 4: *"Winners and losers: A comparison of populations of native and invasive Erodium species' fitness under different water availability scenarios"*, is based on a manuscript prepared for submission to Oikos. This chapter looks at patterns of phenotypic plasticity under different water availabilities and water dose applications. The chapter investigates the effect of changing the frequency of the stressful and favourable treatments (where treatments involve changing only water availability, only water dose or a combination of both) on the adaptive value of plasticity in different traits. Furthermore the paper assesses the effects of these scenarios on two fitness proxies: total biomass and number of seeds.

Chapter 5: *"Adaptive responses to water availability and competition in native and invasive Erodiums - are two stresses one too many?"*. This chapter looks at patterns in costs and limits to adaptive phenotypic plasticity under different water availabilities and competition for light. As with Chapters 2 and 3 the differences between the invasive and native species and populations from the dry and wet end of the water gradient are investigated.

Chapter 6: *"Discussion"*, provides a discussion of key results and application of findings for future studies on phenotypic plasticity and invasiveness and the role of plasticity in adaptation of plants to climate change.

Appendix 1: *"Plant phenotypic plasticity in a changing climate"*, is a published manuscript in Trends in Plant Science (Nicotra *et al.*, 2010) of which I am a co-author. The manuscript outlines key concepts in phenotypic plasticity for the ecology and agricultural fields to consider in better understanding and preparing for plant responses to climate change.

Appendix 2: *"Adaptive phenotypic plasticity in plant water use"* is a published manuscript in Functional Plant Biology (Nicotra & Davidson, 2010) of which I am a co-author. The manuscript provides a literature review of our current knowledge regarding the adaptive value of phenotypic plasticity in water-use efficiency.

***Appendix 3:** "Beware: alien invasion. Where to next for an understanding of weed ecology?" is* an invited commentary *published manuscript in* New Phytologist (Davidson & Nicotra, 2012). The manuscript provides a review of Dawson *et al.*, (2012) but also outlines future directions for the study of phenotypic plasticity and the role of such plasticity in plant invasions and species adaption to changing climates.

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Chapter 2 Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis.

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Distribution of work

Professor Michael Jennions assisted with selection of appropriate meta-analysis statistics for the study. He also assisted by reading and commenting on final proofs for submission.

Dr Adrienne Nicotra contributed to forming the initial concepts for the paper and by reading and commenting on final proofs for submission.

In discussion with Adrienne I developed the ideas for the study, sourced all of the data, formatted the data, conducted all of the analyses and wrote the initial draft alone. I then worked with Adrienne and Michael to edit the final manuscript for submission. In addition, I received comments from three anonymous reviewers as well as the editor, Jessica Gurevtich, these comments helped revise the methodology and clarify the writing.

2.1 Abstract

Do invasive plant species have greater phenotypic plasticity than non-invasive species? And, if so, how does this affect their fitness relative to native, non-invasive species? What role might this play in plant invasions? To answer these long-standing questions we conducted a meta-analysis using data from 75 invasive/non-invasive species pairs. Our analysis shows that invasive species demonstrate significantly higher phenotypic plasticity than non-invasive species. To examine the adaptive benefit of this plasticity, we plotted fitness proxies against measures of plasticity in several growth, morphological and physiological traits to test whether greater plasticity is associated with an improvement in estimated fitness. Invasive species were nearly always more plastic in their response to greater resource availability than non-invasives but this plasticity was only sometimes associated with a fitness benefit. Intriguingly, non-invasive species maintained greater fitness homeostasis when comparing growth between low and average resource availability. Our finding that invasive species are more plastic in a variety of traits but that non-invasive species respond just as well, if not better, when resources are limiting, has interesting implications for predicting responses to global change.

2.2 Introduction

Phenotypic plasticity is defined as the change in phenotypic expression of a genotype in response to environmental factors (Bradshaw 1965; Schlichting 1986) and has been shown to have significant evolutionary consequences (Murren *et al.* 2005; Schlichting 2004). Plasticity is adaptive if the phenotypes produced in response to a change in the environment result in higher average fitness across both environments than either fixed phenotype would (van Kleunen and Fischer 2005).

The idea that high phenotypic plasticity has contributed to the success of invasive plants was proposed almost half a century ago (see Baker 1965). The theory makes intuitive sense because it is based on several plausible assumptions. First, an invasive plant usually arrives in a new area with few individuals (and generally relatively low genetic diversity) and faces an environment that differs from that in which it evolved. High levels of phenotypic plasticity should enable a colonising species to cope with, and become established, under these novel conditions (Schlichting and Levin 1986). Indeed, ecological breadth has been shown to be positively correlated with plasticity in some species (Sultan 2001; but see Pohlman *et al.* 2005). Second, the ability to take advantage of environmental fluctuations through adaptive phenotypic plasticity is likely to affect not only a plant's ability to become established in a new environment but also its ability to outcompete the existing vegetation, i.e. its success as an invader (Murray *et al.* 2002; van Kleunen and Richardson 2007).

In a manner analogous to the novel environmental conditions that are experienced upon invasion of a new habitat, increasing temperatures, higher CO_2 levels and associated climate changes over recent decades have introduced novel environmental conditions. This might favour more phenotypically plastic species (see Chown *et al.* 2007) and result in an increased competitive ability of invasive plants over co-occurring native, non-invasive species (Dukes 2007).

Many studies have suggested that invasive species have higher levels of phenotypic plasticity, but direct empirical tests of this theory are less common (Hulme 2008) and previous reviews have not achieved quantitative conclusions (see Daehler 2003; Richards *et al.* 2006). Richards *et al.* (2006) drew on Baker (1965) to pose three testable scenarios regarding the importance of phenotypic plasticity in plant invasions and the role of plasticity in maintaining fitness across different environments (*Figure 2.1*). These are that relative to a non-invasive species, an invasive species might be a:

a) "Master-of-some" if phenotypic plasticity in response to more favourable conditions enables a larger increase in fitness.

b) "Jack-of-all-trades" if phenotypic plasticity in response to stressful conditions enables greater fitness homeostasis (i.e. a smaller decline in fitness).

c) "Jack-and-master", when characteristics of a) and b) are combined such that phenotypic plasticity in response to more favourable environments enables greater fitness, and plasticity in response to more stressful conditions permits a smaller decline in fitness (i.e. greater fitness homeostasis).

It is now possible to gather sufficient data to empirically test the proposed scenarios about the relationship between adaptive phenotypic plasticity (*Figure 2.2*) and biological invasion. The power of the 'Jack-of-all-trades' and 'Master-of-some' scenarios is that they provide guidelines about the expected relationship between plant traits and fitness proxies (*Box 2.1*). Comparing the relationship between the degree of trait plasticity and the change in fitness proxies of the invasive plant with that of a co-occurring non-invasive plant in response to a given shift in resource availability enables us to quantify the relative importance of plasticity in providing invasive species with a competitive advantage over non-invasive species.

2.2.1 Hypotheses

In this study we conducted a meta-analysis to synthesise the published literature and test three specific hypotheses regarding the role of phenotypic plasticity in plant invasions:

1) that invasive species show greater phenotypic plasticity across various growth, morphological, physiological and fitness traits compared with co-occurring non-invasive species (tested using the "Overall plasticity analysis");
2) that increased plasticity in growth, morphological and physiological traits is correlated with *higher fitness gains* in invasives compared to non-invasives when plasticity is measured as the difference between a *high resource supply* environment and average conditions (tested using the plasticity and fitness analysis; *Figure 2.3*);
3) that increased plasticity in growth, morphological and physiological traits is correlated with *lower fitness losses* in invasives compared to non-invasives when *low resource supply* environment and average conditions (also tested using the plasticity and fitness analysis; *Figure 2.3*)

2.3 Methods

2.3.1 Data selection

We conducted a literature search on Web of Science and Cab Abstracts for the terms [invas* or nonnat* or non-nat* or alien* or weed or nonindig* or non-indig*] and [nat* or indig* or endemic]. All published records up until May 30 2009 were searched. We limited results to the topics of plant science and weeds. In addition a cited literature search on Richards *et al.* (2006) was conducted in Web of Science to the same date. Studies were then individually assessed and retained if the following conditions were met:

- The study included at least one non-invasive and one invasive species. Native species were all presumed to be non-invasive and hereafter are referred to under the general title "non-invasive". The authors' definitions of invasive were accepted in all cases as they did not vary greatly and generally related to nationally agreed criteria.

- Both the invasive and non-invasive species had to each be grown in at least two standardized conditions that differed in resource availabilities.
- Data could be obtained for the means, standard deviations and sample sizes for measurement of focal traits for both the invasive and non-invasive species in two or more conditions.

2.3.2 The Database

Suitable data were available from 46 studies measuring 362 individual traits across 150 species that were assigned into 75 species pairs (see Appendix S2.1). Within a given study, one or more pairs consisting of an invasive and non-invasive species were generated to maximise the phylogenetic relatedness within pairs. It was not possible to pair species across studies because the environmental conditions that were manipulated, and the traits that were measured differed greatly among studies. The pairing of species allowed us to investigate *differences* in plasticity between species that can more readily be attributed to invasiveness status because we have partially removed variation in phenotypic plasticity that is due to systematic differences among studies (e.g. methodology, exact levels of treatments). A database of all species pairs was created.

To construct the database of all species pairs, we first categorised the growing conditions into eight 'resource treatment' types based on analysis of the most common types used in the available studies. These were manipulation of: (1) nutrients (including different elements e.g. nitrogen, phosphorous etc); (2) light; (3) water; (4) competition or density; (5) disturbance; (6) CO_2 enrichment; (7) presence/absence of climbing substrate; and (8) presence/absence of soil biota and/or mycorrhiza. Second, we categorised the response traits for plasticity measures into 11 categories: (1) water use efficiency (WUE); (2) photosynthetic rate; (3) biomass; (4) relative growth rate (RGR); (5 and 6) tissue nitrogen or phosphorous content; (7) root biomass; (8) shoot length; (9) specific leaf area (SLA); (10) root to shoot ratio (R:S); and (11) nitrogen use efficiency (NUE). Third, we noted the extent of phylogenetic relatedness between the pair of non-invasive and invasive species, which we defined as: (1) congeners; (2) confamilial; or (3) less closely related. Fourth, we noted whether the growth-form of the invasive was: (1) herb; (2) grass; (3) shrub; or (4) tree or (5) vine. And fifth, we divided the invaded habitat into eight types: (1) grassland; (2) forest; (3) scrub-, shrub- or heath-land; (4) tropical forest; (5) wetland; (6) desert; (7) tree-shrub mix; and (8) disturbed land or agriculture.

As described above, if we had data on trait plasticity for more than one invasive and one non-invasive species in a given study, species were paired to provide the closest phylogenetic matches between the invasive and non-invasive species. In each study a species was only represented in a single species pair. Of the 46 available studies, five had two species pairs and eight had more than two species pairs. We treated species pairs as independent data points for the purposes of most analyses. In so doing, we make the reasonable assumption that there is no systematic variation among studies in the likelihood that they will report greater plasticity in invasive than non-invasive species. Biologically, this assumption is well justified because plants of different species were grown separately and were not in competition. The only exception is seven field-based studies in which natural competition occurred.

Likewise, when two or more types of environmental manipulations were imposed on the same species pair, we calculated separate effect sizes for each resource treatment. Of the 46 studies, 14 studies measured responses to two treatments and 10 studies to more than two treatments. Finally, we calculated separate effect sizes for each response trait for each species pair for each treatment type. Thirteen studies recorded two response traits per species pair per treatment and 23 recorded three or more response traits.

Plasticity was not explicitly measured or specifically reported in the majority of the studies used in our meta-analysis as the traits were originally measured for other purposes. This has the advantage of avoiding any publication bias directly associated with our main hypothesis (i.e. towards only publishing positive results). This should reduce any associated "file drawer" problems (Rosenthal 1979). It does not, however, avoid the issue of a 'research bias' whereby data is more often collected from certain species (Gurevitch and Hedges 1999). For example, the available species pairs might over-represent commercially important invasive species (although inspection of our species list does not support this claim) or be biased towards species which are more easily grown in greenhouse experiments (e.g. short-lived herbs and grasses). As with most research fields, these caveats about the availability of data in the current literature should inform interpretation of our meta-analyses (Jennions *et al.* in press).

The available studies rarely used clones or full-siblings so genotypes *per se* were imperfectly replicated across experimental environments. Phenotypic difference between treatments could therefore be due to both genetic differences in plants assigned to each treatment and phenotypic plasticity. If, however, plants from a given population (or species) express consistently different phenotypes in the different environments, phenotypic plasticity can still be analysed. Clearly, the resultant plasticity estimates will have greater uncertainty than those based on measurement of replicated genotypes (see Funk 2008). On the other hand, however, the use of only a few genotypes could result in a poor sample of the available mean level of plasticity within a species if there are moderate to high levels of genotypic variation in phenotypic plasticity within a species. Perhaps most importantly, however, there are no obvious bias in how individual plants were assigned to growing treatments (i.e. no propensity to assign certain genotypes to specific treatments), so there should be no systematic bias in the resultant measure of the difference in plasticity.

2.3.3 Data analysis

2.3.3.1 Calculation of effect size for plasticity and general analysis

A commonly used effect size is Hedges' *d* (i.e. *J*-corrected Hedges' *g sensu* Rosenberg *et al.* 2000). This is the difference between two means divided by an estimate of their pooled standard deviation, using *J* to correct for any bias due to low sample size (equations in Rosenberg *et al.* 2000). We first calculated Hedges' *d* for a specific trait and specific treatment for each species. In other words, how much did the measured trait differ between the two environmental treatments when expressed in the unitless measure of standard deviations? We used the *absolute* value of Hedges' *d* as our measure of the plasticity for a given species (hereafter we refer to $|d|$ which is a species' effect size for plasticity, as "*P*"). We calculated the absolute difference in means rather than the difference in any one direction (e.g. *P* = high resource mean – low resource mean) for two reasons. First, because an adaptive trait change in a given environment could require either an increase or a decrease in trait value. For some environmental changes there might even be multiple adaptive response strategies such that a shift in some traits may potentially be adaptive in either direction (e.g. fast growth in response to a drought to reach reproductive maturity quickly or slower growth to prolong lifespan). Second, Hypothesis 1 is simply whether invasive species are more plastic than non-invasive species. As such it is essential, by definition, to compare the absolute change in phenotype between invasive and non-invasive species. We calculated P using the mean and standard deviation for each treatment extracted from summary tables, figures (using Graphclick, Arizona Software, 2008) or by contacting the author directly.

Next, to test whether invasive species have higher phenotypic plasticity than non-invasive species we calculated an effect size for the difference in plasticity between a pair of invasive (i) and non-invasive (n) species ($P_{diff} = P_i - P_n$). The effect size we used to calculate the difference in plasticity for each focal trait differs from the type that the reader might be familiar with because it is the *difference* between two effect sizes, rather than the difference in means from two populations (Hedge's *d* – used for our estimate of P_i and P_n – are examples of the latter, Borenstein *et al.* 2009). An example of the type of effect size we used is Cohen's *q* which is the difference between two effects size that are Fisher Z_r transformations of correlation coefficients. The variance of the difference between two uncorrelated effect sizes (e.g. our P_{diff}) is simply the sum of their variance (see Borenstein *et al.* 2009, p228). If the value of $P_{diff} > 0$ it indicates that the invasive species showed a greater degree of phenotypic plasticity (i.e. P_i, the absolute change in phenotypic means between the two treatments) than the non-invasive species.

We calculated the mean value of P_{diff} in Metawin 2.0 using a random effects model. Given modest to low sample sizes when data was subdivided to consider specific treatment types or focal traits we used the non-parametric, bias-corrected, bootstrapped 95% confidence interval to assess the significance of results. If the confidence interval for P_{diff} excluded zero, we consider the effect to be significant at the 0.05 level. For the overall plasticity analysis we tested whether invasive species display higher average phenotypic plasticity than non-invasives (hypothesis 1) by calculating the mean value of P_{diff} for all available traits and assessing whether it was significantly greater than zero. We also calculated the total heterogeneity (Q_T) in effect sizes. Although the probability test associated with Q_T is not relevant for a random effects model, it helps highlight which models might benefit from the inclusion of moderator variables that could account for variation in P_{diff}. We assessed the significance of five moderator factors: response trait type, treatment type, growth-form, invaded habitat type and level of relatedness using the test statistic $Q_{between}$ (Q_b) with the associated *P*-value as a guide (for details see Gurevitch *et al.* 2001; Borenstein *et al.* 2009).

2.3.3.2 Correction for possible non-independence of effect sizes

Some species pairs contributed two or more effects sizes (P_{diff}) because more than one trait was measured and/or more than one treatment applied to the same pair. We therefore pooled effect sizes where possible to reduce the potential non-independence. The extent to which we could pool effect sizes varied with the moderator variable (trait, treatment-type, relatedness of invasive and non-invasive, invaded habitat type and growth-form). To assess the influence of trait type we were unable to pool the effect sizes and thus used the individual species pair trait responses (hereafter referred to as "individual traits" analysis, *Figure 2.4a*). For the assessment of the effect of treatment on the relative plasticity of invasive and non-invasive species we pooled traits within treatments for each species pair (hereafter referred to as the "treatment mean" analysis, *Figure 2.4b*). For the remaining moderator variable categories (growth-form, invaded habitat type and relatedness of the invasive and non-invasive) we calculated a single composite effect size for each species pair by pooling effect sizes across traits and treatments (hereafter referred to as the "species mean" analysis, *Figure 2.4c*).

Although we assume, based on how plants were grown, that estimates of the species mean for different species pairs are independent when measured in the same study, we did conduct a sensitivity analysis to test this assumption. To do so, we repeatedly calculated the mean value of P_{diff} after randomly selecting a single P_{diff} estimate from each of 46 studies (hereafter this analysis is referred to as "random trait" analysis). We then ran 50 iterations of the randomization process to calculate the 95% confidence interval for the mean. Additional iterations were unnecessary due to the small range in the resultant estimates of the mean for P_{diff}.

We compared the summary statistics for P_{diff} for all four analysis types: individual traits, treatment means, species means and random trait. The outcome was qualitatively very similar (see Results). This justifies retention of an analysis based on P_{diff} of the individual traits because presenting trait-specific results is more biologically informative. It should be noted that the use of a composite effect size to remove non-independence between P_{diff} estimates requires the assumption that the estimates are all measuring the 'same' general property of interest. So, for example, a difference in plasticity between an invasive and non-invasive species of the same magnitude is treated as equivalent estimate of plasticity regardless of whether the trait is photosynthetic rate or biomass. Although this might seem unwarranted it is consistent with there being no *a priori* reason to weight some traits as more important than others and the main conceptual task being quantification of overall phenotypic plasticity.

To calculate the pooled mean effect sizes discussed above (species mean and treatment mean) we first calculated the weighted mean for P_{diff} for each species using the appropriate set of P_{diff} estimates. We used a standard fixed effects model so that it was also possible to calculate the variance of P_{diff} taking into account any correlation between effect sizes (Mengersen & Jennions in press). For the variance of this mean, we calculated the variance of the mean of X_i effects each with a variance of V_i and V_{ij} where V_i and V_{ij} represent the covariance between X_i and X_j, and r_{ij} represents the correlation between X_i and X_j (only one combination of a given pairing is required) as:

$$V_{mean} = (\sum_{i=1}^{m} V_i + 2\sum_{i,j} V_{ij}) / m^2 = (\sum_{i=1}^{m} V_i + 2\sum_{i,j} (r_{ij}\sqrt{V_i}\sqrt{V_j})) / m^2 \qquad \text{(Eqn 1)}$$

(Modified from Borenstein *et al.* 2009, p228)

If the variance differed between effect size estimates, we multiplied each V_i in the first sum by $(W_i/Wmean)$ and each term in the second sum by $(W_{i*}\ Wj)/W_i\ Wj$ *mean* (i.e. give greater weighting to estimates from traits with lower variances) ($W_i = 1/V$). The correlation between different traits is usually unknown, so we calculated two values where we either set $r = 0$ (i.e. assumes each trait provides a fully independent estimate of phenotypic plasticity for the species pair in question) or $r = 1$. This acts as a form of sensitivity analysis bracketing the upper and lower boundaries. For calculation of means and standard deviations and confidence intervals we report only the results of $r=1$ as this is the more statistically conservative measure.

The role of the five categorical moderators (trait, treatment type, relatedness of the invasive and non-invasive species, invaded habitat type and growth-form of the invasive) that might account for variation in effect sizes were investigated by calculating Q_b. The P-values for Q_b were Bonferroni adjusted for these five tests so that $P_{critical} = 0.05/5 = 0.01$.

2.3.3.3 Plasticity and fitness analysis

We tested hypotheses 2 and 3 by assessing the relationship between phenotypic plasticity in growth, morphological and physiological traits (hereafter referred to as focus traits) and our fitness proxies. Each study was examined individually to determine whether it included any measures that could be used as fitness proxies. We considered variables directly related to fecundity (number of seeds or flowers, reproductive biomass, germination rates of second generation), survival and biomass to be indicators of fitness. Total biomass was used as a fitness proxy because greater vegetative size is often associated with higher reproductive output (Weiner *et al.* 2009). The final set of focus traits used to assess plasticity for the plasticity and fitness analysis were: WUE, tissue nitrogen content; tissue phosphorous content, shoot length; SLA; R:S; and NUE.

The available data meant that the original hypotheses of Richards *et al.* (2006) had to be simplified. We substituted 'resource availability' for an environmental gradient progressing from stressful to favourable. In many cases data was only available for two levels of resource availability, whereas the hypotheses originally proposed by Richards *et al.* (2006) require data from at least three points along an environmental gradient. Resource levels were used to distinguish environments rather than the categories: "favourable" and "stressful", as the former can be objectively defined and are independent of the species-specific responses (e.g. high light could, depending on the species, be either favourable or stressful). As described below, comparisons were dependent upon both species responding in the same direction to the change in resource availability.

When a study compared average to high resource availability the situation was classified as a Master-of-some scenario (hypothesis 2). If a study compared low and average resource availability the situation was classified as a Jack-of-all-trades scenario (hypothesis 3). The original hypotheses included a third strategy of 'Jack-and-master' that we were unable to test because it requires at least three points along a resource gradient and there are very few such studies available that met our initial conditions for inclusion in the database.

"Average" conditions were based on information provided in the original paper. Interpretation was relatively simple for field-based and common garden studies as meteorological data was usually available to demonstrate which year represented average conditions for climatic treatments. For field nutrient, competition and other treatments representative sites were used and similar sites modified for the elevated and/or reduced resource treatments. For glasshouse studies we were more reliant on the author's interpretations. For example, average conditions for water or nutrient treatments in glasshouses often required authors to decide what soil moisture availability or nutrient level represented average field conditions. For nutrients this often involved fertilizer being added to obtain similar nitrogen/phosphorous levels to field soils although many authors used actual field collected soil. When such soil was unmodified we assumed this represented the "average" condition. In general the definition of average conditions was explicitly specified in the methods sections of the papers. If it was not possible to determine 'average' conditions, the studies were omitted from the plasticity and fitness analysis.

We calculated Hedges' *d* for each species for each fitness proxy as the difference between the average and altered resource treatment (i.e. either higher *minus* average or lower *minus* average). An effect size for each fitness proxy (P_{diff_fit}) for the species pair was then calculated as the difference in the value of Hedges' *d* between the paired species (i.e. invasive *minus* non-invasive species). P_{diff_fit} is a measure of the extent to which invasive and non-invasive species differed in the fitness change that occurs following an environmental change. If $P_{diff\_fit} > 0$ then the invasive species had a larger fitness gain in response to an *increase* in resources than the non-invasive species *or* a smaller reduction in fitness in response to a *reduction* in resources. We only calculated P_{diff_fit} for a species pair if both species responded in the same direction to the change in resource availability. This ensured that the favourable environment was the same for both species enabling a meaningful comparison of P_{diff_fit}. Furthermore, this approach ensures that a resource increase is synonymous with a shift to a favourable environment, and a resource decrease to a shift to a stressful environment.

For the analysis of responses to an increase in resources, we had P_{diff_focus} estimates from 36 studies of 59 species pairs representing 182 species pair trait combinations. Data to calculate P_{diff_fit} was available from 24 studies representing 34 species pairs and 45 different species pair fitness trait combinations. For the analysis of a response to a decrease in resources, we had P_{diff_focus} estimates from 22 studies of 26 species pairs representing 86 different species pair trait combinations. Data for P_{diff_fit} was available from 13 studies representing 16 species pairs and 18 response traits. As with the overall plasticity analysis we analysed data using the individual traits as well as the species means for P_{diff_focus} and P_{diff_fit}.

Separate scatter plots of P_{diff_fit} on P_{diff_focus} were generated for each resource scenario using those species pairs for which both P_{diff_focus} and P_{diff_fit} could be calculated. This analysis used lower level trait estimates rather than species pair means to ensure a sufficient sample size ($n = 52$ and 24 to test hypotheses 2 and 3 respectively)). For hypothesis 2 and 3 to be supported the majority of the data should fall in the upper right quadrant of the correlation graph. This is consistent with the invasive species showing greater plasticity ($P_{diff\_focus} > 0$) coupled with the appropriate fitness response (i.e. $P_{diff\_fit} > 0$ so either a bigger increase in fitness in response to added resources or smaller decrease in fitness in responses to a decline in resources for invasive than non-invasive species).

2.4 Results

2.4.1 Overall plasticity analysis

The summary effect size for the analysis using the individual traits, treatment means, species means, and random trait analysis all yielded highly concordant results. In all cases, there was a significantly positive mean for P_{diff} indicating that invasive species have greater phenotypic plasticity. The estimated mean ranged from P_{diff} = 0.42 to 0.67 standard deviations (*Table 2.1*). Using multiple data-points from some studies therefore did not have a strong effect on the estimated mean.

Invasive species had greater phenotypic plasticity for all 11 traits (*Table 2.1*). Furthermore, the 95% bootstrap confidence intervals indicated that the effect size was significantly positive for five traits: WUE, biomass, root biomass, R:S ratio and NUE, and only marginally non-significant for photosynthesis ($P \approx 0.05$, *Figure 2.5, Table 2.1*). The magnitude of the difference in phenotypic plasticity between invasives and non-invasive species did not depend on which of the 11 response traits was measured ($Q_B = 3.13$, df = 10, $P = 0.978$).

The effect of treatment type was examined using the treatment means (excluding treatments with fewer than five effect size estimates). Treatment explained a reasonable amount of the heterogeneity in effect sizes ($Q_B = 10.97$, df = 3, $P = 0.012$), and was only marginally non-significant if multiple testing was taken into account ($P_{critical} = 0.01$). The mean effect size was positive for all four treatments, and significantly so for light and water (*Table 2.2*), meaning the greater overall P_{diff} was not biased by certain treatment types but rather invasive species were more able to respond plastically to a suite of environmental changes.

Finally, using the species means we tested whether P_{diff} estimates differed depending on the plant growth-form, invaded habitat type or the level of relatedness between the invasive and non-invasive species. None of these three moderators explained a significant portion of the heterogeneity in effect sizes (Growth form: $Q_B = 1.84$, df = 4, P = 0.765, Habitat: $Q_B = 5.77$, df = 4, P = 0.217 and Relatedness: $Q_B = 2.02$, df = 2, P = 0.363) (*Table 2.3*).

In sum, irrespective of how the data was subdivided or the level of analyses used to estimate the mean effect size, the consistent conclusion was that invasive species show greater phenotypic plasticity ($P_{diff\ fit} = 0.4$ to 0.7) than non-invasive species. Hypothesis 1 was therefore supported.

2.4.2 Plasticity and Fitness analysis

2.4.2.1 Response to an increase in resources

The subset of data available to test the response to an increase in resources (hypothesis 2) was consistent with the finding that invasive species are more plastic in focus traits than non-invasive species (mean $P_{diff\ focus} > 0$; *Table 2.4*). The 'Master of some' hypothesis predicts that higher plasticity in invasive species is associated with a greater increase in fitness in response to an increase in resources (hypothesis 2). This should result in the majority of data being in the upper right quadrant of the scatter plot. In our analyses, however, the greater plasticity of invasives did not correlate with greater improvements in fitness. There was a weak trend (i.e. $P_{diff\ fit} > 0$) but invasive species did not display a significantly higher average gain in fitness than non-invasive species (*Table 2.4*). Even if the analysis is confined to the 57% of cases where the invasive species showed greater plasticity (i.e. $P_{diff\ focus} > 0$), these are almost evenly divided into those where $P_{diff\ fit}$ is above and below zero (*Figure 2.6a*). The Master-of-some hypothesis was therefore not supported.

2.4.2.2 Responses to a decrease in resources

The Jack-of-all-trades hypothesis predicts that invasive species show both higher plasticity in focus traits and a smaller decline in fitness in response to a reduction in resources (hypothesis 3). This should result in most data being in the upper right quadrant of the correlation graph. Again, the subset of data used to test the response to a decrease in resources agrees with the wider pattern that invasive species are more plastic than non-invasive species. The mean estimate of $P_{diff\ focus}$ was only slightly lower than that from the full dataset, although it was marginally non-significant ($P \approx 0.06$, probably due to the modest sample size; *Table 2.4*). In contrast, the mean value of $P_{diff\ fit}$ was significantly *less* than zero, indicating that invasive species had a significantly *larger* decline in fitness than non-invasive species when resources were reduced (*Table 2.4*). This can be interpreted as meaning that non-invasive species show greater fitness homeostasis. Even if the analysis is confined to the 63% of cases where the invasive species showed greater plasticity, in most cases $P_{diff\ fit}$ is still less than zero (*Figure 2.6b*). We can therefore actively refute the Jack-of-all-trades hypothesis.

2.5 Discussion

Our results indicate that invasive species do show greater phenotypic plasticity than non-invasive species. Perhaps surprisingly, this conclusion is not dependent on the type of response trait measured or the level of relatedness between invasive and non-invasive species. The higher plasticity of invasive species only sometimes resulted in their showing a greater gain in fitness than non-invasive species in response to a resource increase. In contrast, non-invasive species were more likely than invasive species to show an adaptive fitness response – that of fitness homeostasis – in response to a decrease in resources.

2.5.1 Overall plasticity

Our meta-analysis strongly supports the general claim that invasive species are more phenotypically plastic than co-occurring non-invasive species across a wide range of growth, morphological, physiological and fitness related traits across several different types of environmental variation (*Figure 2.5*). This result is concordant with the argument that pioneering plants and plants that colonise new sites have inherently higher phenotypic plasticity than later settlers (Bazzaz 1979, 1996). The degree of phylogenetic relatedness between matched pairs of invasive and non-invasive species did not significantly affect our estimate of the effect of invasiveness on plasticity (*Table 2.3*). The most tightly controlled analysis is based on studies where species were paired at the genus level, and this produced the largest effect size estimate. The higher observed plasticity of invasive species is therefore not due to a taxonomic bias with invasive species being preferentially derived from clades with a tendency to show greater phenotypic plasticity than those from which non-invasive species were sampled.

When traits were assessed separately, invasive species had significantly higher phenotypic plasticity than non-invasive species for 6 of 11 traits, and the trend was in the same direction for all 11 traits (*Figure 2.5, Table 2.1*). This is an interesting result given that it is generally argued that phenotypic plasticity is trait specific (Givnish 2002). Superficially this finding may suggest that the use of a composite measure of phenotypic plasticity (e.g. the species means used here) or a single trait can provide an effective indicator of relative differences in plasticity. We urge caution in extending this result, however, as it is likely to be dependent on the traits chosen being relevant to the specific nature of the investigation (e.g. treatment imposed, experiment set-up and species).

2.5.2 Plasticity and fitness analysis

It is difficult to draw conclusions about the adaptive significance of phenotypic plasticity, especially with respect to its role in successful plant invasions, unless the fitness consequences of phenotypic changes are measured (Nicotra & Davidson 2010; Richards *et al.* 2006). To this end we tested the hypotheses of Richards *et al.* (2006) and Baker (1965) that greater plasticity provides a fitness advantage to invasive species in response to environmental changes. We found that despite invasive species generally showing greater phenotypic plasticity in focus traits when resource availability increased (a substitute for the Master-of-some scenario) this higher plasticity did not correlate with greater fitness gains.

When resources shifted from average to lower levels (the Jack-of-all-trades scenario), invasive species were still more plastic than non-invasive species ($P \approx 0.06$) but they rarely showed greater fitness homeostasis than non-invasive species as predicted by the Jack-of-all-trades hypothesis. In fact, non-invasive species were significantly *more* likely to demonstrate fitness homeostasis indicating the generally superior relative response of non-invasive species to poorer conditions. This is a surprising result, but provides a message of hope for restoration efforts and future scenarios under climate change. Of course, this presupposes that the absolute fitness (i.e. population growth rate) of invasive and non-invasive species is fairly similar under average conditions (because effect sizes measure the change in fitness relative to that under average conditions).

Greater fitness homeostasis by non-invasive species under more stressful conditions where resources are limited is in line with Grime (1979)'s "stress tolerator" plants. However, the C-S-R strategy (Grime 1979) is generally interpreted as suggesting that plants with a "stress-tolerator" phenotype will be characterised by relatively low levels of phenotypic plasticity. Indeed, the notion that plants are unable to allocate resources to change their phenotype in response to an environmental change when resources availability is, on average, low is common in the plasticity literature (e.g. see deWitt *et al.* 1998). However, Funk (2008) reported high phenotypic plasticity in both native, non-invasive and invasive plants from environments which on average are resource-limited, likewise, our plasticity values were of a similar magnitude for the response to an increase as for the response to a decrease in resources for both the invasive and non-invasive species.

2.5.3 Data considerations

Although our results do not support the hypothesis that greater plasticity confers a fitness advantage to invasives, there are some caveats about our assessment of the relationship between plasticity in focus traits and fitness. First, in most studies, plants were grown in individual pots in the absence of competition. Second, we considered only the relative change in trait values, with no correction for any difference in mean trait values between invasive and non-invasive species. A recent meta-analysis of several of the traits measured in our meta-analysis found that, on average, invasive species showed higher mean values than pair-matched non-invasive native species (van Kleunen & Weber *et al.* 2009). For example, invasive species were larger and had higher SLA values. These larger trait values could mean that even when the relative fitness response to resource change is smaller in response to a resource increase (or the decline is greater in response to a resource decrease) for the invasive species compared to the non-invasive species, the average net fitness of the invasive might still be higher. It seems plausible that the combination of higher means and greater plasticity in many traits for invasive species could confer a significant fitness advantage when competing for resources (particularly when they are non-limiting), but that in the absence of competition this advantage is minimal.

We can identify two further constraints that limit our current ability to fully test the hypothesis which future research could alleviate. First, we did not exclude traits based on any *a priori* judgements (e.g. mechanistic models of plant function) about whether they were likely to display adaptive plasticity to a specific resource treatment. It is unlikely that plasticity in all traits is adaptive, and it has been suggested that the majority of phenotypic plasticity is actually selectively neutral (van Kleunen and Fisher 2005). Neutral plasticity can arise from environmental constraints that limit trait expression (Scheiner 1993) but do not affect fitness, or from linkage with other traits. Such linkage of traits is unimportant if the change in the linked trait is also adaptive but can complicate matters if it is not. Inclusion of all measured traits, even if they actually display neutral plasticity, was however, appropriate in both our overall plasticity analysis and our plasticity and fitness analyses to avoid introducing potential bias. In addition, the available data are limited so reducing the number of traits examined would have resulted in tests with very low statistical power. Future studies should therefore focus on measuring plasticity in traits that are assumed to have a strong effect on fitness, which ideally should be empirically tested.

Second, fitness proxies in our analysis were related to biomass or fecundity-related measures. Only 15% of studies provided any information on reproductive output or performance of the second generation (see Appendix S2.1). The paucity of accurate measures of fitness is a widespread problem in most areas of evolutionary ecology (review: Hunt & Hodgson 2010). Baker (1965, 1974) actually proposed that higher plasticity confers an advantage to invasive species over non-invasive species through seed production (larger increases in production in response to favourable conditions and lower relative decreases under more stressful conditions). It was not possible to directly assess Baker's 1965 proposal as very few studies measured seed production. Such an enquiry would be valuable, however, given the importance of propagule pressure in many invasion processes (Burke and Grime 1996; Brown *et al.* 2003; Davis *et al.* 2000; Lockwood *et al.* 2005).

In addition to methodological constraints, the results of our fitness and plasticity analyses might be partially explained by the theory that higher plasticity evolves in populations of an invasive species after colonisation of the novel habitat rather than being a characteristic that preselects species as potential invaders (see Richards et al 2006, Bossdorf *et al.* 2005 and refs within). It is usually proposed that the evolutionary response would be very rapid and occur during the establishment stage (Richards *et al.* 2006). It is possible, however, that evolution of higher phenotypic plasticity could occur at a more moderate pace, or that higher plasticity evolves rapidly but that selection against traits that display maladaptive plasticity takes longer. This could explain why non-invasive species showed less plasticity but the plasticity that was expressed was more often associated with a smaller decline in fitness (i.e. non-invasives had a greater proportion of adaptive plasticity) compared to invasive species in response to a reduction in resources (*Figure 2.5a, b*). The invasion histories of all the species used in this analysis are unknown but such information could enable analyses of the relationships between an invasive species' residency time, and the proportion of phenotypic plasticity in key traits that are an adaptive response to changes in resource availability. In addition, direct comparisons of phenotypic plasticity in invasive species between their native and introduced ranges could help to resolve how often there is evolution of adaptive plasticity post-colonisation by invasive species.

2.5.4 Implications under climate change

The results of the fitness and plasticity analyses have important implications for predicting how invasive and non-invasive species might respond to projected climate changes and rising CO_2. It has been suggested that adaptive phenotypic plasticity confers greater tolerance to changing conditions (Ghalambor *et al.* 2007), either by enabling species to tolerate new environments and persist long enough to adapt, or by directly facilitating evolution through genetic assimilation (Waddington 1951, West-Eberhard 2005). Indeed, recent studies on arthropods (Chown *et al.* 2007) and birds (Charmantier *et al.* 2008) suggest that higher phenotypic plasticity is advantageous in tolerating novel conditions associated with climate change. As demonstrated in *Figure 2.6*, however, showing greater plasticity did not always elevate relative fitness.

Some environmental changes, such as higher CO_2, lead to increased resource availability. Our analyses suggest that invasive species were marginally more likely to respond with adaptive plasticity to such an increase (*Figure 2.6a*). Even if the higher plasticity of invasive species in response to increased resources only resulted in a greater fitness increase than that seen in non-invasive species half of the time, this suggests that we have an increasingly large weed problem on our hands. However, other global climate changes will create more stressful environments (e.g. increased rainfall variability) favouring species that maintain fitness homeostasis. This ability was more often seen in non-invasive than invasive species (*Figure 2.6b*). Of course, species that exhibit an adaptively plastic response to both favourable conditions and greater environmental stresses should thrive, particularly under climate change. There is, however, little evidence for species that display such a Jack-and-master phenotype (Richards *et al.* 2006).

2.5.5 Future Directions

Plasticity studies in glasshouses and field/common garden studies can yield important and relevant information for management of agricultural and conservation areas, but only if the manipulation of resource conditions is biologically meaningful. Studies are most useful when multiple conditions are selected that represent a range of resource levels that span very favourable to highly stressful resource levels. Ideally the extremes should include levels predicted to be potential future conditions (Hulme 2008; Schlichting 2008). This is particularly important if we are to make inferences about the value of phenotypic plasticity and its effect on species performance under future environmental conditions - due to greater urbanisation, climate change or increased carbon dioxide levels (IPCC 2007). Such information would enable better assessment of the hypotheses of Richards *et al.* (2006).

As discussed above, our analyses of how plasticity affects fitness have to be interpreted with caution due to the limited availability of appropriate fitness data. It is major challenge to decide what measurement to use as a proxy for fitness, or whether the same trait instead should be assessed for adaptive plasticity. Without good fitness measures it is impossible to know if phenotypic plasticity in focal traits is adaptive. Future studies should therefore include explicit measures of fitness (albeit in variables based on measures of proxy traits). In the case of annual and short-lived perennial plant species estimates of fitness based on a plant's lifetime fecundity and, if possible, offspring viability would be particularly valuable.

2.5.6 Conclusion

Our meta-analysis indicates that invasive species generally have greater phenotypic plasticity than co-occurring non-invasives. This result is consistent across several traits and a range of resource conditions and is robust to the accuracy with which pairs of species are matched based on phylogenetic relatedness. Even so, the extent to which this greater phenotypic range facilitates survival under rapidly changing environmental conditions remains largely unknown. Further, in our dataset non-invasive species, were found to maintain fitness homeostasis better than invasives under resource limited or stressful conditions.

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2.7 Boxes, Figure and Tables

Box 2.1 Plasticity and Fitness

As J.B.S. Haldane once noted: "Fitness is a bugger" (Hunt & Hodgson 2010). To determine whether a plastic response is adaptive a researcher must regress plasticity in the focal trait against some estimate of fitness. This is difficult, because fitness is almost impossible to measure directly (e.g. Head *et al.* 2005; Hunt & Hodgson 2010). Thus, to explore the relationship between trait plasticity and fitness we must make a distinction between those aspects of the phenotype that are considered to be components of, or proxies for, fitness and other traits where the relationship with fitness is less clear (see *Figure 2.2*). Ideally, measuring fitness requires a demographic approach whereby one tracks a population over multiple generations to monitor the rate of increase in a trait or the relative number of descendents derived from each organism in the original generation. Sometimes researchers use lifetime reproductive output as a measure of fitness; although even this is flawed as it fails to account for potential variation in the reproductive value of offspring (e.g. Head *et al.* 2005). In most cases, however, logistic constraints force researchers to rely on various fitness indices or proxies as a measure of fitness e.g. traits closely related to size or fecundity (see table 4.3 in Hunt & Hodgson 2010). Researchers then assume that these components of fitness are positively correlated with net fitness; where net fitness is the relative ability of an individual (or population) to survive, reproduce and propagate genes in a given environment. This is a compromise, but it is a basic reality that is common to almost all studies of selection.

Selection will always act to maximise fitness in a given environment, thus plasticity in net fitness is unlikely to be adaptive (Scheiner 1993, Sih 2004). Plasticity in individual components of fitness (e.g. biomass, seed weight etc), however, can be under direct selection and can elevate average net fitness across environments (see Weiner 2004) because the relative contribution of different fitness components to net fitness often varies across environments. For example, low fecundity in one year for a perennial plant might reflect diversion of resources to survival or vegetative growth that ultimately boosts lifetime fitness (Sih 2004). Another example might be lower seed production in many species in response to water stresses (Freeman *et al.* 1981). In an agricultural setting, Sadras *et al.* (2009) found that wheat lines that had greater plasticity in yield (where yield is measure of fitness for agriculture crops) in response to water availability had higher average yields across years than genotypes that did not show phenotypic plasticity in yield across conditions. Similarly, Scheiner (1993) reported selection for different rates of reproductive output in different native environments due to a trade-off between reproduction and survival.

In the present study we examined plasticity in a broad range of traits for our overall plasticity analysis. However, when explicitly testing the Jack-of-all-trades and Master-of-some hypotheses we had to distinguish between measures that were reasonable proxies or components of fitness (fitness proxies) and those growth, morphological and physiological traits that we were testing for adaptive plasticity. For simplicity's sake we describe morphological, physiological and growth traits as 'focus traits' for our effect size estimates and consider their plasticity relative to our fitness proxies. We recognise that these distinctions are not clear-cut, and often studies of plasticity do not identify a measure of fitness against which the adaptive value of a plastic trait response can be tested. We therefore stress the importance in future studies of carefully choosing to measure explicit and meaningful components of fitness to better evaluate the adaptive value of plasticity in other traits.

Table 2.1 Results of the meta-analysis comparing plasticity of invasive species to non-invasive species. The effect sizes are reported for four different methods of analysis: using the individual trait data for each treatment and species pair (individual trait), combining trait data within treatments for each species pair (treatment mean) and combining trait data to all treatments within species pairs (species mean). Data is also reported for a randomization test where one trait from each study was randomly selected for each of 50 iterations (random trait). The data for each of the traits for the individual trait analysis is also provided. * = mean is statistically significant at 0.05 level.

Analysis	**df**	**Mean effect size**	**Bootstrap CI**
Individual trait	361	0.668	0.417 to 0.895 *
Treatment mean	138	0.509	0.272 to 0.749 *
Species mean	74	0.548	0.322 to 0.768 *
Random trait	49	0.686	0.446 to 0.927 *
Trait			
Biomass	91	0.629	0.145 to 1.318 *
N content	31	0.742	-0.054 to 1.606
NUE	16	1.004	0.392 to 1.701 *
P content	10	0.343	-0.142 to 0.847
Photosynthesis	26	0.830	-0.007 to 1.729
Root biomass	39	0.760	0.214 to 1.334 *
RGR	21	0.976	-0.014 to 2.176
Root:shoot	61	0.601	0.090 to 1.123 *
Shoot length	26	0.131	-0.617 to 0.832
SLA	12	0.480	-0.290 to 1.713
WUE	18	0.488	0.080 to 1.049 *

Table 2.2 Results of the overall plasticity meta-analysis comparing plasticity in invasive species to non-invasive species using mean effect sizes per treatment of each species pair within a study (treatment mean). * = mean is statistically significant at 0.05 level.

Treatment	**df**	**Mean effect size**	**Bootstrap CI**
competition	24	0.185	-0.434 to 0.689
light	22	1.285	0.747 to 1.888 *
nutrient	50	0.300	-0.085 to 0.694
water	11	0.833	0.074 to 1.666 *

Table 2.3 Results of the overall plasticity meta-analysis comparing plasticity in invasive species to non-invasive species using mean effect sizes of each species pair within a study (species mean). Summary effect sizes are provided for the following categorical explanatory variables: growth-form, invaded habitat type and level of relatedness between the invasive and non-invasive species. * = mean is statistically significant at 0.05 level.

Category	**df**	**Mean effect size**	**Bootstrap CI**
By invasive species growth-form			
grass	22	0.397	0.057 to 0.735 *
herb	34	0.649	0.302 to 1.055 *
shrub	4	0.356	-0.386 to 1.084
tree	4	0.477	-0.176 to 0.968
vine	6	0.912	-0.127 to 2.014
By habitat			
disturbed land	9	0.459	0.223 to 0.824 *
forest	30	0.762	0.248 to 1.231 *
grassland	17	0.428	-0.053 to 0.934
scrub/heath	11	0.188	-0.194 to 0.643
wetland	4	1.133	0.161 to 1.945 *
By species pair level of relatedness			
genus	17	0.755	0.284 to 1.262 *
family	23	0.361	0.060 to 0.584 *
less-related	32	0.582	0.241 to 0.944 *

Table 2.4 Results of the plasticity and fitness meta-analysis comparing invasive species to non-invasive species using species means (mean effect sizes of species pairs within each study). * = mean is statistically significant at 0.05 level.

Treatment type	**Trait type**	**df**	**Mean effect size**	**Bootstrap CI**
Resource increase	Focus traits	58	0.414	0.151 to 0.701 *
	Fitness proxies	33	0.474	-0.237 to 1.279
Resource decrease	Focus traits	21	0.387	-0.021 to 0.828
	Fitness proxies	15	-4.177	-9.986 to -1.052 *

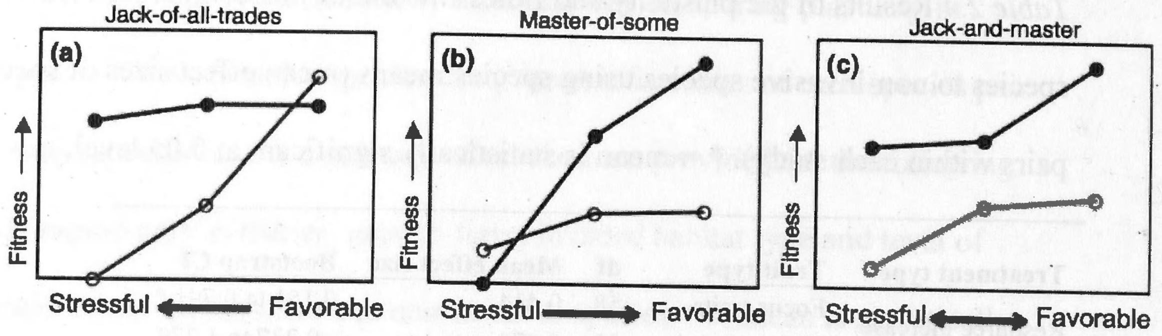

Figure 2.1 Black lines represent invasive species, grey lines represent non-invasive species. a) invasive species have more robust fitness in the face of stressful environmental conditions (Jack-of-all-trades), b) invasives are better able to respond with increased fitness in favourable conditions (Master-of-some) and c) fitness norm-of-reaction of invasives has characteristics of both robustness and responsiveness (Jack-and-master). Adapted from Richards *et al.* 2006 with permission.

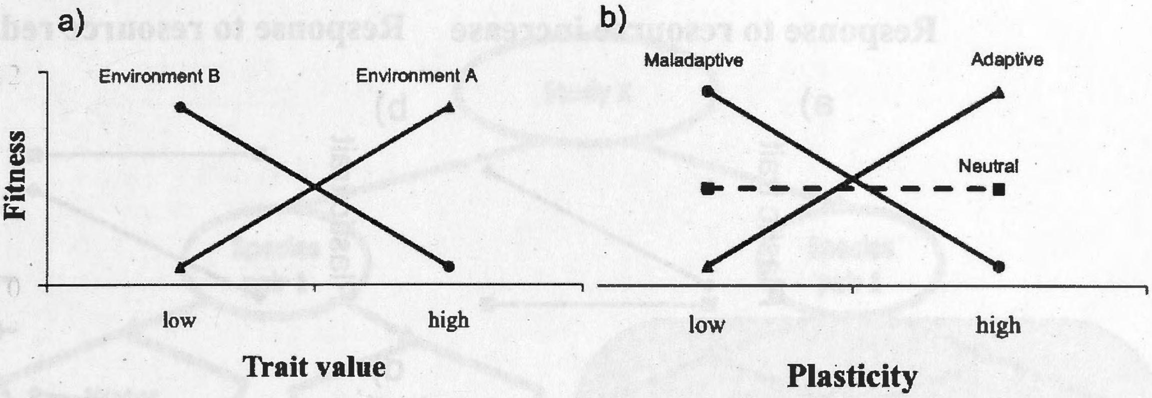

Figure 2.2 Two methods for assessing the adaptive value of plasticity. In method a), fitness is plotted against different values of a phenotypic trait of interest for multiple environments. Plasticity in the trait has the potential to be adaptive if different trait values confer the highest fitness in each environment. Alternatively, adaptive plasticity may be assessed by regressing the fitness of an organism against its average plasticity in a trait of interest as in b). (Adapted from Nicotra *et al.* (2010) and van Kleunen and Fischer (2005) with permission).

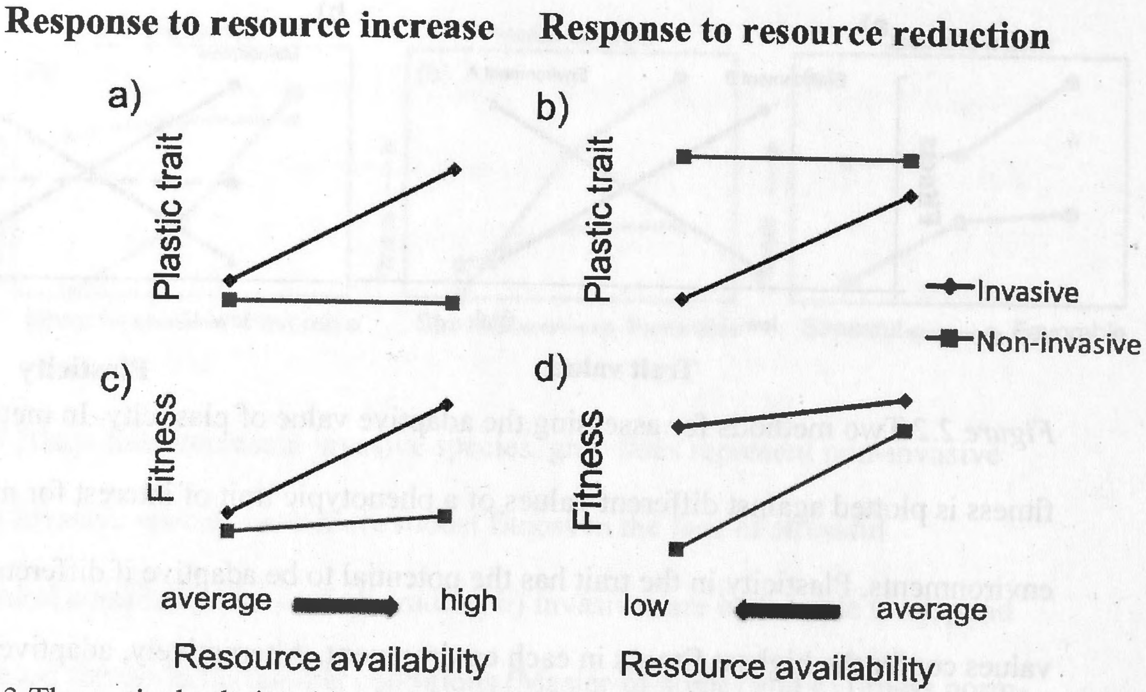

Figure 2.3 Theoretical relationships between trait values and resource availability and the corresponding fitness outcomes for non-invasive and invasive species. In both the increase-in-resources (a, c) and decrease-in-resources (b, d) scenarios the invasive species responds more plastically to the change in resource availability than the non-invasive species does (a, b) and this is associated with higher average fitness (c, d).

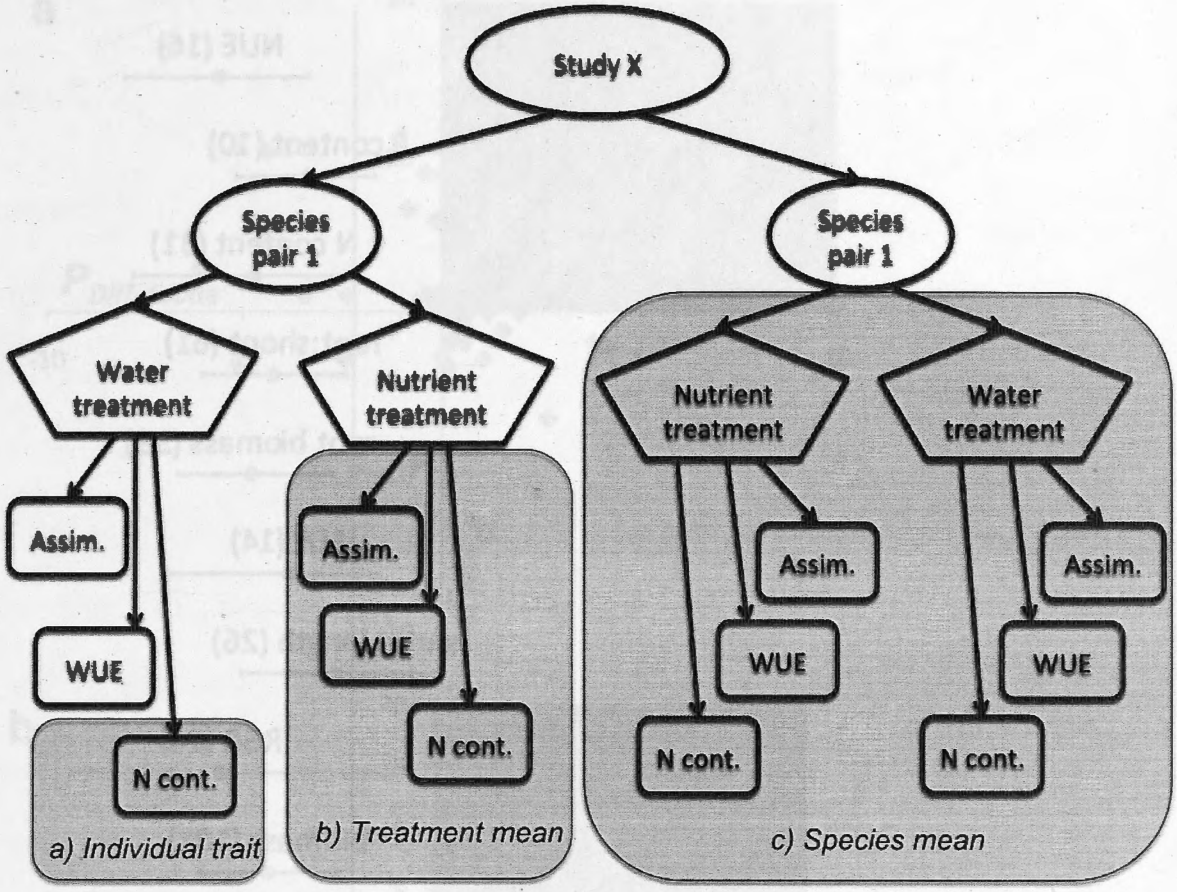

Figure 2.4. Hypothetical example of the hierarchical approach used to analyse the difference in plasticity between invasive and non-invasive species (P_{diff}). The Individual trait (a) was used to assess the effect of trait on P_{diff}, the treatment mean (e.g. in Study X there would be 12 individual traits); the treatment mean (b) was used to assess the effect of treatment type on P_{diff} (e.g. in study X there would be four treatment means); and the species mean (c) was used to assess the effects of invaded habitat type, growth-form and relatedness of the invasive and native species in the species pair on P_{diff} (e.g. in study X there would be two species means).

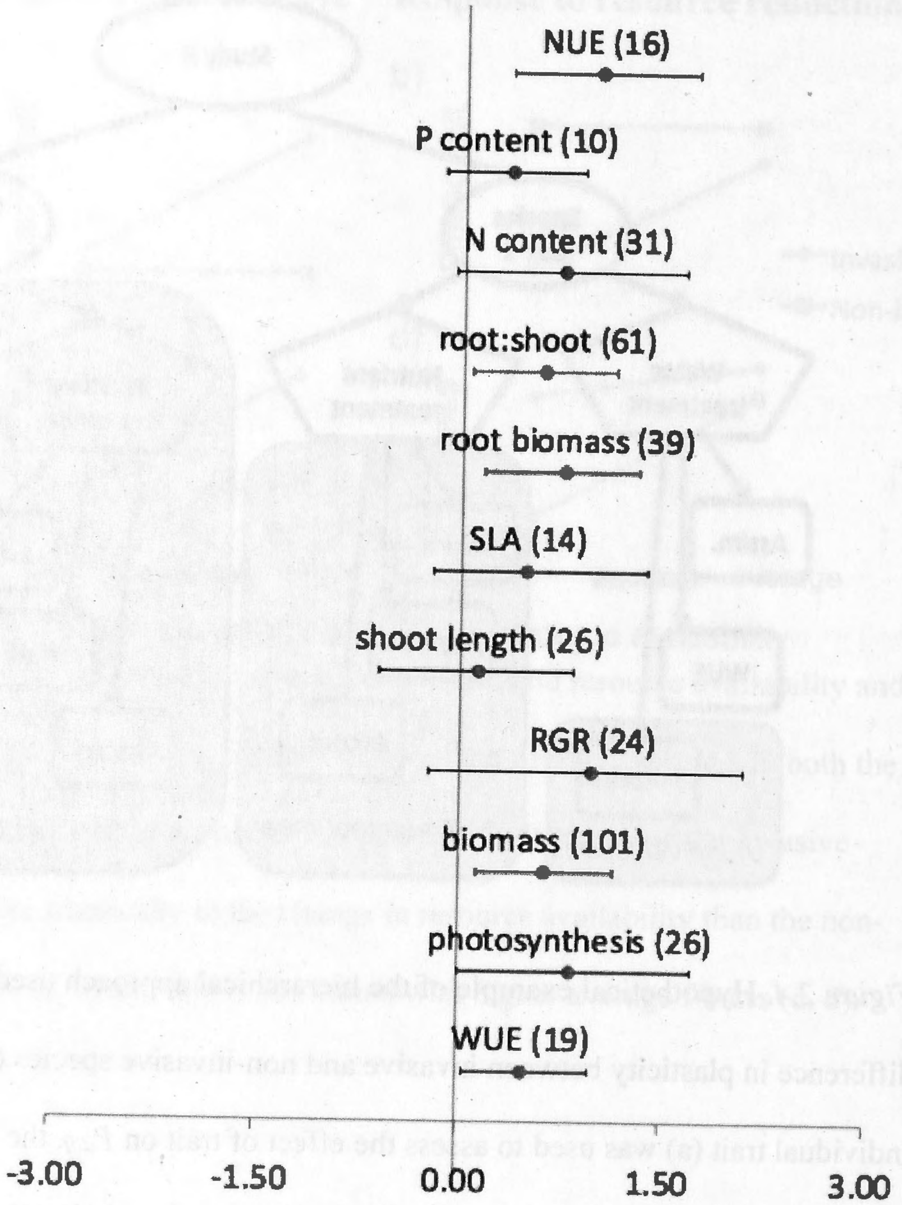

Figure 2.5 The mean difference in plasticity between invasive and non-invasive species for 11 traits using effect sizes for individual trait with separate effect sizes for each resource treatment. A positive mean effect size indicates that the invasive species has greater average plasticity than the non-invasive species. The bars represent 95% confidence intervals. The number of effect sizes used to calculate the mean is shown in parentheses.

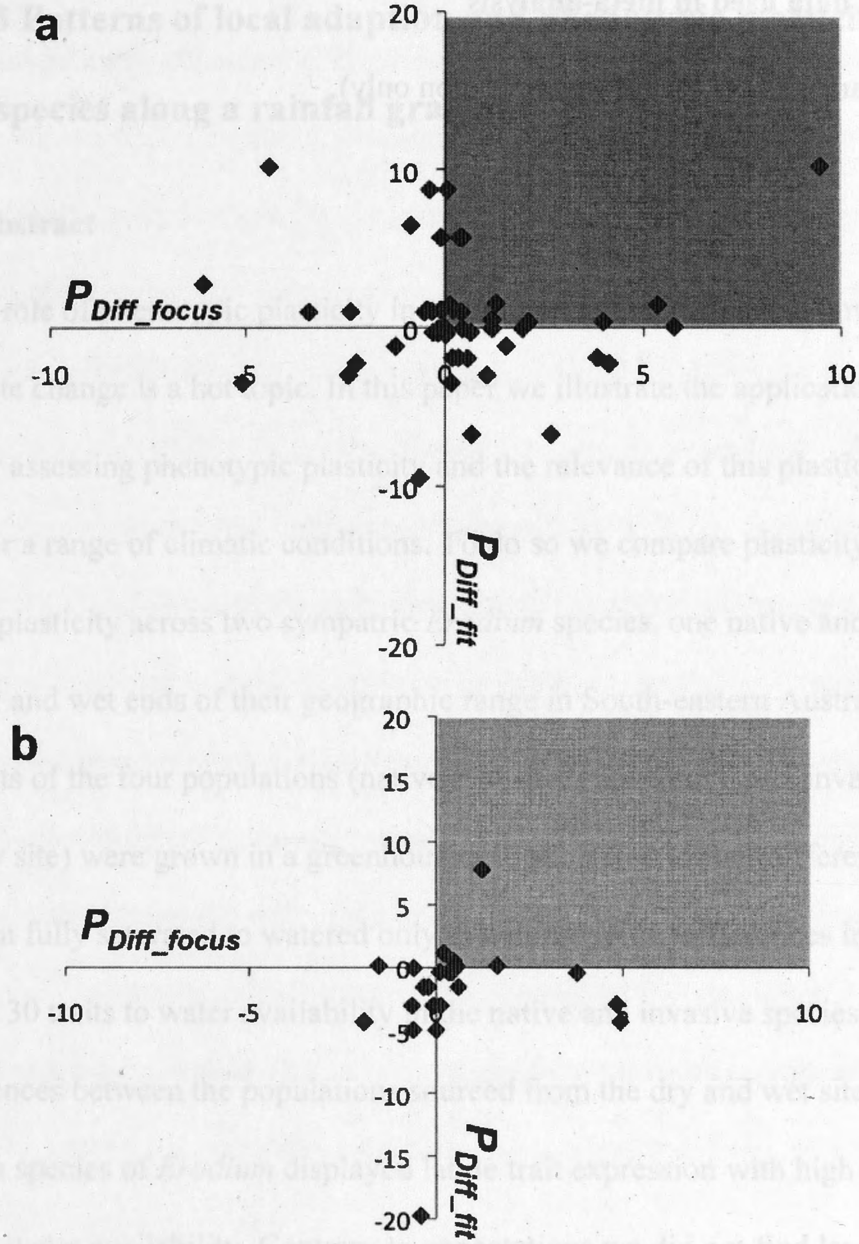

Figure 2.6 The relationship between P_{diff_focus} (relative plasticity of invasive versus non-invasive species) and P_{diff_fit} (relative change in fitness of invasive versus non-invasive species) in response to a) increases in resources (Master-of-some) and b) decreases in resource availability (Jack-of-all-trades). Positive P_{diff_focus} values indicate the invasive species is more plastic and positive P_{diff_fit} values indicate the invasive species has a better fitness response to a change in resources. The shaded quadrant is where points are expected to cluster if the Master-of-some and Jack-of-all-trades hypotheses are supported.

Appendix S2.1 data used in meta-analysis

See separate Excel sheet (digital version only).

Chapter 3 Patterns of local adaption and phenotypic plasticity in sympatric *Erodium* species along a rainfall gradient

3.1 Abstract

The role of phenotypic plasticity in enabling plants to withstand novel conditions in situ under climate change is a hot topic. In this paper we illustrate the application of several methods for assessing phenotypic plasticity and the relevance of this plasticity to populations fitness under a range of climatic conditions. To do so we compare plasticity patterns in phenotypic plasticity across two sympatric *Erodium* species, one native and one invasive from both the dry and wet ends of their geographic range in South-eastern Australia.

Plants of the four populations (native-wet site, native –dry site, invasive wet-site, invasive dry site) were grown in a greenhouse and subjected to four different water treatments ranging from fully saturated to watered only at wilting point. Differences in patterns of plasticity in 30 traits to water availability in the native and invasive species were compared as were differences between the populations sourced from the dry and wet-sites.

Both species of *Erodium* displayed labile trait expression with high phenotypic plasticity to water availability. Contrary to expectations we did not find large differences between the species. We did, however, find large differences between populations. In both species plants from populations from the dry end of the water gradient (the more heterogeneous site) showed greater adaptive plasticity than those from the wetter site. We discuss the significance of our findings and with respect to key considerations for future studies of plant populations under changing climatic conditions.

3.2 Introduction

Phenotypic plasticity refers to the influence of the environment on an organism's morphological, anatomical and developmental expression (Scheiner, 1993). Studies have shown plasticity to be both heritable and in some cases adaptive (Bradshaw, 2006). Plasticity may evolve in response to temporally or spatially heterogeneous conditions (Schlichting and Pigliucci, 1998), and potentially can facilitate tolerance of novel environmental conditions, particularly in sessile organisms such as plants (Ghalambor et al. 2007). However, empirical evidence of a link between species range size or environmental heterogeneity and plasticity are mixed (e.g. Pichancourt and van Klinken, 2012; Pohlman et al. 2005; Sultan, 2001).

It is also frequently suggested, and indeed empirical evidence supports the notion, that invasive species – those which have successfully colonised novel environments - have higher levels of phenotypic plasticity than non-invasive species (Daehler, 2003; Davidson, et al. 2011; but see Palacio-López and Gianoli, 2011). Yet it remains unclear whether this higher plasticity is directly responsible for a species' invasiveness or is an artifact of also being pioneering species. As such, it is not known whether plants with inherently higher phenotypic plasticity will be better placed to tolerate and adapt to new conditions (Nicotra et al. 2010; Davidson and Nicotra 2012).

Plasticity may be selected for if an organism experiences varying, but predictable, environmental conditions. However, if the contrasting environmental conditions are sufficiently spread through space or time, canalisation may occur instead, resulting in different ecotypes in each of the environments (Pigliucci et al. 2006). Thus, we may expect higher levels of adaptive plasticity under conditions likely to be encountered by populations but little or even maladaptive responses to conditions not frequently experienced by a population (e.g. low rainfall for a wet site population or a new environment for an invading species). Alternatively costs of plasticity (deWitt et al. 1998) may lead to canalization of traits and the evolution of ecotypes, and as such some researchers suggest that plasticity may be more common when genetic variation is low (Scheiner, 1993).

In order to address these key questions about the role of plasticity in species' relative abilities to tolerate and adapt to changing conditions we need to understand how and when selection will favour plasticity compared to a fixed phenotype. In other words, we need to be able to quantify adaptive plasticity rather than all plasticity. In this paper we use a model species pair consisting of co-occurring con-generic species, one invasive the other native, to explore four relatively simple means of assessing plasticity in key functional traits at various levels of water availability and then test its adaptive value. While there are several studies comparing the plastic responses of co-occurring native or non-invasive species and invasive species, measurements are generally taken at only two resource levels reflecting current conditions. Furthermore adaptive plasticity is rarely measured, rather the adaptive value of plasticity is inferred based on differences in fitness. If we are to understand the value of plasticity under changing conditions we need to explicitly measure adaptive plasticity at various resource treatments. By growing plants in a full range of conditions (Hulme, 2008) we can expose cryptic genetic variation (Ghalambor et al. 2007) and unveil hidden reaction norms (Shlichting and Pigliucci, 1998).

Richards et al. (2006) outline a number of scenarios contrasting the relationships between plasticity and fitness in invasive and native species. By quantifying the adaptive value of plasticity across resource supply gradients these can be tested. For example will plasticity be favoured when conditions become more stressful – enabling fitness homeostasis under these conditions – i.e. a "Jack-of-all-trades" phenotype? Or alternatively is plasticity favoured when conditions become more favourable enabling an increase in fitness in response to higher resource availability i.e. a "Master-of-some"? We may expect differences in response to resource conditions between populations based on colonisation history. For example, Davidson *et al.* (2011) found that native species tended to be more likely to display "Jack-of-all-trades" phenotypes than co-occurring invasive species.

In our model species pair we measured a suite of physiological, leaf morphological, size, growth and phenological traits relevant to water use in herbaceous annuals (30 in total, see Methods and Appendix S3.1). We measured the response of these traits, along with a fitness proxy – seed number – at four different levels of water. Furthermore, we compared the response of populations sourced from a dry site and from a wet site along a naturally occurring rainfall gradient for both species. Comparing the responses of these four populations to the different water availability treatments provided an opportunity to explore different methods to answer three common ecological questions regarding plasticity.

First, we asked whether there are differences in the expression of plasticity, and particularly adaptive plasticity, between natives and invaders or between plants sourced from different environments? Based on previous research and theory, we hypothesized that trait plasticity (and adaptive plasticity) would be higher in the invasive species (H1) and in plants sourced from the more heterogeneous site – the dry site (H2). Second, we asked whether there are differences in the extent of local adaptation, as indicated by differences between the wet and dry site populations, between a native and an invader? We hypothesized that the native species would show greater site differentiation than the more recently arrived invasive (H3), but as a corollary, predicted there would be no tradeoff between local adaptation in traits and plasticity therein for either species or sites (H4). Finally, we asked whether the expression of either adaptive or maladaptive plasticity vary between the different intervals along an experimental water gradient? We had several working hypotheses, based on previous research and theory, first, that the native species would exhibit more adaptive plasticity than the invasive species (H5), and that this difference might be more pronounced at the lower water availability treatments (following findings of Davidson *et al.* 2011 that native species are more likely to respond with adaptive plasticity to stressful conditions), second that the invasive would be more likely to show maladaptive plasticity (given the limited time for evolution to purge such responses, H7). The related hypothesis from the site perspective were that plants from the dry site would be more likely to display adaptive plasticity than those from the wet site (H7), particularly at the lower water availability treatments (as plants from this site would be more likely to have evolved phenotypes to cope with such condition) whereas plants from the wet site would be more likely to show maladaptive plasticity at low water than plants from the dry site (H8).

We test each of these hypotheses with our model species pair. While a single study on species pair cannot provide broadly generalisable answers regarding plasticity in invasive species or under climate change, it does provide a useful model to look at differences in plasticity between populations and to trial various methodologies for assessing the adaptive value of that plasticity. We hope that this model study provides a framework for future studies to assess general patterns in the ecological importance of plasticity in different populations and its potential adaptive value under future conditions.

3.3 Materials and Methods

Erodium crinitum is an annual herb native to Australia and found from temperate to semi-arid habitats. *Erodium cicutarium* is native to the Mediterranean region. It is invasive in large areas of the United States, parts of Asia and southern Australia. It reaches a smaller maximum size than *E. crinitum* even under favourable conditions (usually less than 50 cm high compared to up to 1 m for *E. crinitum*). Both species, like most *Erodium,* can freely self-pollinate (Fiz et al. 2008) and seeds are distributed by mechanical dispersal (Cox and Conran, 1996). In many ways both can be considered to have many attributes of pioneer species. By comparing a native and invasive species with similar ranges along a rainfall gradient, we avoid confusing differences due to range size with those related to invasiveness and ensure both species are likely to have evolved phenotypes that can endure a broad range of water availabilities.

Seeds of both species were collected in the Spring of 2008 from two sites (one semi-arid and one temperate). The semi-arid Mildura site (hereafter referred to as the dry site), is located in inland New South Wales (NSW) and has an average annual precipitation of 290 $mmyr^{-1}$. The temperate site, Canberra (hereafter referred to as the wet site), is located within the Australian Capital Territory (ACT), in central eastern NSW, it has an average annual precipitation of 620 $mmyr^{-1}$. As well as the significant difference in total rainfall, he dry site also experiences a more heterogeneous rainfall than the wet site (heterogeneity of rainfall is defined as the coefficient of variation of average annual rainfall, www.bom.gov.au).

Collections were made at >12 locations for each species at each site, the latitude and longitude of each location were recorded. Locations within sites were at least two kilometres apart (and usually greater than five kilometres) to maximise genetic variation, seeds were collected from one to five mother plants at each location. We assume that geographic distance is a good proxy for genetic relatedness in *Erodium* species because seeds of this genus have limited dispersal distance (Stamp, 1989) but occupy very similar sites environmentally within each species-site population.

3.3.1 Experimental set-up

Seeds were scarified with sandpaper and germinated on one per cent agar at 20˚C day/10˚C night in a growth chamber. Seedlings were transplanted into seedling trays filled with Osmocote® seed raising mix and placed in a greenhouse where they were watered daily for the first week and every second day thereafter. The seedlings were watered weekly with half strength Thrive Soluble All Purpose Plant Food fertiliser (Yates, Regents Park, 0.5mlL^{-1}). The greenhouse temperature was kept between 20˚C and 35˚C during the day and 5˚C and 15˚C at night. At the five-leaf stage, seedlings were transferred into nine cm diameter 70 cm long PVC pipes. Seedlings were chosen from as many populations as possible so as to maximise genetic diversity.

The tubes were filled with a mix of 10 per cent mushroom compost, 15 per cent clay mix (Canberra collected subsoil) and 75 per cent sand. The soil mix was treated with steam prior to use to kill any fungal spores. The mix had a slightly alkaline pH (~7.5) similar to that of soils where the species is found. Low phosphorous slow release Osmocote® Native Gardens fertiliser (17.9N:0.8P:7.3K) was mixed through the soil at the recommended rate ($5gL^{-1}$).

Four water treatments were imposed ranging from fully saturated to watered-only-at-wilting-point. A vapour-pressure deficit (VPD, MicroGrow Inc, city) controlled irrigation system delivered the three higher watering regimes. The system calculates VPD using real-time humidity and temperature information; it then accumulates VPD credits since the time of last watering – adjusting for any increase in apparent VPD. The fourth water treatment was delivered by hand as watering occurred only at wilting point. Wilting point was declared when >50% of the plants in the treatment had lost turgor and had severely drooping leaves. The watering rates were: fully saturated daily (high water: HW ~5250 $mLweek^{-1}$), watered approximately twice per week (medium water: MW ~2625 $mLweek^{-1}$), watered approximately once per week (low water: LW ~750 $mLweek^{-1}$) and watered approximately once per month (very low water: VLW, watered at wilting point, average of ~163 $mLweek^{-1}$), noting that the VPD system accounts for higher water usage as plants increase in size and thus watering frequency increased slightly over the experiment. The pots were watered to saturation at each watering event. This method of completely saturating tubes at varying time intervals were employed rather than maintaining soil at a set soil water content as it enables a slow dry-down of the soil profile in the tubes which more closely mimics natural events where the soil is saturated following rain and slowly dries down.

There were ten replicates of each combination of the two species, two sites and four water treatments (160 plants). Plants were arranged in five blocks to control for variation in light and temperature. As some measures were only conducted on five replicates we split each block into four quadrants and randomly selected two of the quadrants for the replicates to be used for all measures. Plants were then randomly assigned within each set of two quadrants for each block. Out of the 160 plants, one plant had to be removed due to transplant error.

3.3.2 Measurements

Thirty traits were measured in total. Traits were chosen based on literature assessments of the key physiological, leaf morphological, size, phenological and growth traits for herbaceous annual plants and logistical constraints. Traits included: $F_VF_M^{-1}$, assimilation (A), instantaneous water-use efficiency (WUE_i), A_{Sat} (asimilaition at saturating CO_2), WUE_{iSat}, stomatal density (abaxial), stomatal density (adaxial), leaf dissection index (LDI), leaf mass per area (LMA), leaf water content, leaf biomass, aboveground biomass, root biomass, total biomass, root:shoot biomass ratio (R:S), final diameter, final height, final leaf length, final leaf number, juvenile growth increment (GI) diameter, juvenile GI height, juvenile GI leaf length, juvenile GI leaf number, mature GI diameter, mature GI height, mature GI leaf length, mature GI leaf number, number of seeds and time to flowering (further information at Appendix S3.1). Growth measurements such as the number of leaves, diameter encompassing 90 per cent of the plant, height and length of the longest leaf (or compound leaf in the case of *E. cicutarium*) were measured on all ten replicates at two weeks (time of transplantation to tubes), eight weeks (time of transition to reproductive phase) and 14 weeks (beginning of senescence for dry treatments). GIs were then calculated by subtracting the natural log measurements for growth at two weeks from the measurement at eight weeks and dividing by 42 days to get the juvenile GI for each of the growth measurements. A similar process was conducted using measurements at 14 weeks and at eight weeks to get GI mature. Phenological measurements included the time (measured to the nearest week) at which flowering and seeding commenced and were measured on all ten replicates as was the fitness proxy seed number. All other measures were conducted on five replicates. Further details on each of the traits are available at Appendix S3.1.

3.3.3 Statistical analyses

3.3.3.1 Analysis of variance

Data were normalised using log transformations as necessary to ensure residuals met assumptions. Analysis of variance (ANOVA) tests were performed on all variables using datasets with and without outliers. Where the removal of outliers significantly changed the outcomes of the ANOVA the most conservative result was chosen. ANOVAs were performed on the full dataset set as well as analysing each species individually. Factors assessed in the ANOVAs included the water treatments, species, site and all two-way interactions, block was also included as a fixed factor. There was insufficient replication of collection location to include this in the ANOVA, instead mantel tests were conducted to test the influence of mother collection location(explained below). Significant interactions between treatment and species would indicate that one species had significantly greater plasticity than the other, the same applies for site*treatment interactions. Individual analyses for each species were conducted to enable identification of differences in trait responses and site patterns within the species.

All of the ANOVAs were conducted on 30 traits, the resulting probability statistics were then adjusted for multiple testing using the Benjamini –Hochberg correction, a corrected value of ≤0.05 was considered significant. This correction method was chosen as it provides a reasonable balance between type I and type II errors (Benjamini and Hochberg, 1995).

3.3.3.2 Mantel tests

We did not have sufficient replication to include collection location within site to include as a term in the ANOVA however, we waned to test the influence of the geographic position of collection location on traits, To do this partial mantel tests were performed assessing water effects on plant traits controlling for collection location We conducted the partial mantel tests on each species-site combination (e.g. native/wet, native/dry) separately. The partial mantel tests used two distance matrices, the first based on the water amounts in each treatment and the second based on geographic distance between collection locations in the field. Partial mantel tests are a more conservative test of treatment effect than ANOVAs, however, as they are unable to attribute variance to more than two co-variables in any one test they were used only to check the assumptions of the ANOVAs but not replace them.

We also conducted mantel tests on each species to measure the effect of wet and dry site collection locations, independent of growth conditions, on traits to assess genetic differentiation and thus potential local adaptation. The mantel and partial mantel analyses were performed using the Ecodist package in the R statistical program (see Goslee and Urban, 2007). A probability of ≤0.05 was considered to indicate significant geographic (and most likely) genetic influences on traits.

3.3.3.3 Plasticity index

To quantify plasticity for tests of relationships between plasticity and fitness, a modified version of the plasticity index (PI e.g. Valladares et al. 2000) was chosen, *Equation 1*.

$$\frac{|(x_1 - x_2)|}{|\max(x_1, x_2)|} \qquad \textit{Equation 1}$$

where: x_1, x_2 = trait value in treatment 1 and treatment 2.

When calculating PI, the mean value of a genotype/population/species of a set of treatments is generally used for the trait values and treatment 1 and 2. In this study, PI was calculated using normalised data for each treatment for the pair of plants in each block (using only the replicates chosen for physiological measurements so that there was just one treatment replicate for each of the five blocks). By pairing plants within blocks rather than using the mean value for the treatment we maintained replication, thus enabling summary statistical methods such as ANOVA to be performed on the PI values.

We calculated PI for each trait and for each step along the gradient in water treatment (high to medium, medium to low, low to very low water) as well as across the full range of the water gradient (high to very low water). We conducted an ANOVA to assess differences in PI between species and sites at each of the water gradients.

3.3.3.4 Analyses of adaptive plasticity

If plants with higher plasticity perform better than those with less, then plasticity may be adaptive. Alternatively, plasticity could be neutral or maladaptive. We assessed this in two ways, which each show subtly different things. Firstly, we assessed potential selection for plasticity in each of the four species by site combinations by regressing traits against seed number, the fitness proxy and looking for contrasting slopes of the relationship between traits and fitness (indicating potential selection for adaptive plasticity) at the different water treatments. Secondly, we regressed actual plasticity expressed (calculated as PI) against average fitness and compared the number of significant positive (adaptive plasticity) and negative (maladaptive plasticity) relationships for each water treatment interval and species by site combination as well as at the species level (across both sites) and site level (across both species).

For the first analysis all traits were regressed against the fitness proxy seed number. Normalised data were used for these regressions to ensure residuals met the assumptions of linear analysis. Separate regressions were produced for the intervals: high water to medium water, medium water to low water and low water to very low water. Analyses were done at each water interval rather than using water amount as a continuous variable for two reasons. Firstly the sample size of five would not permit statistically rigorous non-linear relationships to be fitted. Secondly we wanted to explore the shape of the relationship between the different intervals and believe that creating a smooth relationship across treatments could mask the true shape along the gradient, given many biological responses are triggered at a threshold rather than responding gradually to changing conditions. Analyses were conducted using all species and site combinations as well as assessing the sites and species separately.

We were interested in traits that displayed contrasting selection across the gradient, as indicative of selection for adaptive plasticity. Data were only considered where $R_{adj}^2 \geq 0.30$, as this generally corresponded to a probability of the null hypothesis of <0.05, furthermore we considered that a relationship which explained less than 30 percent of the variation with a sample of size of only five was not particularly biologically meaningful. Chi-squared tests were used to determine the statistical significance of differences in the numbers of traits displaying selection for plasticity as indicated by differences in slope between populations against the null hypothesis that the number of traits would be equal between populations. We also tested for significant differences in the number of traits displaying potential selection for plasticity between sites and species.

For the second analysis of adaptive plasticity, linear regressions of PI against average seed number (our fitness proxy) were conducted. Average fitness for any given PI was calculated as the mean of the fitness of the two plants used for the PI calculation. Correlations were considered significant when $P<0.05$ and $R_{adj}^2 \geq 0.30$. This was assessed at the population level as well as assessing differences at a site (with species combined) and species (with sites combined) level. A significant positive relationship indicated that plasticity should be favoured by selection (adaptive plasticity) and a significant negative correlation that selection should act against plasticity (maladaptive plasticity). Chi-squared tests were used to determine if the number of traits with significant positive relationships and the number of traits with significant negative traits varied significantly between the species or sites. The null hypothesis was that the proportion of traits with a significant positive relationship between trait PI and average fitness should be the same for each of the species and also for each of the sites, the same applied to the distribution of trait PIs with significant negative correlations with average fitness.

3.4 Results

For the individual species and site ANOVAs twenty-two of the 30 traits measured exhibited plasticity (measured as a significant water treatment effect) in at least one species or site (when results of the mantel test were also included twenty-three traits in total exhibited a significant treatment effect). The effects of the water treatment on key traits were in the expected direction (Table 3.1). Plants had lower photosynthetic rates and higher water-use efficiency when water supply was limiting (Table 3.1, Figure 3.1, see also Appendix S3.2 for the mean and standard deviation of all traits at each water treatment for each population). Several leaf morphological traits including stomatal densities and LDI did not respond significantly to water availability, although LMA was generally smaller at lower water availability, (Table 3.1). Plants also had lower juvenile and mature phase growth rates when water availability was lower (Table 3.1, Figure 3.1). Our fitness proxy, seed number was greatly affected by the water treatment for all populations (Table 3.1, Figure 3.1, see also the full ANOVA results at Appendix S3.3). Seed number also displayed a significant site effect ($Pr_{adj}<0.001$) and species*site interaction, ($Pr_{adj}<0.001$ Appendix S3.3) indicating that the populations differed in fitness.

Overall the results of the partial mantel tests were again largely congruent with the results of the ANOVAs (Table 3.1), suggesting genetic effects did not strongly influence trait response in most cases. The main exceptions to this were that F_V/F_M, juvenile diameter and juvenile height increment were significant in the ANOVA but not the partial mantel tests for any of the four populations. In contrast R:S only showed significant response to water availability in the partial mantel tests for each population but not in the ANOVAs (R:S was larger at lower water availability, Table 3.1).

3.4.1 Differences in the number of traits expressing significant plasticity in key traits in response to water.

Contrary to our predictions (H1), there was little difference in the number of traits exhibiting plasticity in response to water either between the two species (18 and 19 traits displaying significant treatment effects for the invasive and native respectively) or between plants grown from seed sourced from the wet site versus the dry site (H2, 17 traits each, Table 3.1). The responses for six of these traits representing one of each of the major groups:

Table 3.1 Direction of change in trait for all traits with significant Benjamini-Hochberg adjusted probabilities from ANOVA analyses for water treatments. Italicised text indicates that results of partial mantel test differed from those of the ANOVA (1= significant in ANOVA but not the partial-mantel, 2= significant in the partial-mantel but not ANOVA), native: *Erodium crinitum*, invasive: *E. cicutarium*, wet = seed sourced from the Canberra site, dry = seed sourced from the Mildura site. HW= high water, VLW = very low water, NS= not significant, GI = growth increment.

Trait	***invasive***	***native***	***wet***	***dry***
a. Physiological traits				
F_V/F_M	NS	NS	*HW>VLW*[1]	NS
A (Carbon assimilation) ($\log_2$)	HW>VLW	HW>VLW	NS	*NS*[2]
Instantaneous water-use efficiency	VLW>HW	VLW>HW	VLW>HW	VLW>HW
A_{sat} (Log$_2$)	*NS*[2]	HW>VLW	NS	*NS*[2]
WUE_{isat} (ln)	VLW>HW	VLW>HW	VLW>HW	VLW>HW
b. Leaf morphological traits				
Leaf biomass (ln)	HW>VLW	HW>VLW	VLW>HW	VLW>HW
Final leaf length (ln)	HW>VLW	*HW>VLW*[1]	VLW>HW	*VLW>HW*[1]
Leaf Mass per Area (LMA)	NS	NS	HW>VLW	HW>VLW
c. Size traits				
Aboveground biomass (ln)	HW>VLW	HW>VLW	*NS*[2]	*NS*[2]
Root biomass (ln)	HW>VLW	HW>VLW	HW>VLW	HW>VLW
Root:shoot biomass ratio ($\log_{10}$)	*NS*[2]	NS	NS	*NS*[2]
Final diameter (ln)	*HW>VLW*[1]	HW>VLW	HW>VLW	HW>VLW
Final height (ln)	HW>VLW	HW>VLW	HW>VLW	HW>VLW
Final leaf number (ln)	HW>VLW	HW>VLW	HW>VLW	HW>VLW
d. Growth traits				
Juvenile GI diameter	*HW>VLW*[1]	*HW>VLW*[1]	*HW>VLW*[1]	*HW>VLW*[1]
Juvenile GI height	*HW>VLW*[1]	*HW>VLW*[1]	*HW>VLW*[1]	*HW>VLW*[1]
Juvenile GI leaf length	HW>VLW	HW>VLW	HW>VLW	HW>VLW
Juvenile GI leaf number	HW>VLW	HW>VLW	HW>VLW	*HW>VLW*[1]
Mature GI diameter	NS	HW>VLW	NS	*HW>VLW*[1]
Mature GI height	*NS*[2]	HW>VLW	*HW>VLW*[1]	*NS*[2]
Mature GI leaf length	NS	NS	NS	NS
Mature GI leaf number	HW>VLW	*NS*[2]	NS	HW>VLW
f. Fitness proxy				
Total biomass (ln)	HW>VLW	HW>VLW	HW>VLW	HW>VLW
Number of seeds (ln)	HW>VLW	HW>VLW	HW>VLW	HW>VLW

physiological, leaf morphological, size, biomass and growth rate traits as well as seed number are shown in Figure 3.1.

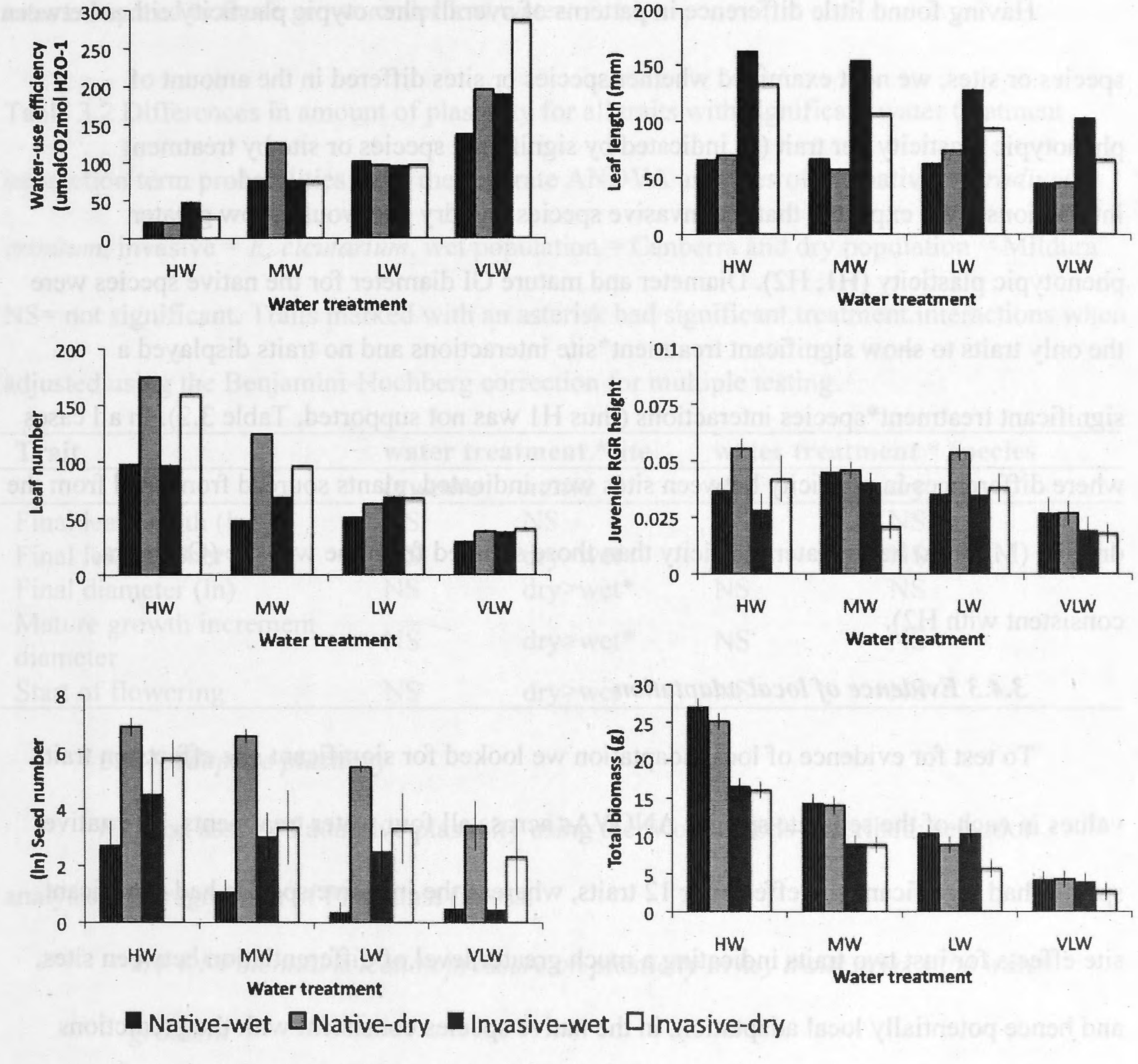

Figure 3.1 Reaction norms of selected traits with significant treatment effects for the separate analyses of each site (dry site = Mildura, wet site = Canberra) and species (native = *Erodium crinitum*, invasive = *E. cicutarium*). Graphs use back transformed values of mean and standard error from the normalised data where relevant. HW = high water (~5250mLweek-1), MW = medium water (~1500mLweek-1), LW = low water (~750mLweek-1) and VLW = very low water (~163mLweek-1). Error bar = + one standard error.

3.4.2 Differences between species and sites in the magnitude of the plastic responses to water availability.

Having found little difference in patterns of overall phenotypic plasticity either between species or sites, we next examined whether species or sites differed in the amount of phenotypic plasticity per trait (as indicated by significant species or site by treatment interactions). We expected that the invasive species and dry site would show greater phenotypic plasticity (H1, H2). Diameter and mature GI diameter for the native species were the only traits to show significant treatment*site interactions and no traits displayed a significant treatment*species interactions (thus H1 was not supported, Table 3.2). In all cases where differences in plasticity between sites were indicated, plants sourced from seed from the dry site (Mildura) had greater plasticity than those sourced from the wet site (Canberra, consistent with H2).

3.4.3 Evidence of local adaptation

To test for evidence of local adaptation we looked for significant site effects on trait values in each of the separate species ANOVAs across all four water treatments. The native species had significant site effects for 12 traits, whereas the invasive species had significant site effects for just two traits indicating a much greater level of differentiation between sites, and hence potentially local adaptation, in the native species consistent with the predictions (H3, Table 3.3). The mantel tests for an effect of geographic distance on trait values generally agreed with the ANOVA results for site effects (Table 3.3).

There was no evidence that traits which displayed strong genetic differentiation had limited plasticity. In the native species, of the 14 traits which displayed significant site effects nine of these (64%) responded plastically to water (i.e. had a significant water treatment effect, Tables 3.1 and 3.3). Similarly for the invasive species, both traits which displayed a significant site effect also displayed significant plasticity (Tables 3.1 and 3.3). The trait differences due to site effects tended to be consistent with the phenotypic change associated with the water treatment. For example plants from the wet site had higher A_{sat} rates compared to the dry site origin plants for the native species. Additionally, plants grown under high water had higher A_{sat} rates compared to those grown under low water.

Table 3.2 Differences in amount of plasticity for all traits with significant water treatment interaction term probabilities from the separate ANOVA analyses of the native = *Erodium crinitum*, invasive = *E. cicutarium*, wet population = Canberra and dry population = Mildura. NS= not significant. Traits marked with an asterisk had significant treatment interactions when adjusted using the Benjamini-Hochberg correction for multiple testing.

Trait	**water treatment *site**		**water treatment * species**	
	invasive	***native***	***wet***	***dry***
Final leaf length (ln)	NS	NS	NS	NS
Final leaf number (ln)	NS	dry>wet	NS	NS
Final diameter (ln)	NS	dry>wet*	NS	NS
Mature growth increment diameter	NS	dry>wet*	NS	NS
Start of flowering	NS	dry>wet	NS	NS

3.4.4 Adaptive plasticity

We next assessed adaptive plasticity using the two methods described: selection analysis, and regression of PI against fitness.

3.4.4.1 Potential selection pressure on plasticity in key traits across the water gradient

When we assessed the potential selection for plasticity by regressing traits against seed number we found evidence of contrasting direction of selection between water treatments for 20 traits encompassing a total of 40 incidents (Table 3.4). The populations displayed quite different patterns with relatively little selection for adaptive plasticity in the native wet population but quite a lot in the native and invasive dry populations (Table 3.4). This greater level of potential selection for plasticity in plants sourced from the dry compared to the wet site was significant when tested using Chi-squared statistics, consistent with H2 (Chi squared probability = 0.01, Table 3.4). Although the invasive species displayed potential selection for adaptive plasticity more frequently than did the native species this difference was not statistically significant (Chi squared probability = 0.42). Potential selection for plasticity occurred across the water gradient (e.g. when high to medium water availability was assessed.

Table 3.3 Direction of change in trait for all traits with significant Benjamini-Hochberg adjusted probabilities from ANOVA analyses of site effects. Italicised text indicates that results of the mantel test differed from those of the ANOVA (1= significant in ANOVA but not the mantel, 2= significant in the mantel but not ANOVA). HW= high water, VLW = very low water, wet = Canberra, dry= Mildura, NS= not significant, GI= growth increment.

Trait	***Invasive***	***Native***
a. Physiological traits		
F_V/F_M	*NS*^{2}	NS
A_{sat} (log$_2$)	NS	wet>dry
b. Leaf morphological traits		
Leaf dissection index (sqrt)	NS	dry>wet
Leaf biomass (ln)	wet>dry^{1}	NS
Final leaf length (ln)	wet>dry	NS
Leaf Mass per Area (LMA)	NS^{2}	NS
c. Size traits		
Root biomass (ln)	NS	wet>dry^{1}
Root:shoot biomass ratio (log$_{10}$)	NS	wet>dry
Final diameter		dy>wet
Final height (ln)	NS	dry>wet
Final leaf number (ln)	NS	dry>wet
d. Growth traits		
Juvenile GI height	NS	dry>wet
Juvenile GI leaf length	NS	dry>wet
Juvenile GI leaf number	NS	dry>wet^{1}
Mature GI leaf length	NS	NS^{2}
Mature GI leaf number	NS	dry>wet
e. Phenological traits		
Start of flowering	NS	wet>dry
Start of seeding	NS	wet>dry
f. Fitness proxy		
Number of seeds (ln)	NS	dry>wet

right down to when low to very low water availability was assessed) and there was no particular bias for certain populations to express selection for plasticity at one end of the water

Table 3.4 Summary of key traits' relationships with seed number, nat = native species: *Erodium crinitum*, inv= invasive species: *E. cicutarium*, wet =wet site: Canberra, dry= dry site: Mildura. ln indicates natural log transformation of trait values. HW=high water, MW = medium water, LW=low water and VLW = very low water. +/- indicates a positive relationship under the first treatment (top line of column) but a negative relationship under the second treatment (bottom line of column. -/+ indicates the reverse relationship pattern.

		Direction of selection					
Data	**Trait**	***HW MW***	***HW LW***	***HW VLW***	***MW LW***	***MW VLW***	***LW VLW***
inv-wet	$F_VF_M^{-1}$	+/-			-/+		
inv-wet	Stomatal density (adaxial)						+/-
inv-wet	Leaf water content						+/-
inv-wet	Leaf mass area^{-1}			+/-			
inv-wet	Leaf biomass (ln)						-/+
inv-wet	Root:shoot biomass ratio (log$_{10}$) (R:S)		+/-*	+/-*			
inv-dry	Total biomass (ln)			+/-			+/-
inv-dry	R:S						-/+
inv-dry	Diameter	+/-					
inv-dry	Height		+/-*	+/-*			
inv-dry	Juvenile growth increment (GI) diameter		+/-		-/+	-/+	
inv-dry	Juvenile GI height	+/-	+/-				
inv-dry	Mature GI leaf length					+/-	+/-
inv-dry	Mature GI diameter				-/+		
nat-wet	Leaf length						+/-
nat-wet	Juvenile GI diameter		+/-				
nat-wet	Mature GI leaf number				+/-		
nat-dry	Assimilation$_{sat}$		-/+*				
nat-dry	Leaf dissection index				-/+		
nat-dry	Leaf water content			+/-		+/-	
nat-dry	Leaf length		-/+	-/+			
nat-dry	R:S						-/+
nat-dry	Leaf number	-/+			+/-	+/-	
nat-dry	Juvenile GI leaf number		+/-*				
nat-dry	Mature GI leaf number						+/-
nat-dry	Mature GI leaf length			-/+			
nat-dry	Mature GI height	-/+					

*$P<0.05$

availability treatments (Table 3.4). Thus the results do not support the hypothesis that plasticity in the dry site origin plants will be more common at low water availability (H6).

Table 3.5 Summary of trait plasticity (PI) – average fitness (seed number) relationships at different water availability intervals, nat= native species: Erodium crinitum, inv= invasive species: E. cicutarium, wet =wet site: Canberra, dry= dry site: Mildura. ln indicates natural log transformation of trait values. HW=high water, MW = medium water, LW=low water and VLW = very low water, GI = growth increment.

Water interval	**Population**			
	inv-wet	***inv-dry***	***nat-wet***	***nat-dry***
a) Positive PI-Fitness relationships (adaptive plasticity)				
HW-MW				stomatal density (abaxial)
MW-LW	juvenile GI leaf number		leaf mass area^{-1} flowering time	
LW-VLW	leaf number			leaf number mature GI leaf number
HW-VLW		diameter		
b) negative PI-fitness relationships (maladaptive plasticity)				
HW-MW	juvenile GI leaf length stomatal density (adaxial)	mature GI leaf number		assimilation root biomass
MW-LW		juvenile GI height	mature GI leaf number	
LW-VLW		leaf length juvenile GI leaf length		
HW-VLW	water-use efficiency, mature GI height	leaf mass area^{-1}		mature GI diameter

3.4.4.1 Comparisons of potential adaptive and maladaptive plasticity to water availability

When we regressed PI against average fitness and compared the number of significant positive (adaptive plasticity) and negative (maladaptive plasticity) relationships we found a total of eight significant cases of adaptive plasticity and 13 significant cases of maladaptive plasticity (Table 3.5). There were too few traits displaying adaptive plasticity in Table 3.5 to assess for differences between species (H1 and H5) or site of origin (H2 and H8) either across all water treatments or at specific water intervals. However, consistent with H6 the invasive species displayed maladaptive plastic response more frequently than did the native populations, but this difference was not statistically significant (Chi squared probability = 0.17). We predicted that the wet site populations would display maladaptive plasticity more often than the dry site populations particularly at very low water. Wet site plants were no more likely to display maladaptive plasticity at the low end of the gradient or anywhere else as such H8 is rejected.

3.5 Discussion

The *Erodium* species displayed significant responses to water in 22 out of 30 traits (Table 3.1), creating a good opportunity to assess patterns of adaptive plasticity. In this discussion, we firstly describe the key differences between the two species and the two sites in their plastic response to water availability. Next we describe key differences in local adaptation of the plants to the dry and wet sites. We then look at the proportion of phenotypic plasticity that is adaptive and whether we can identify any patterns relating to species, site or location along the water treatment gradient where plasticity tends to play an adaptive role. Finally we consider what the results tell us about adaptive plasticity in general and considerations for future studies.

3.5.1 Differences in plasticity and adaptive plasticity between species (H1)

The native and invasive *Erodium* species responded in very similar ways to the water treatments (Tables 3.1 and 3.2, Figure 3.1) despite significant morphological differences and very different lengths of time to adapt to local conditions in Australia. Similarly there were no significant differences between species with respect to adaptive plasticity (Tables 3.4 and 3.5). We therefore reject H1 that trait plasticity and adaptive plasticity will be higher in the invasive species.

The results are not in accord with the generally held theories of Baker (1965) that invasive species will display greater phenotypic plasticity than native species. A recent meta-analyses on plasticity of invasive and native species (Davidson et al. 2011) did, however, find general overall support for this theory. One possible explanation for the lack of differences in plasticity between the two species in this experiment may relate to the characteristics of the native species chosen. *Erodium crinitum*, the native in this study, is a widely distributed species encompassing a broad environmental niche in a highly variable climate. In contrast, many other comparisons of native and invasive species have selected native species that occupy a relatively narrow niche. It has been suggested that species with wider distributions will have relatively higher phenotypic plasticity in key traits whereas more narrowly distributed species will be characterised by having specialised phenotypes with relatively little plasticity (e.g. Sultan, 2001).

It could also be that both *Erodium* species are early successional pioneers and that plasticity is a characteristic of pioneering plants, not invasives *per se* (Bazzaz, 1979). *Erodium crinitum* does readily establish from a seedbank in disturbed areas. The ability to establish in disturbed areas may be a separate quality to invasiveness as *E. crinitum* has been introduced multiple times to Japan but never established (Thomas, 2007). The higher plasticity in invasive species found by Davidson et al. (2011) could also be related to the invasive species qualities as pioneering plants compared to their native counterparts and this deserves further attention.

3.5.2 Differences in plasticity and adaptive plasticity between site of origin (H2)

There was weak support for greater overall plasticity in the dry site populations compared to the wet site populations (H2) with three traits displaying a greater magnitude of response in the dry site plants (Table 3.2) but no difference in the total number of traits that responded plastically to water (Table 3.1). However, when only plasticity that is adaptive was compared, H2 was supported as adaptive plasticity was displayed in more traits in plants from the more heterogeneous dry site compared to those from the wet site (Tables 3.4 and 3.5).

3.5.3 Patterns of local adaptation (H3 and H4)

Twelve traits showed a significant site effect for the native species compared to just two for the invasive species. This provides evidence for greater local differentiation (and potential adaptation) of the native species compared to the invasive species (Table 3.3) consistent with H3. This is to be expected given the greater time of residence of the native species and thus increased time under selection to maximise fitness in both the dry and wet sites. Scheiner (1993) proposed several theories relating to the genetic mechanisms underlying phenotypic plasticity. One of these was that plasticity would be greater when homozygosity was higher. This theory has frequently been interpreted to mean that plasticity should play a larger role when there is limited genetic variation. Thus, it has sometimes been suggested that plasticity will be higher in species with less ecotypic differentiation. The study did not support the idea that ecotypic differentiation was an alternative to phenotypic plasticity as there was no evidence that the greater level of genetic differentiation in the native species had any impact on the level of phenotypic plasticity observed, consistent with H4.

3.5.4 Patterns of adaptive plasticity (H5-8)

We found evidence of selection for adaptive plasticity in several size and growth traits, but only a few physiological or leaf morphological traits. Although plasticity was commonly detected across traits, species, sites and water conditions, cases of adaptive plasticity were far less common. Furthermore, there were no obvious patterns regarding which trait was plastic or under which circumstances. It thus seems likely that neutral and maladaptive plastic responses are very common in natural populations (van Kleunen and Fisher, 2007).

In general, smaller plants were favoured under the lower water availability scenarios and larger plants were favoured when water was more abundant (i.e. there was evidence for adaptive plasticity for plant size traits). This perhaps reflects a shift in phenology with faster transfer of vegetative biomass to reproductive biomass (in order to complete the lifecycle quickly) under more stressful conditions (Aronson et al. 1992). However, there were as many cases of maladaptive plasticity in size traits as there were cases of adaptive plasticity (Tables 3.4 and 3.5). It is possible that although smaller size at reproduction is favoured under stressful conditions, plants with extremely small biomass are unable to convert sufficient energy to seed production and therefore perform poorly. Thus the smallest plants had poorest performance and consequently plasticity towards small size at low water was not favoured in these cases. This suggests that the reaction norm for plant size is not linear and that plants with highest fitness at the low water scenarios were those with an overall "medium" size. Due to constraints associated with replication number, we were only able to fit linear relationships however, the true relationship may be curved (e.g. a negative parabola) rather than straight.

We predicted that we would detect a greater proportion of adaptive plasticity in the native species compared to the invasive species, particularly at low water availability (H5). Such a pattern did occur (Table 3.5) but was not clustered at the lower water availability scenarios as predicted (H5).

Davidson et al. (2011) found that native species tended to do relatively better than invasive species under stressful conditions. However this result was over multiple species pairs. It is likely that many such pairs included an opportunistic weed, which benefits from plasticity through a "Master-of-some" response to additional resources rather than a "Jack-of-all-trades" response by maintaining fitness in stressful environments (Richards et al. 2006). *Erodium cicutarium*, however, occurs at very low water availabilities in its native range in the Mediterranean as such it is potentially more a stress tolerator (Grime, 1979) than other, more typical, opportunist weed species. These characteristics of the invader may put the species on a more even playing field and, as with the overall amount of plasticity, dilute the differences typically seen between native and invasive species. While we did not find significant differences in overall levels of plasticity or adaptive plasticity, the invasive species did express maladaptive plasticity slightly more often than the native species perhaps reflecting the shorter residency time and thus shorter time for selection to act on the invasive phenotype.

Contrary to predictions (H7), the plants of dry site origin showed adaptive plasticity at all points of comparison along the water gradient rather than greater adaptive plasticity at the more stressful scenario. This perhaps highlights the importance for semi-arid plants to be able to respond to variable rainfall (Aronson et al. 1992) leading to a "Jack-and-master" type phenotypic response, which combines stress tolerance and rapid growth in good conditions (Richards et al. 2006). The dry site, Mildura as well as having lower rainfall than Canberra, also has a more variable rainfall. Greater adaptive plasticity in plants of the dry site is thus consistent with the hypotheses of greater selection for plasticity in more heterogeneous environments (Gianoli and Gonzalez-Teuber, 2005, Schlichting and Pigliucci, 1998 but see Nicotra et al. 2007). This greater plasticity would seem to be responsible for the dry site populations' overall greater seed production at each water interval (Figure 3.1, Appendix S3.2).

3.5.5 Conclusions and future directions

Assessing differences in plasticity by looking at significant interactions between treatment and population is a commonly used method in plasticity studies. However, it only tells us part of the story regarding the role of plasticity in a plant's ability to cope with different environments. Which traits and the number of traits displaying plasticity are arguably as important as the magnitude of plasticity expressed. However, neither the number of plastic traits nor the magnitude of plasticity matters if traits displaying plasticity are not related to the plant's fitness. It is critical that studies assess the adaptive value of plasticity by measuring the relationship between plasticity and fitness rather than simply inferring that if a population has higher average fitness and higher average plasticity the two are related. The present study is admittedly a small one focusing on a single species pair and assessing plasticity at a population rather than lineage scale, but we hope that the approach we have taken illustrates a framework for how to test hypotheses about the adaptive value (or otherwise) of plasticity. Future research could build upon this framework in several ways.

In this study we were only able to assess linear relationships between our plasticity measure and fitness proxy due to constraints related to sample size. Given the results discussed above with respect to plant site and fitness it would be interesting to investigate non-linear relationships between plasticity and fitness, such as would lead to disruptive selection for example.

We found that plasticity was relatively common (the majority of traits had a significant treatment effect). However, when we investigated the treatments at different water availability intervals many traits were plastic only at particular points along the water gradient or even switched from adaptive to maladaptive plasticity depending on which water interval was assessed. Therefore future studies ought to measure trait responses at more than two treatment levels given reaction norms are rarely linear. It is also critical that experiments select treatments which are relevant to the questions being asked and the ecological context of the species being studied and that future conditions under climate change are considered.

In order to conduct true selection analyses genotypes should be replicated and where possible these should be grown in a greenhouse for one generation to eliminate maternal effects. However of arguably greater importance for ecological studies is that the genotypes selected represent the true genetic diversity in the field, otherwise it is impossible to tell if results are truly representative of the natural population or if by chance a very plastic or very non-plastic genotype was selected. In this study we used partial mantel tests to help control for genetic factors given genotypes were not fully replicated and this may prove a useful method for future studies.

We selected 30 traits relevant to plant water-use to measure. Twenty-two of these traits responded plastically for at least one population. Although the majority of these traits displayed potential selection for adaptive plasticity, adaptive plasticity was only realized in a handful of traits. It would be useful to gain a better *a priori* understanding of which traits' plasticity are likely to meaningfully influence fitness for a given plant community and environmental change.

In our study, site and not species had a far greater influence on the patterns of adaptive phenotypic plasticity to water observed. Thus, it would appear that populations with an ecological context relevant to the environmental change under examination are more likely to be able to respond with adaptive plasticity than those from a more stable environment. This has important implications in making future predictions about species' abilities to cope with novel conditions and highlights the importance of quantifying plasticity at the population level.

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3.7 Appendix S3.1 Descriptions of traits chosen for the water treatment experiment

Table S3.1.1 Descriptions of traits chosen for the water treatment experiment

Trait	**Measurement**	**Expected response to water reduction**	**Reference for stress response**
F_V/F_M	$\frac{Fluoresence_{max} - Fluoresence_{initial}}{Fluoresence_{max}}$	Decrease	Yordanov et al. 2000; Flexas et al. 2000; Maxwell and Johnson, 2000
Assimilation (A)	photosynthesis, (mol $CO_2m^{-2}s^{-1}$) taken at saturating light (1800 PAR) and 400 ppm CO_2,	Decrease	Farquhar and Sharkey, 1982; Tezara et al. 1999 Huber et al. 1984; Flexas et al. 1999
Instantaneous water-use efficiency (WUE_i)	$\frac{A}{g}$ where g = mol $H_2Om^{-2}s^{-1}$	Increase	Nicotra and Davidson, 2010; Hsiao and Acevedo, 1974; Bacon, 2004
High CO_2 carbon assimilation (A_{sat})	As per A but at saturating CO_2 (1000 ppm)	Decrease	Farquhar and Sharkey, 1982; Huber et al. 1984
WUE_{isat}	As per WUEi but at saturating CO_2 (1000 ppm)	Increase	Bacon, 2004; Nicotra and Davidson, 2010
Stomatal density (abaxial)	number/area	Increase then decrease	Xu and Zhou, 2008; Galmés et al. 2007
Stomatal density (adaxial)	number/area	Increase then decrease	Xu and Zhou, 2008; Galmés et al. 2007
Leaf dissection index (LDI)	$\frac{perimeter}{\sqrt{area}}$	Increase	Sack and Tyree 2005; McLellan and Endler, 1998
Leaf mass per area (LMA)	$\frac{weight_{dry}}{area}$ (gcm^{-1})	Increase	Wright et al. 2002; McLellan and Endler, 1998)
Leaf water content	Leaves fresh weight –Leaves dry weight, (g)	Increase then decrease	Morgan, 1984; Siddique et al. 2000
Leaf biomass	Oven dry weight of leaf lamina	Decrease	Schurr et al. 2000
Aboveground biomass	Oven dry weight of leaves and stems, (g)	Decrease	Bazzaz and Grace, 1997

Table S3.1.1 *cont.* Descriptions of traits chosen for the water treatment experiment.

Trait	**Measurement**	**Expected response to water reduction**	**Reference for stress response**
Root biomass	Oven dry weight of roots (g)	Root depth increases but biomass likely to decrease	Chaves et al. 2003; Hayes and Seastedt, 1987
Total biomass	Oven dry weight of roots, stems and leaves (g)	Decrease	Bazzaz and Grace, 1997
Root:shoot biomass ratio (R:S)	$\frac{Biomass_{root}}{Biomass_{aboveground}}$	Increase	Lloret et al., 1999; Chaves et al. 2002
Final diameter	Width (cm) encompassing 90% of plant's horizontal spread	Decrease	Kramer, 1969
Final height	Height (cm) from stem base to highest point	Decrease	Kramer, 1969
Final leaf length	Leaf length (cm) excluding main petiole	Decrease	Schurr et al. 2000
Final leaf number	Number of fully expanded leaves	possibly increase initially to compensate for reduced size	Casper et al. 2001
Juvenile relative growth rate (GI) diameter	$\frac{diameter_{t2} - diameter_{t1}}{t_2 - t_1}$ where: t2 = 8 weeks, t1 = 2 weeks	Decrease	Parsons, 1968
Juvenile GI height	As for diameter but substitute in height	Decrease	Parsons, 1968
Juvenile GI leaf length	As for diameter but substitute in leaf length	Decrease	Parsons, 1968
Juvenile GI leaf number	As for diameter but substitute in leaf number	Increase initially to compensate for reduced leaf size	Casper et al. 2001
Mature GI diameter	$\frac{diameter_{t2} - diameter_{t1}}{t_2 - t_1}$ where: t2 = 14 weeks, t1 = 8 weeks	Decrease	Parsons, 1968

Table S3.1.1 *cont.* Descriptions of traits chosen for the water treatment experiment.

Trait	**Measurement**	**Expected response to decreasing water availability**	**Reference for stress response**
Mature GI height	As for diameter but substitute in height	Decrease	Parsons, 1968
Mature GI leaf length	As for diameter but substitute in leaf length	Decrease	Casper et al., 2001
Mature GI leaf number	As for diameter but substitute in leaf number	Increase initially to compensate for reduced size	Parsons 1968
Number of seeds	Number of awns * 5	Decrease	
Time to flowering	Number weeks until first flower	Decrease	Aronson *et al.*, 1992
Time to seeding	Number weeks until first seed	Decrease	Aronson *et al.*, 1992

All physiological, leaf morphological and size measurements were taken on five of the ten replicates, (one replicate of each species and site combination was randomly selected from each block). Photosynthesis and conductance were measured using a LI-COR® 6400 at ten weeks at 400 μmolm^{-2}s^{-1} and 1000 μmolm^{-2}s^{-1} CO_2 (for A and A_{sat} respectively). The measurements were adjusted for leaf area placed in the IRGA chamber using Image J (http://rsb.info.nih.gov/ij/). Instantaneous water-use efficiency was calculated as assimilation over conductance. Fluorescence measures were taken during week 11 at night (22:00 hrs) to measure the health of photosystem II ($F_VF_M^{-1}$) with a MINI-PAM Photosynthesis Yield Analyzer. The leaf used to measure photosynthesis was also used to measure LMA and LDI. Following licor measurements the leaf was harvested scanned to measure perimeter and projected leaf area of the intact leaf, (Davidson and PrometheusWikicontributors, 2011). LDI was calculated as perimeter/sqrt(area)), using the projected area scans. For calculation of LMA, the leaves were cut into sections for calculation of total (rather than projected) leaf area where there was overlapping between lobes/leaflets. The leaves were then dried in an oven at 60°C for two days (at which time constant weight was achieved) and weighed again. LMA was calculated from the total area and dry weight measures. Water content (fresh weight-dry weight)/fresh weight) was assessed; the production of a latex-like substance from *E. cicutarium* prohibited measurement of saturated weights for calculation of relative water content. Leaf peels were taken on both the abaxial and adaxial surfaces from one leaf on each of the five selected replicates using clear nail polish. The nail polish samples were then viewed under a microscope and a photo taken. Stomatal density was assessed by counting the number of stomata in a 0.3 mm^2 area on three randomly selected locations of the image using Image J.

Harvest for size measures took place at fourteen weeks for five randomly selected replicates. Seed production (the fitness proxy) was also measured at this time. All reproductive material was removed and the number of awns was counted. Seed number was determined by multiplying awn number by five as all awns in a subsample of 100 contained five seeds. The number of seeds on the remaining five replicates were also counted at this time. At 20 weeks the number of seeds on 40 of the remaining 100 plants (two replicates of each species, site treatment) were counted. Seed number at 20 weeks was highly correlated with seed number at 14 weeks (R^2 >0.85) so seed number at 14 weeks was used in all analyses. The leaf blades were removed and dried and weighed separately to the remaining stem and petiole tissue. Roots were washed to remove all dirt prior to being dried. All biomass material was oven dried for three days at 60°C until constant weight was achieved and weighed on an electronic balance.

3.7.1 Appendix S3.1 References

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3.8 Appendix S3.2

Table A3.2.1 Mean and standard deviation of all traits for the native dry site population. n= 10 for final diameter, height, leaf length and leaf number and all growth increments (GI) of these four measures as well as time to flowering, time to seeding and number of seeds. For all other measures n=5.

	High water		**Medium water**		**Low water**		**Very low water**	
	Mean	***StDev***	***Mean***	***StDev***	***Mean***	***StDev***	***Mean***	***StDev***
F_V/F_M	8.39	0.13	8.50	0.16	8.51	0.11	8.28	0.10
Assimilation	3.35	0.15	2.65	0.46	2.87	0.34	2.18	0.35
Instantaneous water-use efficiency (iWUE)	3.04	0.09	4.82	0.97	4.56	0.21	5.27	0.57
$\text{Assimilation}_{sat}$	5.20	0.41	4.79	0.43	5.22	0.35	4.27	0.62
iWUE_{sat}	3.33	0.15	5.82	0.39	5.46	0.69	5.98	0.57
Stomatal density (abaxial)	5.45	0.31	5.37	0.17	5.31	0.35	5.59	0.37
Stomatal density (adaxial)	5.38	0.34	5.06	0.14	5.21	0.24	5.40	0.26
Leaf perimeter to area	3.17	0.12	3.02	0.28	2.96	0.92	3.33	0.77
Specific leaf area (SLA)	0.41	0.21	0.43	0.16	0.41	0.12	0.47	0.13
Water content	2.79	0.46	2.48	0.42	2.86	0.53	2.43	0.25
Leaf biomass	2.15	0.22	1.87	0.21	1.55	0.21	0.79	0.06
Aboveground biomass	3.09	0.24	2.52	0.26	1.74	0.58	1.17	0.19
Root biomass	1.41	0.31	0.92	0.41	0.62	0.11	0.54	0.19
Total biomass	3.22	0.24	2.64	0.28	2.18	0.16	1.45	0.15
Root:Shoot biomass ratio	0.06	0.02	0.06	0.03	0.06	0.02	0.11	0.05
Final diameter	6.62	0.25	6.13	0.23	5.47	1.70	5.10	1.15
Final height	6.07	0.44	5.72	0.32	5.01	1.55	4.65	1.08
Final leaf length	4.20	0.12	4.07	0.47	3.93	1.24	3.65	0.87
Final leaf number	5.00	0.39	4.39	0.52	3.65	1.11	3.17	0.77
8 wk GI diameter	0.05	0.01	0.05	0.01	0.05	0.01	0.04	0.01
8 wk GI height	0.05	0.01	0.05	0.01	0.04	0.02	0.03	0.01
8 wk GI leaf length	0.04	0.01	0.04	0.01	0.04	0.01	0.03	0.01
8 wk GI leaf number	0.05	0.01	0.05	0.01	0.04	0.01	0.04	0.01
12 wk GI diameter	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.01
12 wk GI height	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01
12 wk GI leaf length	0.00	0.01	-0.01	0.02	0.01	0.01	0.01	0.01
12 wk GI leaf number	0.29	0.07	0.21	0.10	0.18	0.09	0.10	0.06
Start of flowering	6.50	0.97	5.90	1.29	5.23	1.72	5.52	1.96
Start of seeding	7.00	1.32	6.33	1.00	5.50	1.73	5.78	1.96
Number of seeds	6.88	0.73	6.54	0.50	5.47	0.46	3.39	1.94

Appendix S3.2 Table A3.2.2 Mean and standard deviation of all traits for the native wet site population. n= 10 for final diameter, height, leaf length and leaf number and all growth increments (GI) of these four measures as well as time to flowering, time to seeding and number of seeds. For all other measures n=5.

	High water		**Medium water**		**Low water**		**Very low water**	
	Mean	***StDev***	***Mean***	***StDev***	***Mean***	***StDev***	***Mean***	***StDev***
F_V/F_M	8.52	0.08	8.59	0.09	8.53	0.12	8.33	0.15
Assimilation	3.48	0.11	3.00	0.16	2.88	0.35	2.59	0.27
Instantaneous water-use efficiency (iWUE)	3.09	0.33	4.33	0.17	4.62	0.48	4.92	0.13
$\text{Assimilation}_{sat}$	5.57	0.11	5.31	0.22	5.15	0.47	5.08	0.18
iWUE_{sat}	3.59	0.40	5.45	0.43	5.49	0.66	5.73	0.29
Stomatal density (abaxial)	5.52	0.25	5.47	0.35	4.99	0.40	5.44	0.24
Stomatal density (adaxial)	5.28	0.26	4.95	0.19	5.20	0.16	5.34	0.13
Leaf perimeter to area	2.64	0.19	2.54	0.14	2.54	0.08	2.55	0.12
Specific leaf area (SLA)	0.45	0.10	0.39	0.07	0.54	0.16	0.42	0.13
Water content	2.43	0.39	2.62	0.20	2.44	0.20	2.45	0.31
Leaf biomass	2.21	0.72	1.85	0.37	1.75	0.13	0.94	0.10
Aboveground biomass	2.99	0.35	2.36	0.34	2.11	0.12	1.18	0.10
Root biomass	2.06	0.37	1.56	0.51	1.12	0.16	0.67	0.19
Total biomass	3.30	0.29	2.66	0.42	2.34	0.12	1.44	0.16
Root:Shoot ratio	0.14	0.05	0.14	0.04	0.11	0.02	0.16	0.04
Final diameter	6.15	0.14	5.93	0.21	5.89	0.10	5.12	1.17
Final height	5.54	0.21	5.56	0.29	5.18	0.23	4.35	1.08
Final leaf length	4.22	0.10	4.25	0.13	4.19	0.12	3.66	0.80
Final leaf number	4.11	0.88	3.82	0.36	3.79	0.19	3.18	0.72
8 wk GI diameter	0.05	0.00	0.05	0.00	0.05	0.00	0.04	0.01
8 wk GI height	0.04	0.01	0.04	0.01	0.04	0.01	0.03	0.01
8 wk GI leaf length	0.04	0.00	0.04	0.00	0.04	0.00	0.03	0.01
8 wk GI leaf number	0.04	0.01	0.04	0.00	0.04	0.00	0.03	0.01
12 wk GI diameter	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.01
12 wk GI height	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.01
12 wk GI leaf length	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.01
12 wk GI leaf number	0.10	0.35	0.14	0.07	0.17	0.04	0.08	0.06
Start of flowering	6.80	1.03	7.60	1.17	7.75	1.04	7.27	1.98
Start of seeding	7.89	1.36	8.43	1.27	8.75	1.26	7.19	2.57
Number of seeds	2.71	1.94	1.08	0.98	0.36	0.80	0.48	1.07

Appendix S3.2 Table A3.2.3 Mean and standard deviation of all traits for the invasive dry site population. n= 10 for final diameter, height, leaf length and leaf number and all growth increments (GI) of these four measures as well as time to flowering, time to seeding and number of seeds. For all other measures n=5.

	High water		**Medium water**		**Low water**		**Very low water**	
	Mean	***StDev***	***Mean***	***StDev***	***Mean***	***StDev***	***Mean***	***StDev***
F_V/F_M	8.42	0.15	8.38	0.18	8.44	0.10	8.36	0.15
Assimilation	3.49	0.25	2.98	0.38	2.93	0.45	2.23	0.35
Instantaneous water-use efficiency (iWUE)	3.37	0.33	4.31	0.59	4.57	0.56	5.66	0.47
$\text{Assimilation}_{sat}$	5.59	0.33	5.25	0.25	5.28	0.51	4.96	0.30
iWUE_{sat}	3.75	0.36	4.65	0.66	5.20	0.68	6.59	0.47
Stomatal density (abaxial)	5.61	0.33	5.41	0.18	5.43	0.30	5.28	0.14
Stomatal density (adaxial)	5.48	0.24	5.36	0.20	5.11	0.25	5.36	0.26
Leaf perimeter to area	4.18	0.85	4.20	0.46	4.26	0.22	4.10	0.28
Specific leaf area (SLA)	0.48	0.18	0.40	0.18	0.26	0.12	0.43	0.24
Water content	2.77	0.76	3.24	0.70	3.41	0.76	2.83	0.82
Leaf biomass	2.01	0.27	1.56	0.40	1.27	0.30	0.62	0.24
Aboveground biomass	2.52	0.26	1.89	0.41	1.50	0.38	0.73	0.28
Root biomass	1.52	0.34	1.01	0.37	0.78	0.45	0.34	0.07
Total biomass	2.78	0.26	2.18	0.17	1.74	0.42	0.97	0.29
Root:Shoot ratio	0.12	0.04	0.10	0.07	0.13	0.07	0.13	0.03
Final diameter	5.98	0.25	5.79	0.23	5.88	0.16	4.94	1.13
Final height	5.27	0.50	4.84	0.35	4.67	0.47	3.78	0.89
Final leaf length	4.91	0.13	4.66	0.23	4.74	0.26	3.98	0.91
Final leaf number	4.72	0.55	4.27	0.43	4.11	0.37	3.31	0.78
8 wk GI diameter	0.06	0.01	0.05	0.01	0.05	0.01	0.04	0.01
8 wk GI height	0.04	0.02	0.03	0.02	0.03	0.01	0.02	0.01
8 wk GI leaf length	0.05	0.01	0.05	0.01	0.05	0.01	0.04	0.01
8 wk GI leaf number	0.04	0.00	0.04	0.00	0.04	0.00	0.03	0.01
12 wk GI diameter	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01
12 wk GI height	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02
12 wk GI leaf length	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01
12 wk GI leaf number	0.26	0.08	0.24	0.07	0.21	0.06	0.10	0.06
Start of flowering	7.10	0.99	8.50	1.08	8.11	1.27	7.74	2.28
Start of seeding	7.70	1.16	8.89	1.27	8.57	1.40	7.24	2.11
Number of seeds	5.79	1.30	3.34	2.60	3.31	2.46	2.31	2.68

Appendix S3.2 Table A3.2.4 Mean and standard deviation of all traits for the invasive wet site population. n= 10 for final diameter, height, leaf length and leaf number and all growth increments (GI) of these four measures as well as time to flowering, time to seeding and number of seeds. For all other measures n=5.

	High water		**Medium water**		**Low water**		**Very low water**	
	Mean	*StDev*	*Mean*	*StDev*	*Mean*	*StDev*	*Mean*	*StDev*
FV/FM	8.55	0.06	8.60	0.20	8.58	0.08	8.25	0.06
Assimilation	3.41	0.20	2.98	0.64	3.13	0.38	2.79	0.23
Instantaneous water-use efficiency (iWUE)	3.86	0.60	4.33	0.62	4.56	0.39	5.24	0.21
Assimilationsat	5.51	0.31	5.34	0.64	5.42	0.63	5.27	0.33
iWUEsat	4.18	1.02	5.02	0.83	5.47	0.86	5.87	0.20
Stomatal density (abaxial)	5.61	0.23	5.26	0.10	5.40	0.21	5.62	0.27
Stomatal density (adaxial)	5.42	0.20	5.12	0.29	5.58	0.13	5.59	0.34
Leaf perimeter to area	3.98	0.49	4.49	0.27	3.99	0.27	3.90	0.22
Specific leaf area (SLA)	0.55	0.19	0.61	0.27	0.49	0.11	0.73	0.12
Water content	2.73	0.50	2.66	0.93	2.60	0.39	2.32	0.86
Leaf biomass	2.18	0.12	1.86	0.22	1.68	0.11	0.99	0.31
Aboveground biomass	2.59	0.13	2.07	0.31	2.12	0.44	1.19	0.27
Root biomass	1.44	0.20	0.97	0.29	1.15	0.35	0.45	0.07
Total biomass	2.81	0.13	2.19	0.29	2.33	0.46	1.35	0.31
Root:Shoot ratio	0.10	0.02	0.10	0.04	0.10	0.04	0.10	0.03
Final diameter	6.00	0.14	5.94	0.21	5.84	0.11	5.13	1.09
Final height	5.37	0.48	5.04	0.27	4.66	0.42	4.28	1.06
Final leaf length	5.02	0.17	5.02	0.10	4.87	0.17	4.21	0.96
Final leaf number	4.35	0.52	4.13	0.21	4.07	0.25	3.44	0.74
8 wk GI diameter	0.05	0.01	0.05	0.01	0.05	0.01	0.04	0.01
8 wk GI height	0.03	0.01	0.04	0.01	0.03	0.02	0.02	0.02
8 wk GI leaf length	0.06	0.01	0.04	0.01	0.05	0.01	0.04	0.01
8 wk GI leaf number	0.04	0.01	0.04	0.01	0.04	0.01	0.03	0.01
12 wk GI diameter	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.01
12 wk GI height	0.03	0.03	0.01	0.02	0.01	0.01	0.02	0.02
12 wk GI leaf length	0.00	0.01	0.01	0.02	0.00	0.01	0.01	0.00
12 wk GI leaf number	0.23	0.09	0.19	0.05	0.18	0.05	0.11	0.04
Start of flowering	8.00	1.58	7.50	2.22	8.50	1.51	6.49	2.39
Start of seeding	8.89	1.69	8.22	2.17	8.86	1.57	6.89	3.05
Number of seeds	4.51	2.90	3.02	1.86	2.50	1.68	0.45	0.90

3.9 Appendix S3.3

Table S3.3.1 Probabilities and Benjamini-Hochberg adjusted probabilities for multiple testing from the full ANOVA of all traits

	Treatment (Tr)		**Site (Si)**		**Species (Sp)**		**Si:Tr**		**Sp:Tr**		**Sp:Si**		**Sp:Si:Tr**	
Trait	***Pr***	***Pr*.**$_{adj}$	***Pr***	***Pr*.**$_{adj}$	***Pr***	***Pr*.**$_{adj}$	***Pr***	***Pr*.**$_{adj}$	***Pr***	***Pr*.**$_{adj}$	***Pr***	***Pr*.**$_{adj}$	***Pr***	***Pr*.**$_{adj}$
F_V/F_M	**0.004**	**0.005**	**0.014**	**0.036**	0.969	0.969	0.120	0.369	0.950	0.954	0.744	0.809	0.486	0.818
Assimilation	**<0.001**	**<0.001**	**0.019**	**0.044**	0.140	0.204	0.112	0.369	0.813	0.936	0.604	0.743	0.288	0.709
Instantaneous water-use efficiency (iWUE)	**<0.001**	**<0.001**	0.352	0.451	0.393	0.503	0.164	0.369	0.819	0.936	0.447	0.641	0.338	0.772
Assimilation$_{sat}$	**0.004**	**0.005**	**0.015**	**0.036**	**0.026**	**0.049**	0.521	0.726	0.582	0.932	0.167	0.315	0.642	0.848
iWUE$_{sat}$	**<0.001**	**<0.001**	0.770	0.838	0.805	0.859	0.214	0.369	0.872	0.938	0.528	0.694	0.385	0.805
Stomatal density (abaxial)	0.243	0.324	0.812	0.838	0.409	0.503	0.767	0.843	0.641	0.932	0.416	0.641	0.069	0.330
Stomatal density (adaxial)	0.364	0.448	0.952	0.952	**0.016**	**0.034**	0.127	0.369	0.589	0.932	0.076	0.197	0.108	0.384
Leaf perimeter to area	**0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.787	0.843	0.268	0.932	0.461	0.641	0.085	0.341
Specific leaf area (SLA)	0.891	0.950	0.162	0.236	0.300	0.401	0.402	0.677	0.922	0.967	0.720	0.835	0.573	0.845
Water content	0.407	0.482	**0.006**	**0.020**	0.083	0.126	0.242	0.369	0.772	0.936	0.139	0.297	0.138	0.437
Leaf biomass	**<0.001**	**<0.001**	**0.002**	**0.006**	0.261	0.363	0.144	0.369	0.627	0.932	0.758	0.809	0.983	0.983
Aboveground biomass	**<0.001**	**<0.001**	**0.027**	0.051	**<0.001**	**0.001**	**0.038**	0.369	0.330	0.932	0.439	0.641	0.833	0.859
Root biomass	**<0.001**	**<0.001**	**<0.001**	**0.002**	**0.022**	**0.044**	0.972	0.972	0.512	0.932	**0.003**	**0.020**	**0.037**	0.299
Total biomass	**<0.001**	**<0.001**	0.201	0.306	**0.001**	**0.002**	0.157	0.369	0.879	0.938	0.076	0.197	0.765	0.848
Root:Shoot ratio	0.343	0.438	**0.023**	**0.045**	0.623	0.712	0.791	0.843	0.183	0.932	**<0.001**	**0.005**	0.513	0.821
Final diameter	**<0.001**	**<0.001**	0.314	0.431	**<0.001**	**<0.001**	**0.028**	**0.005**	**0.039**	0.414	0.168	0.284	**0.035**	0.398
Final height	**<0.001**	**<0.001**	0.323	0.431	**<0.001**	**<0.001**	0.227	0.369	0.559	0.932	0.080	0.197	0.655	0.848
Final leaf length	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	0.055	0.369	**0.025**	0.268	**0.013**	0.051	0.279	0.709
Final leaf number	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.015**	**0.036**	**0.003**	0.091	**0.022**	0.356	**0.004**	**0.018**	0.733	0.845
8 wk GI diameter	**<0.001**	**<0.001**	0.704	0.804	0.308	0.411	0.669	0.843	0.227	0.932	0.753	0.809	0.752	0.848
8 wk GI height	**<0.001**	**<0.001**	0.530	0.652	**<0.001**	**<0.001**	0.067	0.369	0.954	0.954	0.153	0.306	0.484	0.818
8 wk GI leaf length	**<0.001**	**<0.001**	0.212	0.308	**<0.001**	**<0.001**	0.670	0.843	0.333	0.932	0.289	0.487	0.698	0.848
8 wk GI leaf number	**<0.001**	**<0.001**	0.099	0.158	**<0.001**	**<0.001**	0.894	0.923	0.098	0.624	**0.020**	0.062	0.608	0.848
12 wk GI diameter	**0.004**	**0.007**	0.110	0.169	0.050	0.090	0.309	0.615	0.080	0.428	**0.036**	0.106	0.110	0.504
12 wk GI height	**0.003**	**0.005**	0.070	0.118	**0.004**	**0.010**	0.181	0.369	0.579	0.932	**0.015**	0.052	0.065	0.330
12 wk GI leaf length	0.537	0.592	**0.010**	**0.029**	0.858	0.885	0.187	0.369	0.069	0.551	0.542	0.694	0.828	0.859
12 wk GI leaf number	**<0.001**	**<0.001**	**0.001**	**0.004**	0.072	0.115	0.128	0.369	0.789	0.936	0.118	0.269	0.412	0.805
Start of flowering	0.805	0.859	**<0.001**	**0.001**	**<0.001**	**<0.001**	0.223	0.369	0.481	0.932	**<0.001**	**0.004**	**0.012**	0.192
Start of seeding	0.987	0.987	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.235	0.369	0.360	0.932	**0.001**	**0.009**	0.072	0.330
Number of seeds	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.790	0.859	0.724	0.843	0.606	0.932	**0.004**	**0.020**	0.428	0.805

Chapter 4 Winners and losers: the fitness of native and invasive *Erodium species* are differentially affected by water availability scenarios

4.1 Abstract

Phenotypic plasticity may be important in enabling plants to survive under rapidly changing conditions. However, not all plastic responses are adaptive, it is therefore crucial to understand the role of plasticity in maintaining plant fitness under different conditions.

We investigated the response of two species of *Erodium,* one invasive one native, to two forms of variation in water availability: the weekly total of water provided as well as whether this water was provided in saturating or half saturating doses. We also varied the fitness weightings of the different treatment environments in our analysis. The responses of 30 functional traits and their associated fitness were assessed for populations of each species. No clear patterns emerged with respect to adaptive or maladaptive plasticity. Instead traits that displayed adaptive plasticity at one treatment for a population were sometimes maladaptive at another treatment highlighting the importance of context in qualifying the adaptive value of plasticity.

The half saturating dose treatment had a more sever effect on fitness proxies than did the saturating dose treatment of equivalent total water amount. Plants sourced from the wet site had higher fitness in biomass whereas those sourced form the dry site had greater fitness in seed number *weight suggesting different life-history strategies.

The results demonstrate that the choice of fitness proxy and the treatment type and frequency can have dramatic effects on conclusions regarding whether plasticity in key traits is adaptive, maladaptive or neutral.

4.2 Introduction

How well plant populations will be able to adapt to rapid climate change is one of the big ecological questions of this century. The major climatic changes predicted for many Mediterranean and semi-arid systems are that they will become hotter and drier and experience more erratic rainfall (IPCC, 2007). Water availability is likely to be a key determinant of species distributions in these systems.

Water availability is determined not only by the total amount of rain that falls within a month or season but also how that rain is delivered i.e. in several small doses or fewer, larger more saturating doses. Changes in the weekly total and dose affect not only the average soil water content but how the soil water content changes over time as well as throughout the soil column (Schwinning & Ehleringer, 2001). Different water weekly total and dose regimes are likely to favour different plant trait suites (Angert et al., 2007, Jacobsen et al., 2008). Furthermore, as rainfall regimes are dynamic, the frequencies of different weekly total -dose delivery scenarios may influence which trait complexes yield highest average fitness (Schwinning & Ehleringer, 2001).

In addition to influencing average trait expression of plants, water regimes may also influence selection on plasticity in trait expression. Phenotypic plasticity occurs when a genotype's phenotypic expression of one or more traits differs as a function of environments (Bradshaw, 1965, Schlitching, 1986). Such plasticity is considered adaptive if it results in a net increase in average fitness for a given environmental scenario compared to a fixed response. Adaptive plasticity may be particularly important in heterogeneous environments where rainfall varies from year to year. However, not all plasticity is adaptive, in many cases plasticity may have no significant effect on fitness i.e. it is "neutral" and in others a plastic response may result in a net reduction in fitness, "maladaptive plasticity" (van Kleunen & Fischer, 2005).

Davidson et al. (*in review*) investigated plasticity in response to water weekly total in populations of two *Erodium* species (one invasive and one native) sourced from wet and dry sites and found greater levels of adaptive plasticity in the populations sourced from the dry site which experience greater rainfall variability. Contrary to theory and the general findings of Davidson et al. (2011) no support was found for greater overall plasticity in the invasive species for this *Erodium* study species pair in response to changes in water weekly total. They did, however, find some evidence for greater adaptive plasticity of the native species when plasticity was assessed under the more stressful water availability treatments consistent with the findings of Davidson et al., (2011). Such a scenario in which greater plasticity leads to greater fitness homeostasis when environmental condition become less favourable has been coined a "jack-of-all-trades" phenotype (Richards et al., 2006). Which traits displayed adaptive plasticity also differed for each the wet and dry populations of the native and invasive species depending on the point along the water treatment gradient at which plasticity was assessed.

As the preceding discussion demonstrates, it is important that plasticity be assessed at different ranges of the environmental treatment being investigated as the position along an environmental gradient and magnitude of the change could affect the magnitude and adaptive value of the plastic response (Hulme, 2008). It is standard in experiments measuring adaptive plasticity to take the average fitness across the experimental gradient; however, in nature the ratio of these conditions may not be equal. Furthermore, under climate change we may expect the current ratio of conditions to alter e.g. dry to wet years. Donohue et al. (2000) found that the frequency of density environments influenced the adaptive value of trait plasticity in the herb *Impatiens campensis*. Thus the frequency of environments can affect conclusions about the benefits of plasticity because the fitness value of the plastically induced phenotypes compared to a fixed phenotype may not be equal in both environments. For example, If a plastic response results in a more ideal phenotype in one environment compared to a fixed response, but a slightly less optimal phenotype in the other environment, then averaging fitness across the two environments may result in a conclusion that plasticity is maladaptive. However, if the frequency of the environment under with the plastic phenotype's fitness is maximised occurs at twice the frequency of the environment under which the suboptimal phenotype is produced, than plasticity could result in a net increase in fitness compared to the fixed response. In this study we vary the weightings of fitness in each of the water treatments rather than just averaging fitness across treatments enabling us to gain a more complete understanding of the relative fitness of the different populations (van Kleunen & Fischer, 2005).

Measurements of adaptive plasticity require selection of a fitness proxy. Ideally, fitness would be measured over multiple generations, however logistically this is rarely feasible (Hunt & Hodgson, 2010). As such, we investigate whether two common fitness proxies: total biomass and seed number*weight, differ in the patterns of adaptive plasticity identified. Because the *Erodium* species are annual, we would expect plants with larger vegetative biomasses to be able to support greater reproductive output. However, the timing of reproduction (which may respond with different degrees of plasticity to water availability conditions) could influence the efficiency and success of such a vegetative to reproductive conversion (Stephenson, 1981). Such differences in reproductive timing could result in different fitness rankings for different populations depending on the proxy chosen. This is because there is a tendency towards earlier flowering in plant populations from more arid or unpredictable sites but more delayed flowering in populations from more mesic sites (Aronson et al., 1992). Under stressful conditions however, delayed flowering can be a risky strategy as not all reserves stored during the vegetative phase may be successfully converted to reproductive biomass (Ritland, 1983).

This paper builds on the findings from Davidson et al. (*in review*) and explores three key issues with respect to plasticity and water availability.

1) The relative influence of changing water weekly total and dose on fitness and trait expression – in particular the adaptive value of plasticity.

2) How influential the frequency of favourable versus stressful water conditions are in determining the adaptive value of plasticity in key traits.

3) The influence of the fitness proxy chosen on the calculated adaptive value of plasticity in key traits.

In investigating these three key questions we also sought to understand differences between dry and wet site populations of the invasive and native *Erodium* species. More broadly, by studying the adaptive value of plasticity in key morphological, physiological and growth traits in a model species pair we hope to gain insights into the role of plasticity in enabling species and populations to cope with different water availability scenarios. Such knowledge is an important first step in making accurate predictions about the resilience of populations to climate change.

4.3 Methods

The genus *Erodium* comprises annual herbs, commonly occurring as pioneer grassland species. Several *Erodium* species have successfully colonised temperate, Mediterranean and semi-arid regions around the world (see references within: http://www.hear.org/gcw/species/). *Erodium crinitum* is native to Australia, whereas *E. cicutarium* is believed to have arrived in Australia approximately 200 years ago and is native to the Mediterranean region. *E. crinitum* generally grows to a larger size in wet habitats compared to *E. cicutarium* (up to one metre compared to approximately 60 cm in height) although in dry habitats both species usually form small rosettes <15cm high (AMD *pers obs*).

Seeds of both species were collected from two sites in Australia during Spring (Sep-Nov) 2008. Seed was located at two main sties: a “wet” site: Canberra and a “dry “ site: Mildura. Canberra has an average annual rainfall of 620 mm yr^{-1} and is located in inland temperate South-eastern Australia (35.25°S, 149.13°E). The dry site, Mildura is located approximately 1000 kilometres west of Canberra in the semi-arid region of Australia (34.22°S, 142.15°E). Mildura has an average annual rainfall of 290 mm yr^{-1}.

At each of the main sites seeds were collected from approximately twelve locations in order to maximise genetic variation. See was collected form one to four mother plants at each location. Locations within sites were separated by approximately five kilometres. *Erodium* species generally have very limited seed dispersal (Stamp, 1989) and as such geographic distance is a good proxy for genetic relatedness. The potential influence of location was tested using mantel tests and found to be minimal, see Davidson et al. (*in review*).

4.3.1 Experiment set-up

With the exception of the water treatments, the experiment set-up was identical to that of Davidson et al. (*in review*). Plants were grown in 70 cm PVC pipes in a sand:clay subsoil: mushroom compost mix (75:15:10) with slow release Osmocote® Native Gardens fertiliser (17.9N:0.8P:7.3K) mixed through the soil at the recommended rate ($5gL^{-1}$). The tubes were spaced 30 cm apart in steel mesh racks and placed in a greenhouse, which was at kept between 20°C and 35°C during the day and 5°C and 15°C at night.

Because in natural situations water delivery varies not only by weekly total but also in the pattern in which is it delivered, plants were grown under three distinct water treatments. These treatments comprised two different total volumes of water provided per week (hereafter referred to as weekly total) high (5250 mL $week^{-1}$) and low (750 mL $week^{-1}$). In addition to weekly total we also varied the amount of water that was applied during a single watering event (hereafter referred to as dose). There were two different doses, saturated (750 mL $dose^{-1}$, which left all soil in the 70 cm pipe completely saturated) and half-saturated (325 mL $dose^{-1}$). Irrigation was triggered using a Micro-grow vapour-pressure deficit (VPD) system (see Nicotra et al. (2007) for further details of the system). There were three watering treatments altogether; high-sat (weekly total of 5250 mL supplied as daily saturating 750 mL doses), low-sat (weekly total of 750 mL supplied as one dose) and low-½sat (weekly total of 750 mL, supplied as two

325 mL doses). Thus, the low-½sat treatment received the same weekly total as the low-sat, but water was supplied in smaller doses and at twice the frequency – resulting in lower relative water content in the bottom third of the pvc pipes. Soil moisture readings for the lower third of the pvc pipe as taken with a ThetaProbe Soil Moisture Sensor in week two after treatments were imposed were approximately 20-25% soil water capacity in the half saturating dose treatments compared to 50-60% soil water capacity in the saturated treatments.

In total, the experiment included the two species collected from two sites each and three water treatments with ten replicates for each combination resulting in 120 plants. The plants were arranged in five blocks in a randomised block design (two replicates per block) to control for variation in light and temperature.

4.3.2 Measurements

Traits were chosen to cover a range of size, growth, leaf morphology and physiological traits. In total, 31 traits were measured. These traits were: $F_VF_M^{-1}$, Assimilation (A), Instantaneous water-use efficiency (WUE_i), A_{sat}, WUE_{isat}, Stomatal density (abaxial), Stomatal density (adaxial), Leaf dissection index (LDI), Leaf mass per area (LMA), Leaf water content, Leaf biomass, Aboveground biomass, Root biomass, Total biomass, Root to shoot ratio (RSR), Final diameter, Final height, Final leaf length, Final leaf number, Juvenile growth increment (GI) diameter, Juvenile GI height, Juvenile GI leaf length, Juvenile GI leaf number, Mature GI diameter, Mature GI height, Mature GI leaf length, Mature GI leaf number, Number of seeds, weight of seeds and Time to flowering. Further details are available in Davidson et al. (*in review*) with the exception of seed weight. Seed weight was determined by measuring a sample of 100 ripe seeds (or the total number of seeds produced where less than 100 seeds were available) of at least three plants of each of the four populations for each of the three treatments.

4.3.3 Statistical analyses

4.3.3.1 Analysis of variance

Log transformations and square root transformation were applied as necessary to normalise data. Analysis of Variance (ANOVA) tests were conducted to determine the effects of the three water treatments on the fitness proxies: seed number*weight and total biomass and the other 28 measured traits. Species, site, and water treatment were used as fixed factors in the ANOVA. A term for block was also included to account for any variance due to different lighting or temperature across the glasshouse blocks. We investigated the significance of all fixed factors and their two-way interactions (full results at Appendix S4.1). Probabilities were adjusted for multiple comparison using the Benjamini-Hocherg method (Benjamini & Hochberg 1995).

To test whether significant treatment effects for our fitness proxies: seed number *weight and biomass were a due only to a variation in water amount rather than also being caused by the change in water dose, we ran separate ANOVAs for each treatment combination (high-sat vs low-sat, high-sat vs low-½ sat and low-sat vs low-½ sat (Appendix S4.2). There was no significant treatment term for seed number * weight for the low-sat vs low-½sat comparison, as such separate ANOVAs for each of the populations were performed to see if any of the four *Erodium* populations responded significantly to this treatment combination (Appendix S4.3).

4.3.3.2 Plasticity Index analyses

Plasticity was quantified using a modified version of the plasticity index (PI) (e.g. Valladares et al. 2000)

$$\frac{|(x_1 - x_2)|}{|\max(x_1, x_2)|} \quad \textit{Equation 1}$$

where: x_1, x_2 = trait value in treatment 1 and treatment 2.

We calculated PI for each treatment pair in each block for each population instead of using mean trait values, so as to account for any block effect and enable regression analyses against fitness. We calculated PI between all three treatment combinations (high-sat to low-sat, high-sat to low-½sat and low-sat to low-½sat). PI was calculated on all 19 traits that displayed a significant water treatment effect when corrected for multiple testing (Appendix S4.1).

The adaptive value of plasticity is assessed by linear regressions between the plasticity index of each of the traits and relative fitness for each of the two fitness proxies: seed number*weight and total biomass. (Relative fitness was obtained by dividing fitness by the maximum value for that population and treatment). A significant positive slope indicates that plasticity in that trait is adaptive, whereas as significant negative slope indicates plasticity is maladaptive. Regressions were considered significant if $P<0.05$.

We chose to calculate PI by dividing the difference between the trait values in each environment by the maximum trait value rather than just the difference between trait values, to control for the fact that a larger trait value in one environment can result in a larger total difference in trait values between environments (but not necessarily a larger relative change in trait value than a plant with a smaller average trait value). This problem was identified by van Tienderen (1991) who proposed to put both the mean trait value as well as the trait difference between environments in the regression with fitness. This was not possible with our data however, as we found that the trait value and trait difference were highly correlated thus including both in the regression would violate the assumptions of the general linear model (see discussion in Auld et al. 2010). We believe that by calculating PI as we have (*Equation 1*), we adequately address the issue of bias associated with larger trait differences occurring in larger plants.

We varied the ratio of the fitness environments for our regressions. As such, we not only ran regressions with the fitness of each water treatment weighted evenly between treatments as is standard in analyses of adaptive plasticity but analysed regressions of plasticity against fitness with four other fitness weightings. The five fitness weightings were: 90:10 (e.g. high-sat fitness *0.9 plus low-sat fitness *0.1), 75:25, 50:50, 25:75 and 10: 90. We conducted the regressions separately for each of the four species-site populations for each of the fitness proxies: seed number*weight and total biomass.

4.4 Results

4.4.1 Effect of varying water weekly total and dose on plant fitness depends on proxy

Reduction of either water weekly total or dose significantly reduced each of the two fitness proxies: seed number * weight and total biomass (Table 4.1, Figure 4.1). Population differences in response to the water treatments were more pronounced for seed number than for total biomass (Figure 4.1). However only the native-dry population displayed a significant difference in seed number * weight between the low-sat and low- ½ sat treatments (Figure 4.1, Appendix S4.1).

Table 4.1 ANOVA results for water treatment effect on the two fitness proxy responses of the four *Erodium* populations.

Factor	Trait	MS	F	Pr>0
Site	Seed number * weight	124.10	35.54	<0.001
Site:Treatment	Seed number * weight	0.46	0.13	0.72
Species	Seed number * weight	0.00	0.00	1.00
Species:Site	Seed number * weight	53.34	15.27	<0.001
Species:Treatment	Seed number * weight	0.36	0.10	0.75
Treatment	Seed number * weight	49.82	14.27	<0.001
Site	Total biomass	0.89	5.36	0.03
Site:Treatment	Total biomass	0.27	1.60	0.21
Species	Total biomass	1.26	7.56	0.01
Species:Site	Total biomass	0.01	0.07	0.80
Species:Treatment	Total biomass	0.10	0.58	0.45
Treatment	Total biomass	11.22	67.26	<0.001

The dry site populations had highest seed production whereas the wet site populations had highest biomass. However the wet site populations showed greater homeostasis in seed number*weight than did dry site populations, the reverse was true for total biomass. Thus the greater fitness in seed number* weight of the dry site populations, and greater fitness in biomass of the wet site population are due to a "master-of-some" style response (Richards et al.

2006). It should be noted, however, that in absolute terms the dry site populations always out-performed the wet site populations in seed number * weight and the wet site populations always outperformed the dry-site populations in total biomass (Figure 4.2).

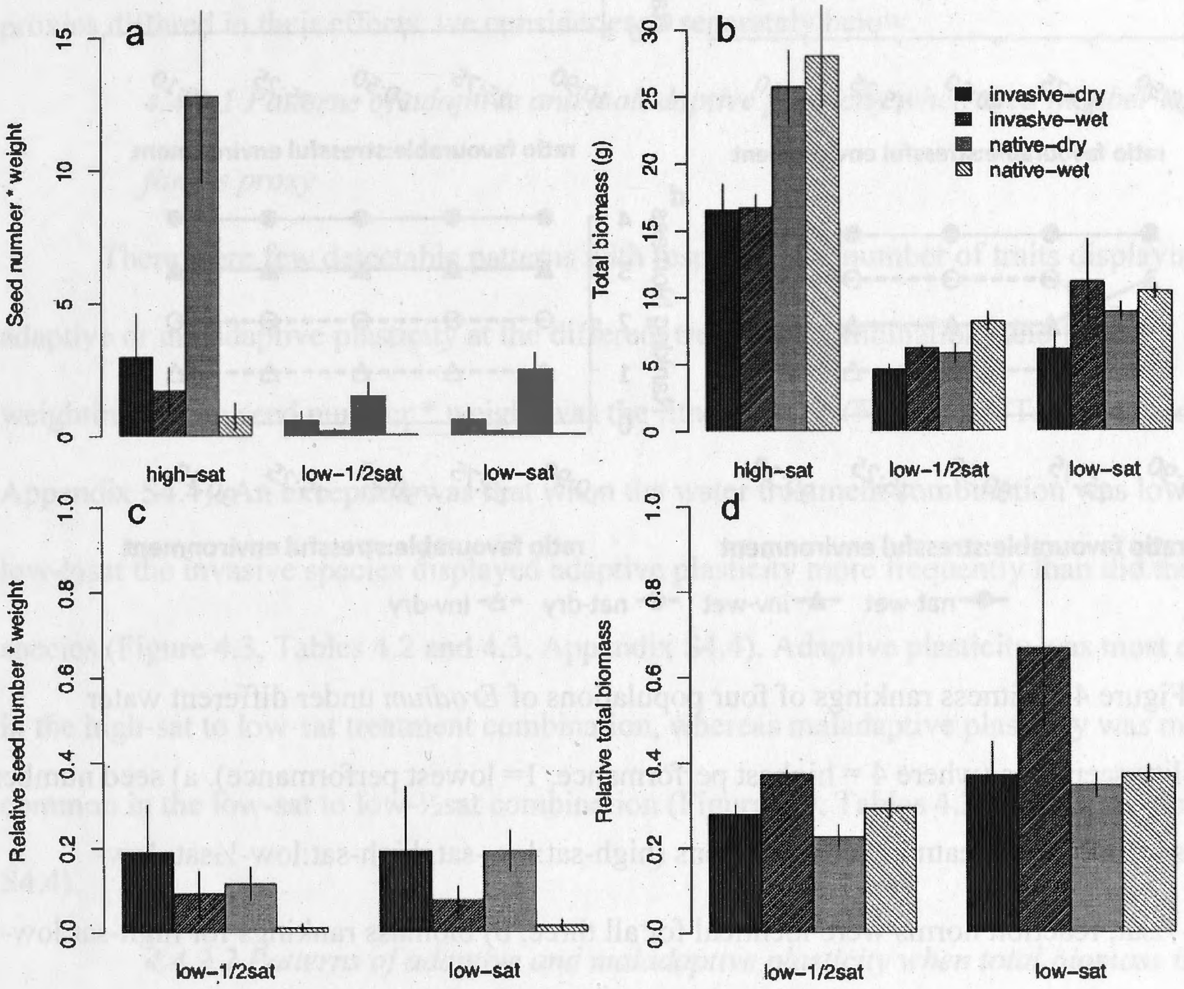

Figure 4.1 Column graphs of fitness a) seed number *weight, b) total biomass c) relative seed number of population compared to high-sat treatment and d) relative biomass of population compared to high-sat treatment of the four *Erodium* populations at three different water availability treatments, nat = native species: *Erodium crinitum* inv= invasive species: *E. cicutarium*, wet = wet site origin: Canberra, dry = dry site origin: Mildura, error bar = ±1 standard error.

The native species had a greater biomass than the invasive species in general, reflected in the significant species effect (Table 4.1). Under the more stressful water scenarios, however, the invasive wet populations actually outperformed the native-dry populations (Figure 4.2 b,c).

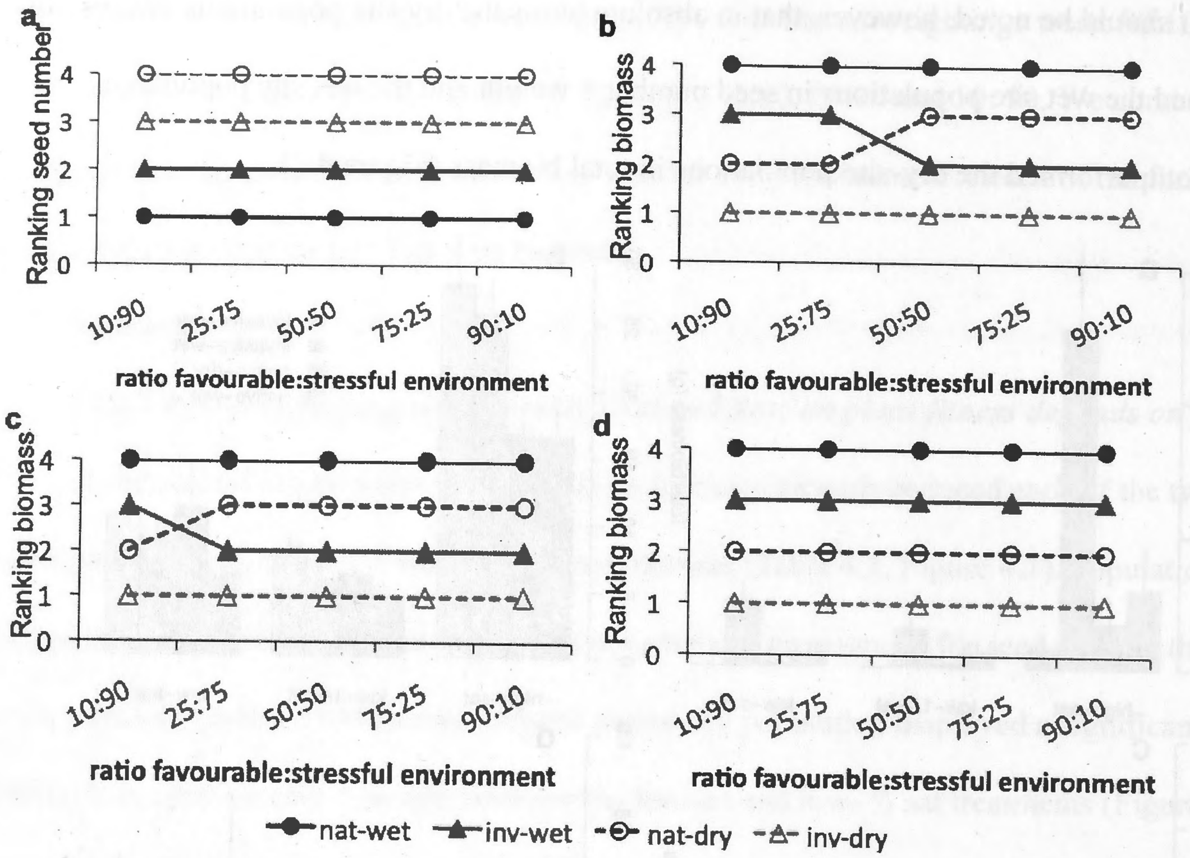

Figure 4.2 Fitness rankings of four populations of *Erodium* under different water availability scenarios (where 4 = highest performance, 1= lowest performance). a) seed number rankings for all three treatment combinations (high-sat:low-sat, high-sat:low-½sat, low-sat:low-½sat, reaction norms were identical for all three, b) biomass rankings for high-sat:low-sat scenarios, c) biomass rankings for high-sat:low-½sat scenarios, d) biomass rankings for low-sat:low-½sat scenario.

4.4.2 Patterns of adaptive and maladaptive plasticity

Having demonstrated that the fitness proxies respond to the different water treatments and that there are significant differences between populations in these responses we now investigate the adaptive value of plasticity in underlying traits for each of these fitness proxies in the four *Erodium* populations. The traits displaying adaptive and maladaptive plasticity often differed between populations. The water availability scenarios were influential (both in terms of the treatment combination: high-sat to low-sat high sat to low-½sat or low-sat to low-½sat, as well as the fitness weightings of these treatments), such that traits that were adaptive at the fitness weightings favoruing the less stressful environment, often were not positively (or significantly) correlated with fitness at the more stressful scenarios (high proportion of either the low –sat or low low-½sat treatments) and vice versa (Appendix S4.4). Because the fitness proxies differed in their effects, we consider each separately below.

4.4.2.1 Patterns of adaptive and maladaptive plasticity when seed number is the fitness proxy

There were few detectable patterns with respect to the number of traits displaying adaptive or maladaptive plasticity at the different treatment combinations and fitness weightings when seed number * weight was the fitness proxy (Figure 4.3, Tables 4.2 and 4.3, Appendix S4.4). An exception was that when the water treatment combination was low-sat to low-½sat the invasive species displayed adaptive plasticity more frequently than did the native species (Figure 4.3, Tables 4.2 and 4.3, Appendix S4.4). Adaptive plasticity was most common in the high-sat to low-sat treatment combination, whereas maladaptive plasticity was most common in the low-sat to low-½sat combination (Figure 4.3, Tables 4.2 and 4.3, Appendix S4.4).

4.4.2.2 Patterns of adaptive and maladaptive plasticity when total biomass is the fitness proxy

There were no strong patterns with respect to which traits displayed adaptive plasticity for any given population or water treatment scenario. Overall maladaptive plasticity was actually more common than adaptive plasticity when biomass was the fitness proxy (Figure 4.3, Tables 4.2 and 4.3, Appendix S4.4). Maladaptive plasticity was most common in the native-dry population. (Figure 4.3, Tables 4.2 and 4.3, Appendix S4.4).

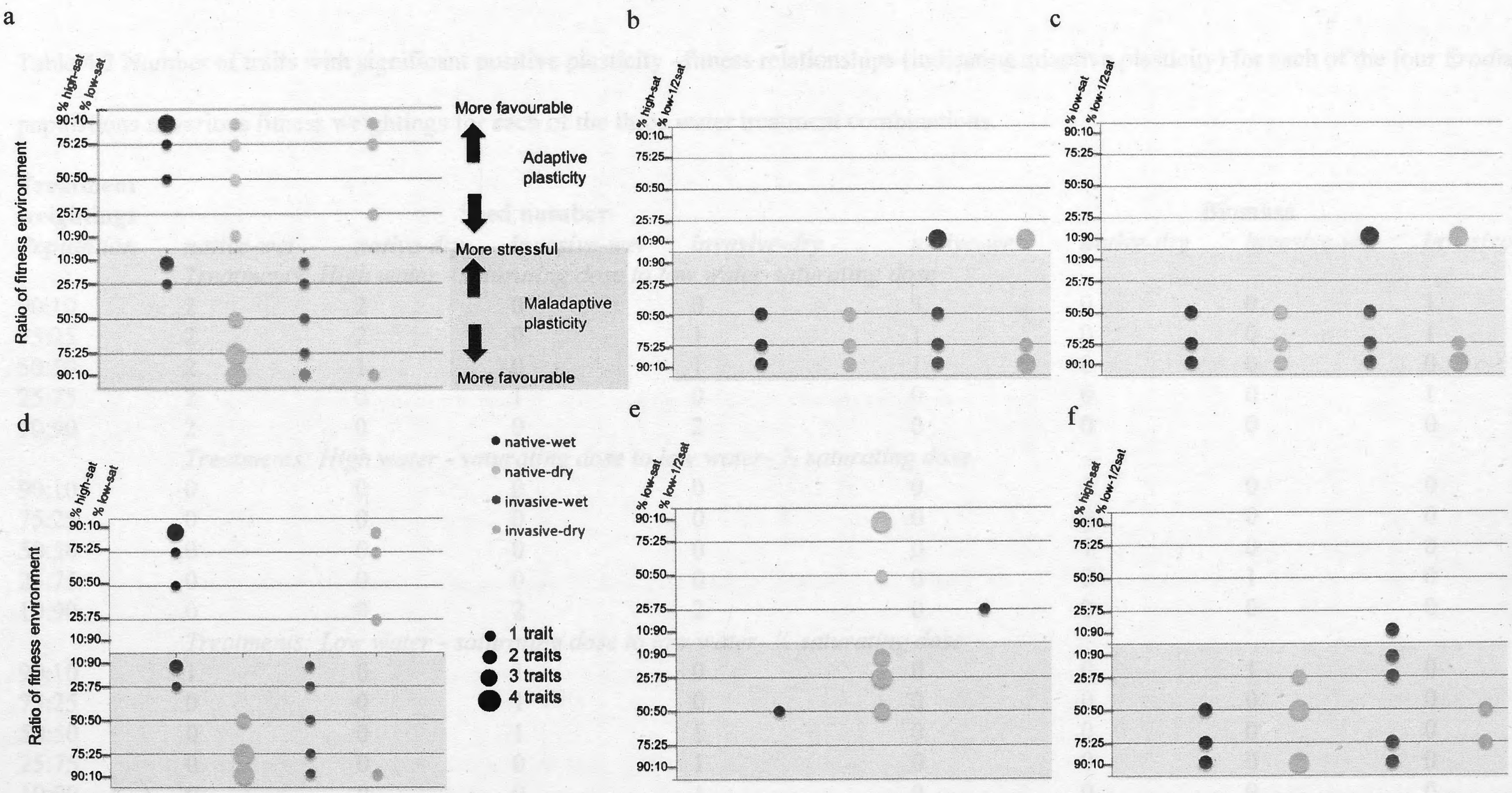

Figure 4.3. Bubble graph depicting the number of traits displaying adaptive plasticity and maladaptive plasticity (shaded area) to a change in water treatment for populations of a native and invasive *Erodium spp* sourced from a wet and dry site. The y-axis represents different weighting of the fitness environments a & d) high water saturating dose (high-sat) to low water saturating dose (low-sat), b & e)

Table 4.2 Number of traits with significant positive plasticity –fitness relationships (indicating adaptive plasticity) for each of the four *Erodium* populations at various fitness weightings for each of the three water treatment combinations.

Treatment weightings	**Seed number**				**Biomass**			
Population	***native-wet***	***native-dry***	***invasive-wet***	***invasive-dry***	***native-wet***	***native-dry***	***invasive-wet***	***invasive-dry***
	Treatments: High water - saturating dose to low water-saturating dose							
90:10	2	2	0	0	3	0	0	1
75:25	2	2	0	1	1	0	0	1
50:50	2	1	0	1	1	0	0	0
25:75	2	0	1	0	0	0	0	1
10:90	2	0	0	2	0	0	0	0
	Treatments: High water - saturating dose to low water- ½ saturating dose							
90:10	0	0	0	0	0	3	0	0
75:25	0	0	0	0	0	0	0	0
50:50	0	0	0	0	0	1	0	0
25:75	0	0	0	0	0	0	1	0
10:90	0	0	2	2	0	0	0	0
	Treatments: Low water - saturating dose to low water- ½ saturating dose							
90:10	1	0	1	0	0	0	1	0
75:25	0	0	1	0	0	0	0	0
50:50	0	0	1	1	0	0	0	0
25:75	0	0	0	1	0	0	0	0
10:90	0	0	0	1	0	0	0	0

Table 4.3 Number of traits with significant negative plasticity –fitness relationships (indicating maladaptive plasticity) for each of the four *Erodium* populations at various fitness weightings for each of the three water treatment combinations.

Treatment weightings	**Seed number**				**Biomass**			
Population	***native-wet***	***native-dry***	***invasive-wet***	***invasive-dry***	***native-wet***	***native-dry***	***invasive-wet***	***invasive-dry***
	Treatments: High water - saturating dose to low water-saturating dose							
90:10	0	1	0	0	0	4	1	1
75:25	0	1	0	0	0	4	1	0
50:50	0	1	0	1	0	2	1	0
25:75	0	2	0	0	1	0	1	0
10:90	0	1	0	2	2	0	1	0
	Treatments: High water - saturating dose to low water- ½ saturating dose							
90:10	1	1	1	2	0	0	0	0
75:25	1	1	1	1	0	0	0	0
50:50	1	1	1	0	1	2	0	0
25:75	0	0	0	0	0	3	0	0
10:90	0	0	0	0	0	2	0	0
	Treatments: Low water - saturating dose to low water- ½ saturating dose							
90:10	0	0	0	1	1	2	1	0
75:25	0	0	0	0	1	0	1	1
50:50	0	0	0	0	1	2	0	1
25:75	0	1	0	0	0	1	1	0
10:90	0	0	0	0	0	0	1	0

4.5 Discussion

We investigated the adaptive value of plasticity in 30 key physiological, morphological and growth traits in wet and dry populations of an invasive and native *Erodium*. In doing so we sought to understand the relative influence of changes in water dose and weekly total water. In addition, we assessed differences between population fitness rankings using two fitness proxies: seed number*weight and total biomass. Finally, we evaluated the effects of changing the frequency of favourable to stressful water scenarios on patterns of adaptive and maladaptive plasticity.

4.5.1 The effect of varying water weekly total and dose

What is perhaps most interesting about the results is that the low-½ sat treatment was more severe than the low-sat treatment (Figure 4.1) despite watering occurring twice as frequently in the low-½sat treatment. There are few other studies examining the effect of both water weekly total and dose in an ecological context. Novoplansky & Goldberg (2001) is one such study and they find significant effects on traits for both water weekly total and dose. They find that the water dose rather than weekly total had the greatest effect on the competitive hierarchies of the species they investigated.

A lower water dose will result in dry soil in the lower part of the soil column in the pots thereby restricting the area in which roots are able to uptake water to the shallower soil. As shallow soil creates a harsher environment due to higher temperatures and drying, plants often need to invest more carbon into their roots in order to produce roots that can withstand these conditions (Schwinning & Ehleringer, 2001). The root architecture of *Erodium* species typically consists of a long tap-root, which most likely is an advantage for following water deep into the soil profile. The long pipes in which the plants were grown took several days to dry out at the bottom but drying in the top part of the pipes occurred relatively quickly. This may explain why the *Erodium* species were more affected by the low-½sat treatment than the low-sat treatment. Indeed, Padilla et al. (2009) found that specific root length i.e. the fineness of roots, was significantly affected by dose but not by water weekly total.

4.5.2 The influence of the chosen fitness proxy

The fitness proxy chosen had a profound effect on which plants were selected as the "winners" with respect to highest average performance across the different water availability scenarios tested. When seed number*weight was the fitness proxy the plants of dry site origin, in particular the native dry site population, significantly outperformed plants from the wet site (Figures 4.1 and 4.2). However, the results were largely reversed when biomass was used as the fitness proxy.

It is often reported that *Erodium crinitum*, the Australian native, is a much larger plant than *E. cicutarium*, the invasive species. Although both wet and dry site populations of the native species maintained higher biomass in favourable water scenarios, when conditions become stressful (such as in the 25% and 10% weightings for the high-sat vs low-sat or low-½sat) the invasive wet site population actually had greater biomass than the dry site origin native plants (Figure 4.2).

These fitness results suggest a trade-off between maintaining vegetative production and reproductive effort. A greater reproductive: vegetative allocation when resources are limited, such as displayed by the dry site populations, is typical of desert and semi-arid plants (Aronson et al., 1992). Seed number is important for species survival – particularly when the probability of any one seed arriving in favourable germination conditions is low, furthermore production of many small seeds may be favoured in annuals such as *Erodium* species when environmental conditions for plant growth and survival deteriorate (Stamp, 1990). However, when there is high aboveground competition, biomass may be critical to maintain space, secure nutrients and access to light and thus be able to effectively reproduce. Indeed, under favourable conditions plants may even delay reproduction and switch to a more perennial or at least biennial phenotype (Bazzaz & Grace, 1997). Although this has not previously been reported in these species we believe it is plausible because previous studies have not compared *Erodium* populations from such divergent high and low rainfall sites as were included in the current study. As such, we advocate the use of seed number and weight or other reproductive measure in preference to biomass as a fitness proxy in semi-arid or otherwise stressful conditions for annual or facultatively annual species where conversion of biomass to reproductive output may not be fully effective.

4.5.3 Patterns of adaptive plasticity

No one trait or group of traits emerged as generally showing adaptive plasticity even when we investigated within a single population or treatment combination. Rather, several traits measured in this experiment displayed adaptive plasticity in a particular circumstance but in many cases these same traits displayed maladaptive plasticity under other circumstances even within a population (Appendix S4.4). Understanding when and in which traits plasticity will be adaptive in plants is important for making predictions about the expansions of invasive plants, adaption of species and populations to climate change and breeding improved food crops in agriculture (Nicotra et al., 2010). Although this study was limited to just four populations encompassing two *Erodium* species, if there were clear patterns in traits these would have been detected. Thus, we may need to think carefully about which traits, and under what conditions, we measure adaptive plasticity if we are to make broad conclusions about its role. The results also suggest that even when plasticity is neutral (neither beneficial to fitness nor detrimental) under some conditions, plasticity in this trait could become important under future conditions.

We expected to find adaptive plasticity in a greater number of traits in dry-site compared to wet-site populations particularly in the more stressful scenarios however this was not supported. If anything, for seed number, the native-dry population displayed the greatest maladaptive plasticity. We also expected a greater number of traits displaying adaptive plasticity in the invasive compared to the native species populations. This was true only when seed number*weight was the fitness proxy and then only for the high-sat to low-½sat and low-sat to low-½ sat treatment combinations. These patterns of adaptive plasticity did not correlate with the differences in fitness between populations. Thus, plasticity in the traits measured here does not appear to be important for maintaining biomass or seed number*weight.

4.5.4 Conclusion

Importantly for future studies, we found that patterns of adaptive plasticity were strikingly flexible; with no consistent patterns either across conditions or populations. The stressfulness of the scenarios had a large impact on fitness rankings and which traits displayed adaptive or maladaptive plasticity. We found that the choice of fitness proxy had a significant influence on the patterns of adaptive plasticity that were detected. In general, we found if a population displayed a greater number of traits with adaptive plasticity this did not necessarily infer greater fitness.

4.6 References

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4.7 Appendix S 4.1

Table S4.1.1 ANOVA results for pair-wise treatment comparisons on fitness proxies

Treatments	Fitness proxy	Factor	DF	SS	MS	F	Pr>0
high-sat vs low-1/2sat	biomass	site	1	21.88	21.88	1.21	0.28
high-sat vs low-1/2sat	seed	site	1	154.61	154.61	14.59	<0.001
high-sat vs low-sat	biomass	site	1	27.75	27.75	1.22	0.28
high-sat vs low-sat	seed	site	1	169.28	169.28	16.32	<0.001
low-sat vs low-1/2sat	biomass	site	1	53.34	53.34	12.07	0.002
low-sat vs low-1/2sat	seed	site	1	13.97	13.97	26.97	<0.001
high-sat vs low-1/2sat	biomass	species	1	361.95	361.95	20.03	<0.001
high-sat vs low-1/2sat	seed	species	1	54.34	54.34	5.13	0.03
high-sat vs low-sat	biomass	species	1	366.52	366.52	16.08	<0.001
high-sat vs low-sat	seed	species	1	69.10	69.10	6.66	0.01
low-sat vs low-1/2sat	biomass	species	1	22.34	22.34	5.06	0.03
low-sat vs low-1/2sat	seed	species	1	3.45	3.45	6.67	0.01
high-sat vs low-1/2sat	biomass	species:site	1	5.11	5.11	0.28	0.60
high-sat vs low-1/2sat	seed	species:site	1	81.99	81.99	7.74	0.01
high-sat vs low-sat	biomass	species:site	1	0.17	0.17	0.01	0.93
high-sat vs low-sat	seed	species:site	1	91.37	91.37	8.81	0.01
low-sat vs low-1/2sat	biomass	species:site	1	2.85	2.85	0.64	0.43
low-sat vs low-1/2sat	seed	species:site	1	5.53	5.53	10.68	0.003
high-sat vs low-1/2sat	biomass	treatment	1	2352.17	2352.17	130.15	<0.001
high-sat vs low-1/2sat	seed	treatment	1	161.17	161.17	15.21	0.001
high-sat vs low-sat	biomass	treatment	1	1422.65	1422.65	62.42	<0.001
high-sat vs low-sat	seed	treatment	1	138.49	138.49	13.35	<0.001
low-sat vs low-1/2sat	biomass	treatment	1	72.36	72.36	16.38	<0.001
low-sat vs low-1/2sat	seed	treatment	1	0.86	0.86	1.66	0.21
high-sat vs low-1/2sat	biomass	treatment:site	1	1.54	1.54	0.09	0.77

Table S4.1.1 *cont* ANOVA results for pair-wise treatment comparisons on fitness proxies

Treatments	**Fitness proxy**	**Factor**	**DF**	**SS**	**MS**	**F**	**Pr>0**
high-sat vs low-1/2sat	seed	treatment:site	1	86.86	86.86	8.20	0.01
high-sat vs low-sat	biomass	treatment:site	1	6.03	6.03	0.26	0.61
high-sat vs low-sat	seed	treatment:site	1	71.89	71.89	6.93	0.01
low-sat vs low-1/2sat	biomass	treatment:site	1	1.64	1.64	0.37	0.55
low-sat vs low-1/2sat	seed	treatment:site	1	0.81	0.81	1.57	0.22
high-sat vs low-1/2sat	biomass	treatment:species	1	183.29	183.29	10.14	0.003
high-sat vs low-1/2sat	seed	treatment:species	1	30.33	30.33	2.86	0.10
high-sat vs low-sat	biomass	treatment:species	1	181.42	181.42	7.96	0.01
high-sat vs low-sat	seed	treatment:species	1	29.62	29.62	2.86	0.10
low-sat vs low-1/2sat	biomass	treatment:species	1	0.33	0.33	0.08	0.79
low-sat vs low-1/2sat	seed	treatment:species	1	0.47	0.47	0.91	0.35

4.8 Appendix S4.2

Table S4.2.1 ANOVA results for treatment effects of low-sat to low- ½ sat water treatment for individual *Erodium* populations for seed number * weight

Population	**SS**	**MS**	**F**	**Pr<0**
native-dry	2.1537	2.1537	6.3455	0.045347
native-wet	0.0000225	0.0000225	0.0567	0.8186
invasive-wet	0.001999	0.001999	0.0577	0.8171
invasive-dry	0	0.00003	0.0001	0.994

4.9 Appendix S4.3

Table S4.3.1 ANOVA results for water treatment effect on all thirty traits. Adjusted probabilities are adjusted for multiple testing using the Benjamini-Hochberg method.

Trait	**MS**	**F**	**Pr**	**Pr$_{adj}$**
$F_VF_M^{-1}$	0.02	1.84	0.181	0.228
Assimilation	2.53	19.40	<0.001	<0.001
Instantaneous water-use efficiency ($_i$WUE)	17.25	57.62	<0.001	<0.001
Assimilation$_{Sat}$	0.39	1.84	0.181	0.228
$_i$WUE$_{Sat}$	33.08	62.00	<0.001	<0.001
Abaxial stomatal density	0.54	7.77	0.008	0.016
Adaxial stomatal density	0.14	2.17	0.148	0.205
Leaf water content	0.52	1.28	0.263	0.318
Leaf dissection index	5.39	18.69	<0.001	<0.001
Specific leaf area	0.00	0.03	0.862	0.887
Leaf biomass	5.03	30.99	<0.001	<0.001
Aboveground biomass	12.12	63.85	<0.001	<0.001
Root biomass	5.71	59.84	<0.001	<0.001
Root to shoot ratio	0.00	0.02	0.887	0.887
Final diameter	3.13	46.35	<0.001	<0.001
Final height	8.12	42.89	<0.001	<0.001
Final leaf length	0.29	5.74	0.018	0.031
Final leaf number	9.48	41.34	<0.001	<0.001
Juvenile Growth Increment (GI) diameter	0.00	10.87	0.001	0.003
Juvenile GI height	0.00	0.60	0.440	0.491
Juvenile GI leaf length	0.00	50.66	<0.001	<0.001
Juvenile GI leaf number	0.00	3.65	0.059	0.085
Mature GI diameter	0.00	4.14	0.044	0.067
Mature GI height	0.00	15.38	<0.001	<0.001
Mature GI leaf length	0.00	5.05	0.027	0.043
Mature GI leaf number	0.10	5.86	0.017	0.031
Start of flowering	0.95	0.65	0.423	0.490
Seed number*weight	49.82	14.27	<0.001	0.001
Total biomass	11.22	67.26	<0.001	<0.001

4.10 Appendix S4.4

Table S4.4.1 Coefficients and probabilities from linear regressions of Plasticity index against relative fitness for each of the treatment combinations and fitness proxies for all traits with significant treatment effects.

see separate excel sheet

Chapter 5 Plastic responses to water availability and competition in native and invasive *Erodium* species - are two stresses one too many?

5.1 Abstract

Phenotypic plasticity enables organisms to express environmentally dependent phenotypes; and therefore may enable organisms to express higher fitness in heterogeneous environments than a fixed phenotype. Such adaptive plasticity is not found as often as one might expect given the advantages it confers. As such it has been proposed that there must be constraints (i.e. costs and limits) to plasticity. Under stressful conditions, such as when multiple resources are limiting, the relative value of responding plastically to one particular environmental factor may be limited and the costs associated with plasticity exacerbated. This theory was tested using two species, a native and invasive *Erodium* species sourced from the dry and wet end of their environmental range in southeastern Australia. Results indicate that the evolutionary history of the populations influenced the patterns of adaptive plasticity and evidence of costs and limits of plasticity in *Erodium* species. For example, plants from populations sourced from the drier, more heterogeneous site displayed more adaptive plasticity towards changes in water availability. Although I did find evidence of constraints to adaptive plasticity, I found little support for the popular theory that limits and costs to plasticity are higher under more stressful conditions.

5.2 Introduction

As sessile organisms, plants must be able to withstand changing environmental conditions. In some circumstances, adaptation will result in a fixed phenotype capable of tolerating the suite of environmental conditions encountered in the habitat. Alternatively, selection may favour phenotypic plasticity – the ability of an organism to alter its morphological, anatomical or developmental expression when environmental conditions change (Scheiner 1993). It is often thought that plasticity will evolve when the environmental conditions change in a predictable manner at a spatial and temporal scale relevant to an individual plant (Alpert and Simms 2002). Whether or not a change in a given environmental factor is detectable and relevant to a plant may depend on the broader environmental context.

It has frequently been argued that because adaptive phenotypic plasticity (plasticity that results in a net fitness benefit for the genotype compared to a fixed response) is not ubiquitous there must be costs and limits associated with either expressing an alternate phenotype or with having the capacity to respond plastically (DeWitt et al 1998). Costs of plasticity exist when a plant exhibiting a plastic phenotype has lower fitness than a plant expressing the same trait value but through a fixed phenotype (DeWitt et al 1998; van Kleunen and Fischer 2005). Limits to plasticity are less well defined than costs: definitions of a limit include environment specific reductions in fitness (van Kleunen and Fischer 2005) as well as the inability to achieve maximum trait values through plasticity (deWitt et al 1998). Furthermore, van Kleunen and Fischer (2005) proposed that some limits such as "developmental range limits" (see deWitt et al 1998) are not really limits but underpinned by costs. Auld et al (2010) take this further and suggest that many hypothesised limits are most likely related to environment specific costs. I concur: the line between costs and limits is blurred and suggests that in many cases it is not necessary to distinguish between costs and limits. In this paper, I refer to constraints on plasticity where a constraint may be underpinned by a cost, a limit or both.

I ask what the effects are of varying the environmental conditions on the expression of plasticity? In particular I look at whether plasticity to one factor is constrained when a second environmental factor is present. Such a constraint on plasticity is often referred to in the literature as "ecological limits" (Valladares et al 2007). Although the net effect of multiple stresses need not mean that a fixed response is favoured over a plastic response; such interactions of stresses can change the selective pressures on traits (McGuire and Agrawal 2005), and thereby limit optimal phenotype production in response to any one environmental factor (see Tonsor and Scheiner 2007). Constraints on plasticity, which may be exacerbated by the presence of multiple stresses, include those related to phenotypic integration (the phenomenon of functionally related traits to co-vary, Pigliucci 2003), both due to genetic factors such as pleitropy and epistasis as well as functional relationships between traits (Sultan and Spencer 2002). The difficulties in correctly interpreting and responding to environmental signals (McGuire and Agrawal 2005) are also likely to amplify when multiple environmental stresses are present. Furthermore, the presence of multiple stresses may limit the resources available to a plant and thereby constrain its ability to respond to environmental signals adaptively (Valladares et al 2007) although see (Funk 2008).

Understanding how multiple environments constrain plasticity is crucial for predicting how and when the expression of plasticity will be constrained in nature (Auld et al 2010). There are, however, relatively few studies investigating constraints on plasticity when multiple stresses are introduced and the majority of these relate to responses to herbivory. For example, Cipollini and Schultz (1999) demonstrated that bean plants with elongated stems – an adaptive response to low light – were more susceptible to herbivory than those without elongated stems.

The combination of low light and water, provides another example of environmental stresses, which, theoretically, should select for opposing phenotypes (Valladares et al 2007, Valladares and Pearcy 1997). For an annual herb in low water environments, selection should favour small plants with low root to shoot ratios (Lloret et al 1999) or other allometric changes such as low vegetative to reproductive biomass ratios (Aronson et al 1992). However, under competition for light, a common adaptive response is for plants to elongate and initially at least, allocate resources towards upward vegetative growth (Dudley and Schmitt 1996; Givnish 1982). It might also be expected that there will be physiological trade-offs between responding to low water and responding to competition for light. Plants subjected to low water availability are expected to increase their water-use efficiency (the amount of carbon sequestered per water molecule lost, Heschel et al 2002; Nicotra and Davidson 2010; Picotte et al 2007). Under competition for light, however, plants should maximize initial growth rates and thus photosynthetic rates (Schmitt 1997) in order to overtop neighbours, most likely resulting in a reduction in water-use efficiency.

The presence of one stress may thus constrain an otherwise adaptive plastic response in a given trait to another stress so that this response becomes maladaptive or neutral. Such constraints may be detected by measuring direct negative selection on plasticity in regressions of plasticity and fitness (e.g. traditional costs analyses as proposed by van Tienderen 1991) or by comparing analyses of adaptive/maladaptive plasticity under different conditions (where maladaptive plasticity is defined as a plastic response that results in a net reduction in fitness across environments compared to a fixed response in the same environments and adaptive plasticity is where the plastic response result in a net gain in fitness, see van Kleunen and Fischer 2005). Alternatively, constraints could be identified by a switch between strong selection for plasticity to a specific change in a chosen environmental factor under one set of environmental conditions; but no selection for plasticity to this same change in the chosen factor under an alternate combination of environmental conditions (e.g. selection for plasticity to water under sunny but not shady conditions, Figure 5.1a.)

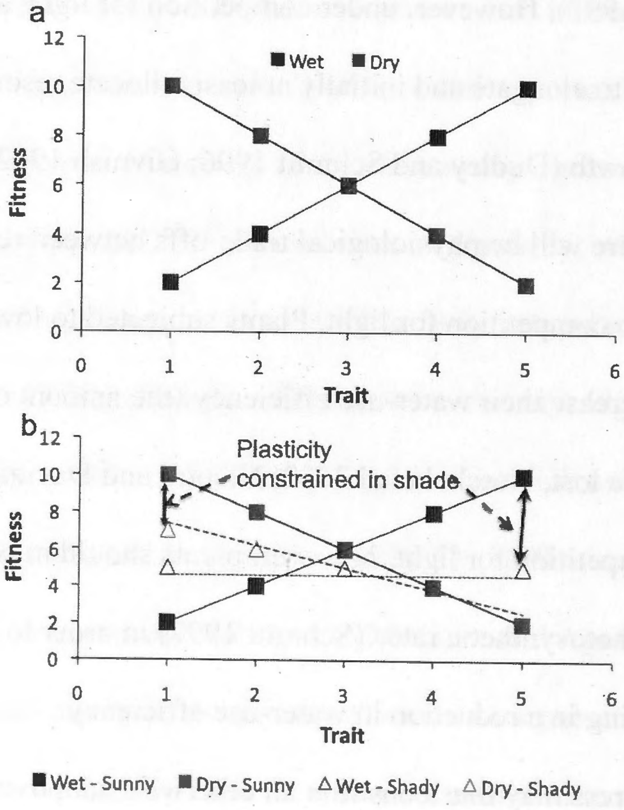

Figure 5.1 Theoretical relationships between a focal trait and fitness for a plant grown in a high water and a low water in sufficient light (a). In b) the plants are also grown at high and low water but in addition this is done both in sufficient light (sunny) as well as low light (shady) conditions. The differences between the maximum trait fitness reached in the shady environments for the high and low light environments represent a constraint on plasticity.

Few studies have found evidence of constraints on plasticity in nature. For example, Auld et al (2010) reviewed the literature and estimated that over half the studies failed to find any significant costs of plasticity. Davidson *et al* (*submitted a, b*) found several incidents of maladaptive plasticity, which could indicate costs. Studies of recombinant inbred lines - which create organisms with novel genetic combinations, not found (nor likely to survive) in nature, have been more successful in general than studies of natural populations in detecting costs (Auld et al 2010; van Kleunen and Fischer 2007). For this reason it is thought selection may have purged populations of genotypes with maladaptive plastic responses, (Agrawal 2002; Weinig et al 2006). However, when plants are introduced to novel conditions, selection will not have had the opportunity to expel maladaptive responses. i.e. there are hidden reaction norms (Schlichting 2008)

I may therefore expect species to respond with adaptive plasticity to conditions found in their natural habitat but maladaptively to those not commonly occurring in their home environment. Weinig (2000) subjected populations of *Abutilon theophrasti* (Velvet leaf) from weedy areas and cornfields to different light conditions. Responding plastically to shading in weedy areas will enhance fitness, as plants are able to overtop weeds by increasing internode length. However, Velvetleaf is not able to overtop corn and as such increasing internode length in cornfields at the expense of other organs is maladaptive. Consistent with these different selective pressures, populations from weedy areas responded plastically to low light conditions but those from cornfields did not (Weinig 2000). Bell and Galloway (2008) found very similar results for plasticity to shade in populations of *Geranium* from different environments.

In this study, I examine the responses of a native *Erodium* (*Erodium crinitum*, Carolin) and invasive (*E. cicutarium*, (L.) L'Hér. ex Ait.) to both limited water and competition. Because the invasive has had less time to adapt to the local conditions one might expect to see a greater number of constraints on plasticity in the invasive species. I also compare the adaptive value of plastic responses between populations from the dry and wet end of the water gradient along which both species occur. Plants from the dry-site populations should have undergone stronger selection to cope with low water availability compared to wet-site populations. In contrast, populations from the wet end of the gradient experience greater aboveground competition and should thus exhibit highest fitness in the competition treatment. Such comparisons enable us to investigate how different longer-term selection pressures have shaped the plants' abilities to respond to simultaneous stresses. In addition, I looked at whether plasticity was more constrained in the more stressful scenarios (low water availability, competition) compared to the more benign scenarios (high water availability, no competition). Such comparisons will help shed light on the theory of ecological limits to plasticity (Valladares et al 2007) and the influence of environmental conditions on the detection of costs.

5.3 Methods

Erodium crinitum and *E. cicutarium* are both annual forbs, which are widely distributed across southeastern Australia in temperate and semi-arid systems. *E. crinitum* is native to Australia whereas *E. cicutarium* was introduced to Australia approximately 200 years ago and is native to the Mediterranean region. Both species are autogamous (Fiz et al 2008) and flowers are generally open for just a single day (*pers obs*). Seeds are formed on 5-10 cm schizocarps with five seeds to an awn and have relatively limited dispersal (Stamp 1989).

Seeds of both species were collected from two locations in southeastern Australia representing the dry and wet ends of their ranges. Canberra (35.25°S, 149.13°W) was chosen as our wet site and receives an average annual rainfall of approximately 690 mm year^{-1}. *Erodium* species are generally found growing in competition with perennial native and exotic grasses and small forbs at this site. In contrast, the dry site, Mildura (34.13°S, 142.15°W), receives an average annual rainfall of 210 mm year^{-1}. At Mildura, the vegetation is much more sparse and *Erodium* plants are found in fairly open areas between saltbushes with only a few other native and exotic annuals as competitors.

5.3.1 Experiment set-up and design

Seeds were collected in the field in the Spring of 2008 and kept in the dark in paper envelopes suspended over silica, the room temperature was kept between 10°C and 20°C. Seeds were sorted and any misshapen or incompletely filled seeds discarded. Seeds were then grown for one generation in the greenhouse to assist in removing maternal effects. The plants were provided with ample nutrients (slow release Osmocote® Native Gardens fertiliser: 17.9N:0.8P:7.3K) and water.

In December 2010, seeds from the greenhouse plants were germinated on agar in growth cabinets. The cabinets were set to a 12 hour day-time/night-time cycle and maintained at a temperature of 20°C in the day and 10°C at night. Once radicals reached at least one centimetre the seedlings were transferred to seedling trays filled with Osmocote seedling mix and placed in a greenhouse. The greenhouse was kept between 20-35°C degrees during the day and 5-15°C degrees at night. In January 2011, seedlings that had reached the five-leaf stage were planted out into 70 cm pvc tubes filled with a soil mix of 75:15:10 subsoil clay:sand:mushroom compost (shade cloth was taped around one end of the pipe). The soil mix was steam treated to kill any pathogens. Slow release Osmocote® Native Gardens fertiliser (17.9N:0.8P:7.3K) was mixed through the soil at the recommended rate ($5gL^{-1}$). Once in their pipes, the seedlings were watered daily for the first week and the low water treatment was then watered every second day for the next week. After this time the high and low water treatments were imposed.

5.3.1.1 Treatments and replicates

There were four treatments altogether consisting of high water (watered to saturation daily) and low water (watered to saturation once a week) treatment in a factorial design with an aboveground competition treatment. The competition treatment was imposed by placing four tubes in a square arrangement so that the plants were forced to compete for light i.e. placed in aboveground competition with one another. I chose not to include belowground competition as this would have potentially altered the available water to each plant. Because I wanted to understand how responding to one stress affected a plant's ability to cope with a second stress it was important that the water availability treatments remained the same between our competition and no competition treatments. Furthermore, *Erodium* plants have long taproots and their competitors under natural conditions usually do not (e.g. grasses), thus I inferred that aboveground competition was more likely to be an important factor. Plants in the no competition treatment were spaced 50 cm apart and had no foliage overlapping with neighbouring plants.

There were five replicates of each of the two species from the two sites for each of the four treatment combinations, resulting in 80 plants altogether. The plants were arranged in five blocks with one replicate of each population by species by treatment combination per block. The plants were arranged randomly within blocks with the exception of the competition treatments, which were placed at one end of the table for logistical reasons.

After 13 weeks I turned off the irrigation system. Plants were watered once more ten days after turning off the water to avoid rapid death of the high water treatment plants. This was done to mimic summer drought, which usually kills off both species in Australia. I allowed plants to slowly die back under water stress rather than harvest them at this point as previous experiments had shown that plants from the wet-site populations continue to accumulate biomass and allocate resources to vegetative growth while water is abundant. It was hoped that by mimicking a summer drought I could encourage plants to convert some of their vegetative biomass to seed production and thereby get a better comparisons of seed production potential of all populations.

5.3.1.2 Measurements

I measured the response of 13 traits to the treatments. Traits were selected to represent a range of relevant morphological, physiological, phenological, and biochemical traits relevant for annual plants in responding to competition for light and limited water availability. The competition treatment inhibited assessment of final biomass as the plants became very tangled and being herbaceous, broke easily. I measured two traits as proxies to aboveground biomass at week 12 (one week before the end of season drought), these included leaf biomass (estimated by weighing ten leaves and extrapolating across the total number of leaves) and projected area. To estimate projected area, I measured the length and width of all branches including leaves and the size of the rosette. I then photographed a representative branch as well as the rosette of each plant and calculated the projected foliage density using image J (http://rsb.info.nih.gov/ij/). I also measured the total number of leaves, diameter (calculated as the average of the maximum and minimum horizontal lengths) and height (taken from the top of the pvc pipe) at week 12. Leaf number was also measured at week four. I calculated relative growth increment of leaf number by dividing the difference between the natural log of the week 12 leaf number and the natural log of the week four leaf number and dividing by 46 (the number of days between measurements).

I measured two leaf traits at week 11. Leaf mass per area (LMA) was measured by dividing the oven-dried mass of five leaves by their total area (scanned when leaves were fresh and analysed to calculate area using image J http://rsb.info.nih.gov/ij/). Leaf dissection index (LDI, the leaf perimeter divided by the square root of its area) was measured using the same five leaves as per LMA (detailed protocol at Prometheus: http://en.wikipedia.org/wiki/Prometheus).

I measured three physiological traits using an infrared gas analyser LI-6400XT (Li-Cor inc Lincoln Nebraska): photosynthesis, instantaneous water-use efficiency (WUE_i, calculated as photosynthesis/ stomatal conductance) and respiration. I selected the youngest, healthy, fully expanded leaf I could reach with the Li-Cor and on this leaf measured respiration and photosynthesis. All physiological measures were taken at a CO_2 concentration of 400 ppm and 1000 PAR. The temperature was maintained close to ambient temperature at 20-22°C during the day and 15°C at night (for respiration). For photosynthesis and WUE_i measurements, humidity was maintained at approximately 70 % and measurements were taken over two days between 8.00 am and 12.00 pm in week ten. Respiration was measured between 9.00 pm (approximately 1.5 hours after sunset) and midnight also in week ten. By taking measurements at night I ensured plants were completely dark acclimated and ran no risk of accidently exposing leaves to light.

I recorded whether plants had produced any flowers or seeds on a weekly basis. Using these records I calculated time to flowering (in weeks). At week 20, I collected all seed awns from plants. Although seeds disperse, the awns remain on the mother plants. Both species consistently produce five seeds per awn (per sobs) thus I calculated seed number by multiplying the number of awns by five. I used seed number as our fitness proxy.

5.3.2 Statistical analyses

Data was normalized using natural log or square root transformations as necessary. Data was then checked diagnostically using histograms, normal plots and box plots.

5.3.2.1 Analysis of Variance (ANOVAs)

Analyses of variance were run on our fitness proxy seed number, using species, site, competition and water treatment as fixed factors and block as a random factor. I investigated all two-way interactions, as well as three-way interactions between species or site with competition and water. A significant two-way interaction between one of these stresses (water or competition) and either species or sites would indicate that the species or site populations respond differently to that stress. A significant two-way interaction between competition and water would indicate that the presence of one stress significantly affects the response to another stress. A significant three-way interaction would indicate that either species or sites were affected differently by the combination of multiple environmental stresses. Diagnostic assessments with box plots were used to identify outliers. ANOVAs were run on data with and without outliers removed. In all cases the most conservative result was retained (the result with highest probability). I corrected for multiple testing using Benjamini-Hochberg correction.

5.3.2.2 Detecting constraints of plasticity

I chose to investigate constraints on plasticity using two methods: trait selection analyses (linear regression of traits against the fitness proxy: seed number) and assessments of adaptive/maladaptive plasticity; under each of the environmental conditions.

5.3.2.3 Plasticity selection analysis

I quantified plasticity using the plasticity index (PI):

$$\frac{ABS(x1-x2)}{ABS(\max(x1,x2))} \quad \textit{Equation 1.}$$

Where: x1, x2 = trait value in treatment 1 and treatment 2.

Instead of using mean trait values, I calculated PI for each treatment pair in each block (see Davidson *et al in review a*). Separate PIs were calculated for low to high water, with and without competition. Similarly, separate PI values were calculated for competition under high water availability and under low water availability. I then calculated average fitness for each calculation of PI. The PI of each of our traits was regressed against average fitness for each of the four sets of PI calculations. Thus I had one PI calculation for each trait, for each population, for each treatment combination. A significant positive regression coefficient was interpreted as evidence of adaptive plasticity and a significant negative regression coefficient as evidence of maladaptive plasticity (Callahan et al 2005; van Kleunen and Fischer 2005, see Figure 5.2). Regression were considered significant if $P<0.05$.

Readers should note that I did not work at the level of genotype within sites, as such trait selection and selection for plasticity tests were conducted at the broader site level for each species. Analysis of selection patterns using multiple genotypes enables the genetic component of the phenotypic variation to be separated from the environmental component. I was, however, unable to do this for logistical reasons.

Results from mantel tests (see Davidson *et al submitted, a, b*) of the genotypes used in this experiment revealed little genetic variation between plants from different mothers within site (wet or dry) populations.

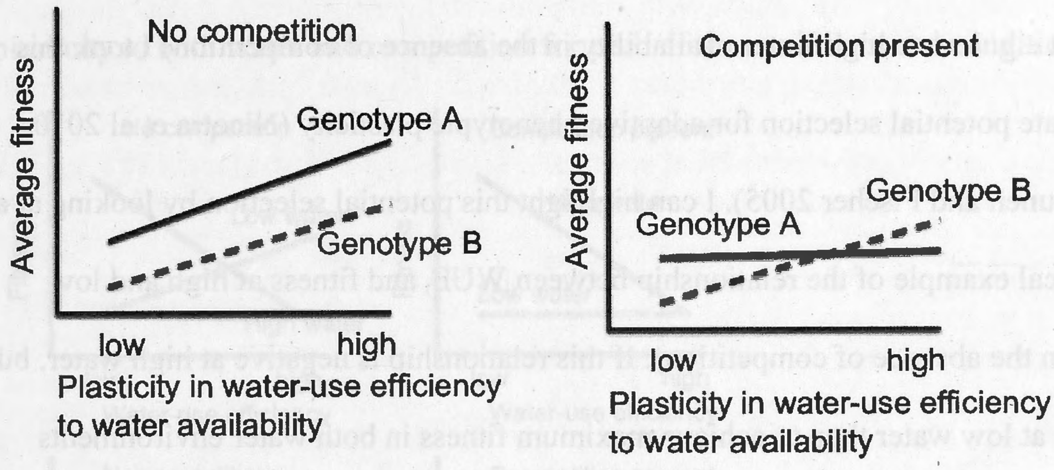

Figure 5.2 Theoretical example of plasticity-fitness regressions for water-use efficiency for responses to a change in water availability for two genotypes (genotype A = full lines, genotype B = dashed lines) in the absence (a) and presence (b) of competition. The presence of competition constrains genotype A's ability to respond plastically to water availability (b) however genotype B is still able to respond plasticity to water irrespective of competition treatment.

5.3.2.4Trait selection analysis

Our data was scaled and centred so that variables had a mean of zero, with a standard deviation of one. I then regressed our scaled traits against our scaled fitness proxy, seed number. Scaling the variables enables direct comparisons of the strength of relationships between traits using the coefficient for each trait from the univariate regression analyses. Firstly I categorised traits in each treatment as either having a zero relationship with fitness (P>0.95) or as being either positively or negatively correlated with fitness.

I compared the trait-fitness regression coefficients in each of the four treatment combinations. Where a trait's relationship with fitness switched direction between treatments from positively related to fitness to negatively related to fitness or vice versa for a change in one environmental variable (while the other was kept constant e.g. low to high water availability in the absence of competition) I took this to indicate potential selection for adaptive phenotypic plasticity (Nicotra et al 2010, van Kleunen and Fischer 2005). I can highlight this potential selection by looking at a theoretical example of the relationship between WUE_i and fitness at high and low water (in the absence of competition). If this relationship is negative at high water, but positive at low water then to achieve maximum fitness in both water environments (when competition is absent), a plant must be plastic in WUE_i in response to water availability (Figure 5.1a).

I had four sets of relationships to investigate for potential selction of palticity. There were: plasticity to water (high water vs low water) under competition, plasticity to water in the absence of competition, plasticity to competition (competition vs no competition) under high water and plasticity to competition under low water. Relationships were categorised as either plastic (contrasting selection on trait values between the two environments under investigation) constant (same direction selection on trait values in each of the two environments under investigation) or zero relationship (at least one trait-fitness relationship out of the two treatments had a trait-fitness regression coefficient with $P>0.95$).

Where a trait relationship was categorised as plastic in response to water availability under one competition treatment but zero under the other; I took this to mean that there was a constraint on the expression of plasticity to water availability in the environment in which there was no relationship (see Figure 5.1b and Figure 5.3).

Likewise, where a trait relationship was classified as plastic to competition under one water treatment but had a zero relationship under the other water treatment; I took this to mean the water treatment constrained the expression of plasticity to competition (Figure 5.3). I compared the number of traits displaying such potential constraints on plasticity to both water and competition for each of the environmental conditions.

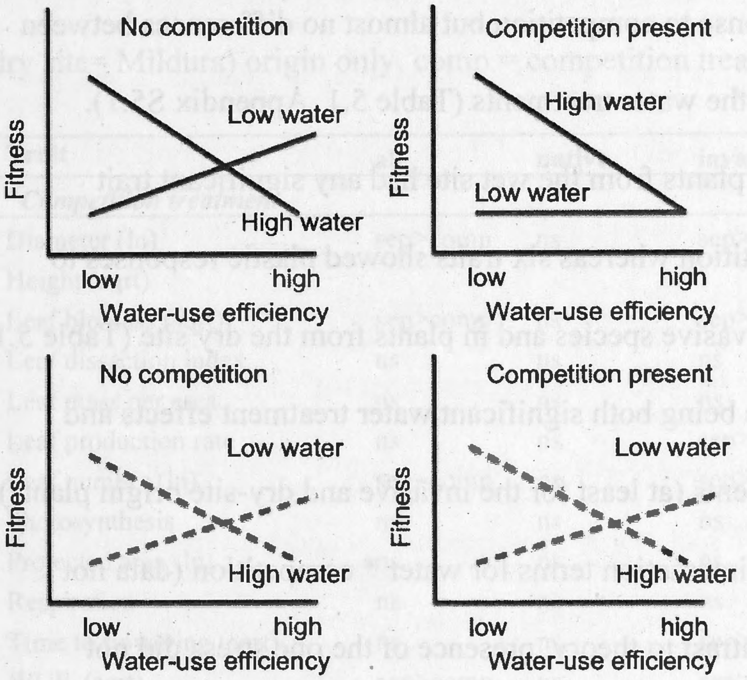

Figure 5.3 Theoretical example of selection regressions for water-use efficiency at high and low water for two genotypes (genotype A = full lines, genotype B = dashed lines) in the absence (a,c) and presence (b,d) of competition. The presence of competition constrains genotype A's ability to respond plastically to water availability (b) however genotype B is still able to respond plasticity to water irrespective of competition treatment.

5.4 Results

5.4.1 Plasticity to water and competition

Plants were bigger, produced more seed and grew faster (high leaf production and photosynthetic rates) under high compared to low water. Plants were also bigger and had more seed when grown separately rather than under competition. Plants from the low water treatment or grown separately also had higher WUE_i (Table 5.1).

Seven out of 13 traits responded plastically to the competition treatment and nine traits were plastic to water availability (Table 5.1, Appendix S5.1) as indicated by significant treatment effects. There were significant differences between both species and sites in their response to competition but almost no differences between species or sites in response to the water treatments (Table 5.1, Appendix S5.1). Neither the native species nor plants from the wet site had any significant trait changes in response to competition whereas six traits showed plastic responses to competition in plants of the invasive species and in plants from the dry site (Table 5.1, Appendix S5.1). Despite there being both significant water treatment effects and significant competition treatments (at least for the invasive and dry-site origin plants) no traits displayed significant interaction terms for water * competition (data not shown). In other words, in contrast to theory, presence of the one stress did not generally alter plastic response to the other. However, for two traits (LDI and height) there was a significant species*water*competition interaction for plants of the dry-site populations ($P<0.05$, Appendix S5.1). In addition time to flowering and seed number had significant three way interactions at $P<0.1$ (using corrected probabilities, Appendix S5.1*)*. This suggests that the presence of one stress did alter the response to another stress for the native dry-site, invasive dry-site or both of these populations. There were no significant three way interactions for the wet-site populations nor were there any significant site* water* competition interactions for the native nor invasive species, meaning the presence of water and competition stress affected these populations in similar ways that did not depart significantly from the responses to either stress individually.

Overall I found little evidence that the presence of one stress has an effect on the trait response to a second stress.

Table 5.1. Outcomes from ANOVA analyses for traits with significant Benjamini-Hochberg adjusted competition and water treatment effects. all = full dataset, native: *Erodium crinitum* only, invasive: *E. cicutarium* only, wet site = Canberra origin only, dry site= Mildura) origin only, comp = competition treatment, sep = separate/no competition treatment, high = high water treatment, low = low water treatment.

Trait	**all**	**native**	**invasive**	**wet site**	**dry site**
Competition treatment					
Diameter (ln)	sep>comp	ns	sep>comp	ns	sep>comp
Height (sqrt)					
Leaf biomass (sqrt)	sep>comp	ns	sep>comp	ns	sep>comp *
Leaf dissection index	ns	ns	ns	ns	ns
Leaf mass per area	ns	ns	ns	ns	ns
Leaf production rate	ns	ns	sep>comp	ns	sep>comp
Leaf number (ln)	sep>comp	ns	sep>comp	ns	sep>comp
Photosynthesis	ns	ns	ns	ns	ns
Projected area (ln)	ns	ns	ns	ns	ns
Respiration	ns	ns	ns	ns	ns
Time to flowering (sqrt)	ns	ns	sep>comp *	ns	ns
WUE$_i$ (sqrt)	sep>comp	ns	sep>comp	ns	sep>comp *
Seed number (ln)	sep>comp	ns	ns	ns	sep>comp
Water treatment					
Diameter (ln)	high>low	high>low	high>low	high>low	high>low
Height (sqrt)	high>low	high>low *	high>low	high>low	ns
Leaf biomass (sqrt)	high>low	high>low	high>low	high>low	high>low
Leaf dissection index	ns	ns	ns	ns	ns
Leaf mass per area	ns	ns	ns	ns	ns
Leaf production rate	high>low	high>low	high>low	high>low	high>low
Leaf number (ln)	high>low	high>low	high>low	high>low	high>low
Photosynthesis	high>low	high>low	high>low	high>low	high>low
Projected area (ln)	high>low	high>low	high>low	high>low	high>low
Respiration	ns	ns	ns	ns	ns
Time to flowering (sqrt)	ns	ns	ns	ns	ns
WUE$_i$ (sqrt)	low>high	low>high	low>high	Low>high	low>high
Seed number (ln)	high>low	high>low	high>low	high>low	high>low

* significant at $P<0.1$ but not $P<0.05$

5.4.2 Fitness responses of populations of native and invasive Erodium to water and competition

The effect of the four treatment combinations on seed production, our fitness proxy, varied greatly between populations. In general, seed number was negatively affected by both water availability and competition, although the affect of the latter was weaker (Figure 5.4). The native dry-site population had the highest seed production in all treatments, and was only marginally affected by competition. Seed number of the invasive dry-site population was relatively consistent across treatments except that it increased under the combination of no competition and high water availability (Figure 5.4) The invasive dry-site plants achieved similar seed numbers to the native dry-site plants except under the competition - high water availability treatment where the native species' seed production was greater (Figure 5.4). Plants of native wet-site population had lowest seed production under all treatments, showing significant negative impacts of both competition and water treatments, although water availability had a far greater effect than did competition (Figure 5.4).

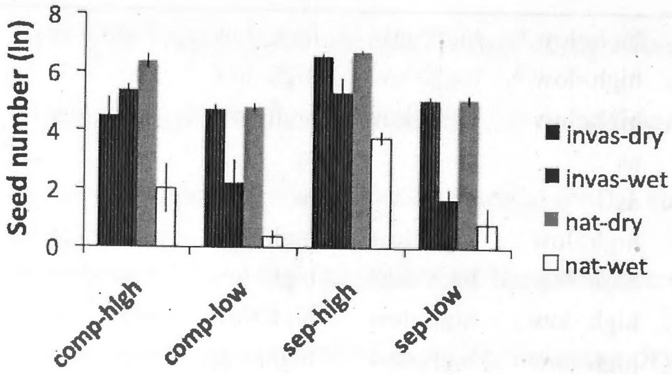

Figure 5.4 Average seed number for plants grown under different water availability and competition treatments for native (nat, *Erodium crinitum*) and invasive (invas, *E. cicutarium*) *Erodium* plants of dry (Mildura) and wet site (Canberra) origins.

5.4.3Treatment effects on patterns of adaptive and maladaptive plasticity

Having established that our fitness proxy (seed number) and several of our traits responded to both water and competition treatment, I next examined the adaptive value of the plasticity in these traits. To do so, I quantified the correlation between PI (our measure of plasticity) and average seed number across conditions to determine which plastic responses were significantly adaptive (higher average fitness for the plastic compared to the fixed phenotype) and which were significantly maladaptive (lower average fitness of the plastic compared to the fixed phenotype). I interpreted maladaptive effects as indicating there were some constraints to producing a plastic phenotype.

There were three instances of adaptive plasticity to competition. Two of these were at high water availability for the native dry-site population and one at low water availability for the invasive dry-site population, thus only dry-site populations displayed significant adaptive plasticity. In addition, there were five cases of maladaptive plastic responses to competition (indicated by plasticity being negatively related to average fitness, Table 5.2). All but one of the cases of maladaptive plasticity occurred for wet-site populations. I expected maladaptive plasticity to be more common when both stresses were present compared to just one, however all five of the maladaptive plasticity instances occurred in the high water treatment and as such the hypothesis was rejected. I also expected maladaptive plasticity to be more common in the dry-site populations compared to the wet-site populations given the ecological contexts of the populations, however this hypothesis was also rejected.

Table 5.2 Traits displaying significant positive (+) or negative (-) coefficients for the regression of plasticity index (PI) of the trait and seed number ($P<0.05$, $R2adj>0.30$), nat = native species: *Erodium crinitum*, inv = invasive species: *E. cicutarium*, wet = Canberra populations, dry = Mildura populations, high = high water treatment, low = low water treatment, comp = competition, sep = no competition.

Trait	**Data**	**PI competition**		**PI water availability**	
		High	***Low***	***Comp***	***Sep***
a) wet populations					
Diameter (ln)	native				
Leaf number (ln)					
WUE_i (sqrt)		- 3.70			
Height (sqrt)	invasive				
Leaf dissection index (LDI)				+ 7.26	
Leaf biomass (sqrt)		- 3.73			
Leaf production rate					
Leaf number (ln)		- 7.69			+ 13.93
Photosynthesis		- 3.95			
Respiration					
b) dry populations					
Leaf number (ln)	native	+ 7.77			
Projected area (ln)				+ 10.05	
RGR leaf number					
Time to flowering (sqrt)					
WUE_i (sqrt)		+ 2.67			
Diameter (ln)	invasive				
Leaf biomass (sqrt)		- 2.66			
Leaf production rate					
Respiration			+ 1.38		

In response to water availability there were three instances of adaptive plasticity and none of maladaptive plasticity. Two of the three cases of adaptive plasticity to water availability occurred under competition and one in the absence of competition. I therefore found no evidence of constraints on plasticity to water availability either when only one stress was present nor when both stresses were present and as such our hypotheses that constraints should be higher when both stresses are present was rejected.

5.4.3.1 Treatment effects on selection for adaptive plasticity

Having demonstrated that fitness and traits responded to our treatments and that there is significant adaptive and maladaptive plasticity in some of these traits, I next explore whether selection is acting to increase or decrease plasticity in our traits and whether this is affected by the presence of a second stress. It is important to note that these analyses assessed the number of traits displaying potential selection for plasticity. They did not consider whether this plasticity is currently realised in the populations nor did they assess the realised fitness benefits of the plasticity. Rather the analyses provide information on whether plasticity may become selected for in the future under the different conditions. I thus compared the patterns of selection under the different treatments to see if selection for plasticity appears to be more constrained in some treatments and whether these patterns differed across our populations.

In the absence of competition, leaf number of the invasive wet-site population was significantly positively related to fitness (seed number) in the high water treatment but negatively associated with seed number under low water availability (Table 5.3). Such contrasting direction of trait selection across treatments indicates potential selection for phenotypic plasticity. However, when competition was present, leaf number of the invasive wet-site population displayed a significant zero relationship with seed number under low water availability (Table 5.3). As such, plasticity of leaf number to water availability was no longer adaptive in the presence of competition.

There were 13 instances of potential selection for phenotypic plasticity to competition under high water. There was selection for plasticity in more traits in the invasive species populations compared to the native species populations (nine traits to

Table 5.3 Patterns of trait regression coefficients with seed number under the different water availability (high or low) and competition (comp = competition, sep = no competition). nat = native species: *Erodium crinitum*, inv = invasive species: *E. cicutarium*, wet = Canberra populations, dry = Mildura populations. P = trait-fitness coefficients across the environments (high to low water or competition to no competition) consistent with selection leading to plasticity i.e. contrasting direction of coefficients in each environment, S = fitness-trait coefficients in same direction across treatments i.e. directional selection, Z = P>0.95 i.e. no relationship in one environment (in all cases there was a relationship in at least one treatment), * = P<0.05 in at least one environment, NA signifies missing data and thus insufficient replicates.

	Native				**Invasive**			
Plasticity	**Plasticity to competition**		**Plasticity to water**		**Plasticity to competition**		**Plasticity to water**	
Competition	***high water***	***low water***	***separate***	***competition***	***high water***	***low water***	***separate***	***competition***
a) Wet populations								
Diameter (ln)	S	S	P	P	S	Z	P	Z
Height (sqrt)	Z	S	Z	P	S	S*	S*	S
Leaf biomass (sqrt)	P*	S	P	S*	S	P	P	S
Leaf dissection index	Z	P*	S	Z	P	S	P	S
Leaf mass area^{-1}	S	S	P	P	S	Z	S	Z
Leaf production rate	S	S	S	S	S*	P	P*	S
Leaf number (ln)	S	S	S	S	S	Z	P*	Z
Photosynthesis	S	S	P	P	P	P	S	S
Projected area (ln)	S	S	P	P	S	P	P	S
Respiration	P	Z	S	Z	P	S	P	S
Time to flowering (sqrt)	S	P	S	P	P	S	S	P
water-use efficiency (sqrt)	Z	S	Z	P	Z	P	S	Z

Table 5.3 *cont.*

	Native				**Invasive**			
Plasticity	**Plasticity to competition**		**Plasticity to water**		**Plasticity to competition**		**Plasticity to water**	
Competition	***high water***	***low water***	***separate***	***competition***	***high water***	***low water***	***separate***	***competition***
b) Dry populations								
Diameter (ln)	P	Z	S	Z	S	S	S	S
Height (sqrt)	P	P	P	P	P	S*	P*	S
Leaf biomass (sqrt)	S*	S	S*	S	P	P	S	S
Leaf dissection index	S*	S	P*	S	S	Z	Z	S
Leaf mass area^{-1}	S*	P*	P*	S*	NA	NA	S	*NA*
Leaf production rate	S	S	S	S	S*	S	S	S*
Leaf number (ln)	S*	S	S*	S	S*	P	P	S*
Photosynthesis	S	S	S	S	P	Z	Z	S
Projected area (ln)	S	S	P	P	Z	P	Z	P
Respiration	S	P	S	P	P	P	P	P
Time to flowering (sqrt)	Z	P	Z	Z	P	S	P	S
Water-use efficiency (sqrt)	S	P	S	P	S	P	S	P

four respectively). Of the 13 instances of selection for plasticity when water availability was high only three traits also displayed patterns of selection for plasticity in response to competition when water availability was low. Under low water availability there were 17 instances of selection for plasticity to competition (Table 5.3). There were therefore slightly more instances of selection for plasticity to competition in the low water compared to the high water treatment contrary to our expectation that selection for plasticity would be greater when only one stress was present. In particular, the native dry-site populations displayed five compared to two traits with potential selection for plasticity to competition when water availability was low compared to when it was high. The exception was the native wet-site population, which showed little plasticity to competition under either water availability treatment. Plasticity in WUE_i in response to competition was often under selection in low water but not high water (Table 5.3).

Plasticity to water was under selection in the no competition treatment in 20 instances. In contrast, in the competition treatment there were just 15 instances of selection for plasticity to water. There were thus more instances of selection for plasticity to water in the no competition compared to the competition treatment, particularly for the invasive wet-site population (noting the native wet-site population was an exception to this generalisation). This is consistent with our expectation that selection for plasticity would be higher when the stress of competition was absent compared to when it was present. There was selection for plasticity to water under competition in a greater number of traits for the native species populations compared to the invasive populations (nine to four respectively). Plasticity to water in leaf number of the invasive species, and leaf biomass of the wet-site populations was under selection in the absence but not presence of competition. However, plasticity in

WUE_i, was under selection for plasticity to water availability when grown in competition but not when competition was absent.

5.5 Discussion

In this paper I investigated evidence for constraints on the evolution of plasticity. Particularly, I asked whether the environmental context of one condition affected the plastic response to an additional factor, contrasting populations of a native and invasive *Erodium* species to multiple treatments. I discuss not only the affects of multiple stresses on overall patterns of adaptive plasticity but also the effects of the ecological histories of our populations (i.e. the influence of the different local environments in which the populations have evolved and differentiated) on their relative fitness.

It has long been hypothesised that plasticity will be more limited in more stressful environments (e.g. see Scheiner and Berrigan 1998; Sultan and Spencer 2002). To test this theory I compared evidence of constraints on plasticity to aboveground competition under both low water availability (a stressful environment) and high water availability (a benign environment). I also compared constraints on plasticity to water in the presence of competition (a stressful environment) and the absence of competition (a benign environment). Although the majority of traits measured were significantly plastic in response to water availability and over half the traits were significantly plastic in response to competition (at least for the native species and wet-site populations) there was no significant interaction between the parameters, thus the presence of one stress did not affect the overall plastic response to a second stress (Table 5.1). This means that the presence of plasticity *per se* was not significantly affected by the presence of a second stress. It does not, however, provide information about whether the adaptive value of the plasticity is altered by the presence of a second stress.

5.5.1 Effect of ecological history on fitness

The second major question of my study related to investigating the effects of the different ecological histories of the populations on their fitness response to the water and competition treatments. I hypothesised that dry-site and wet-site populations of native and invasive *Erodium* species would perform differently to one another, such that populations would maintain fitness in the environments that more closely resemble the types of stress the populations might encounter naturally i.e. competition for the wet-site populations and water limitation for the dry-site populations.

Consistent with my hypothesis, dry-site populations maintained their seed production better under low water availability than did wet-site populations (Figure 5.4). This was expected because selection should have resulted in better adaptation to dry conditions in plants at the lower rainfall site. However, I also expected that wet-site populations would better maintain fitness under competition than dry-site populations. While this was true for the invasive species the opposite was true for the native species. In Australia, grasslands and herbfields (the habitat of *Erodium* species) are dominated by perennial grasses whereas in Spain where *E. cicutarium* originates, annuals dominate. Weinig (2000) demonstrated that the ecological context of populations can influence their responses to shade. Annuals are unlikely to successfully compete with established perennial grasses whereas responding to aboveground competition from other establishing annuals may be worthwhile. As such, an adaptive response to competition from the Mediterranean origin invasive species but not the Australia natives might be expected. Indeed the invasive

(Mediterranean) dry-site populations also maintained fitness under competition when water availability was low and only when water availability was high did competition negatively affect fitness (Figure 5.4).

5.5.2 Patterns of adaptive plasticity and constraints on plasticity

Our final question related to the impact of the ecological histories of our populations on the patterns of adaptive plasticity and number of constraints on plasticity. I anticipated a greater number of constraints to plasticity under environmental conditions that were more novel to the populations. I explore this theory both in relation to responses to aboveground competition and responses to water availability.

5.5.2.1 Constraints on plasticity to competition

I hypothesised that under low water availability plasticity to competition would be more limited then under high water availability due to resource limitations. I also expected the wet-site population to display greater adaptive plasticity to competition than the dry-site populations. However, contrary to our predictions, I found that plasticity to competition was more constrained in high water compared to low water treatments (Tables 5.2 and 5.3). The native wet-site population, however, did not display significant plasticity in any trait to competition (Table 5.1). Furthermore with respect to fitness, this population was unable to maintain seed number under competition (Figure 5.4). As discussed in the previous section, I believe the poor performance of the native wet-site population under competition may be due to its ecological context, I further suggest that this may also explain the lack of adaptive plasticity in this population.

The invasive wet-site population performed relatively well with respect to maintaining seed production under competition and displayed potential selection for plasticity in several traits (Table 5.3). And yet this population still had relatively high levels of maladaptive plasticity. Such maladaptive plasticity may indicate constraints on production of plastic phenotype under certain conditions. It may be, that such constraints are actually far more common than originally thought, indeed Davidson et al (*in submission a b*), found several incidents of maladaptive plasticity in all four *Erodium* species populations studied here in response to changes in water dose delivery and total amount of water.

Although plasticity can be advantageous in coping with environmental change, it is not the only option. Low or no plasticity may result in superior performance if an organism cannot accurately predict the environment (Ghalambor et al 2007). For example, while I detected potential selection for adaptive plasticity to competition in photosynthesis for the invasive wet-site population, individual plants expressing plasticity in photosynthesis performed less well than genotypes with lower levels of plasticity. Thus, despite the potential benefits of expressing plasticity in these traits, the costs associated with expressing such plasticity may result in selection for canalization of these traits (Dechaine et al 2007). It is possible that the invasive species, which has not had a long evolutionary history in Australia, is yet to develop a complete suite of adaptive plastic responses. The success of the invasive species may therefore be underpinned, not by adaptive plasticity, but a generalist phenotype capable of high performance (e.g. Godoy et al 2011; van Kleunen et al 2010) in both the presence and absence of aboveground competition. It should also be noted however, that the presence of adaptive plasticity depends on where along the reaction norm the plasticity is assessed. If, our competition treatment was not severe enough I may have failed to detect an adaptive plastic response in the invasive wet-site population. Although I note that the invasive species had a significant competition effect for six traits in the ANOVA (Table 5.1).

5.5.2.2 Constraints on plasticity to water

The effect of competition on adaptive plasticity and constraints on plasticity to water availability were assessed. The presence of aboveground competition should result in lower light availability and therefore limit resources and thus potentially the ability to express plasticity. Consistent with this theory I found slightly fewer traits displayed potential selection for adaptive plasticity to water in competition compared to when competition was absent. Although there was also one more case of adaptive plasticity to water availability in the competition treatment compared to the no competition treatment (two to one), there was so little significant adaptive plasticity and no significant maladaptive plasticity that is difficult to draw conclusions. Contrary to our expectations, the only two instances of maladaptive plasticity to water occurred in the no-competition treatment.

I expected to find a greater number of constraints on plasticity as well as greater maladaptive plasticity in wet-site populations compared to dry-site populations and in invasive species compared to native species (Tables 5.2 and 5.3). The invasive wet-site population boasted the fewest traits displaying potential selection for plasticity to water when competition was present compared to when it was absent (Table 5.3). However, it displayed two traits with significant adaptive plasticity to water availability, which was greater than any other population (Table 5.3). Despite this, the invasive wet-site plants performed poorly in maintaining seed production under dry conditions (Figure 5.4). Thus, as with plasticity to competition, it would appear that plasticity to water availability in this population did not result in an integrated phenotype, which was able to outperform more fixed genotypes. Indeed plasticity is not the only means by which a population may outperform another population when there is a change in the environment (see Figure 2, Davidson and Nicotra 2012)

5.5.3 Conclusion

Although the adaptive value of the plastic responses were affected by the presence of a second stress, this was not always in the way I expected, nor in ways that explained the different fitness responses of the populations. I did find evidence that the ecological context of our four populations influenced how well they performed with respect to seed production under competition and water stress. However, relatively greater fitness homeostasis under competition and water stress was not always explained by the presence of adaptive plasticity in a greater number of key physiological and morphological traits. Most importantly, I found no support that the phenotypic plasticity to competition or water was constrained when multiple environmental stress were present compared to when only a single stress was present.

5.6 References

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5.7 **Appendix S5.1**

Table S5.1.1 Trait regression coefficients with seed number under the different water availability (high or low) and competition (comp = competition, sep = separate /no competition) treatments for: nat = native species: *Erodium crinitum*, inv = invasive species: *E. cicutarium*, wet = Canberra populations of both species, dry = Mildura populations, RGR = relative growth rate.

			native-wet			**invasive-wet**			**native-dry**			**invasive-dry**		
Competition	**Water**	**Trait**	***Coefficient***	***Pr***	R^2_{adj}	***Coefficient***	***Pr***	R^2_{adj}	***Coefficient***	***Pr***	R^2_{adj}	***Coefficient***	***Pr***	R^2_{adj}
sep	wet	Photosynthesis	0.043	0.791	0.027	-0.369	0.240	0.41	-0.011	0.826	0.019	-0.057	0.367	0.273
sep	wet	water-use efficiency	0.002	0.994	0.000	-0.312	0.777	0.03	0.095	0.188	0.490	-0.093	0.446	0.203
sep	wet	Respiration	0.039	0.738	0.043	0.160	0.624	0.09	-0.064	0.196	0.479	0.054	0.241	0.415
sep	wet	Leaf dissection index	-0.131	0.852	0.022	0.528	0.729	0.04	-0.157	0.048	0.776	-0.191	0.509	0.157
sep	wet	Leaf mass area^{-1}	-0.112	0.548	0.132	0.379	0.123	0.60	0.023	0.707	0.054	-0.037	0.407	0.235
sep	wet	Leaf biomass (sqrt)	-0.178	0.417	0.227	0.541	0.180	0.50	0.095	0.013	0.903	-0.029	0.664	0.071
sep	wet	Leaf production rate	1.037	0.264	0.385	0.923	0.042	0.79	0.190	0.214	0.452	0.232	0.191	0.486
sep	wet	Leaf number (ln)	0.450	0.431	0.215	0.868	0.027	0.84	0.179	0.037	0.811	0.134	0.471	0.184
sep	wet	Diameter (ln)	0.589	0.505	0.160	0.491	0.104	0.64	0.046	0.652	0.121	-0.034	0.842	0.015
sep	wet	Height (sqrt)	-0.002	0.994	0.000	0.261	0.708	0.05	0.014	0.742	0.041	0.189	0.327	0.313
sep	wet	Projected area (ln)	0.102	0.522	0.148	-0.194	0.525	0.14	-0.024	0.503	0.161	0.009	0.902	0.006
sep	wet	Time to flowering (sqrt)	-0.089	0.620	0.092	-0.243	0.331	0.30	-0.003	0.976	0.000	-0.012	0.898	0.006
sep	dry	Photosynthesis	-0.417	0.718	0.050	-0.417	0.437	0.21	-0.148	0.396	0.246	0.011	0.935	0.003
sep	dry	Water-use efficiency	0.463	0.422	0.223	-0.126	0.888	0.00	0.207	0.108	0.632	-0.015	0.874	0.010
sep	dry	Respiration	0.131	0.821	0.020	-0.426	0.139	0.57	-0.060	0.731	0.045	-0.021	0.800	0.025
sep	dry	Leaf dissection index	-0.329	0.542	0.136	-0.052	0.852	0.01	0.447	0.700	0.090	-0.004	0.987	0.000
sep	dry	Leaf mass area^{-1}	0.686	0.274	0.372	0.106	0.571	0.11	-0.163	0.033	0.825	-0.053	0.418	0.628
sep	dry	Leaf biomass (sqrt)	1.080	0.158	0.538	-1.428	0.133	0.58	0.239	0.558	0.126	-0.196	0.271	0.376
sep	dry	Leaf production rate	0.381	0.622	0.091	-0.676	0.462	0.19	0.183	0.401	0.241	0.139	0.471	0.184

Table S5.1.1 *cont*

			native-wet			**invasive-wet**			**native-dry**			**invasive-dry**		
Competition	**Water**	**Trait**	***Coefficient***	***Pr***	***R^2_{adj}***	***Coefficient***	***Pr***	***R^2_{adj}***	***Coefficient***	***Pr***	***R^2_{adj}***	***Coefficient***	***Pr***	***R^2_{adj}***
sep	dry	Leaf number (ln)	1.253	0.086	0.679	-0.274	0.784	0.02	0.151	0.597	0.104	-0.574	0.205	0.464
sep	dry	Diameter (ln)	-0.686	0.667	0.070	-0.179	0.762	0.03	0.236	0.098	0.653	-0.138	0.674	0.067
sep	dry	Height (sqrt)	-0.107	0.890	0.008	0.864	0.015	0.89	-0.106	0.113	0.622	-0.224	0.009	0.926
sep	dry	Projected area (ln)	-0.435	0.143	0.565	0.144	0.462	0.19	0.100	0.447	0.203	-0.040	0.523	0.148
sep	dry	Time to flowering (sqrt)	-0.529	0.192	0.484	-0.189	0.350	0.28	0.073	0.574	0.117	0.223	0.050	0.770
comp	wet	Photosynthesis	0.412	0.364	0.276	1.264	0.125	0.59	-0.067	0.805	0.024	0.537	0.280	0.365
comp	wet	Water-use efficiency	-0.167	0.716	0.051	0.022	0.942	0.00	0.084	0.710	0.053	-0.409	0.685	0.062
comp	wet	Respiration	-0.604	0.274	0.373	-0.212	0.051	0.76	-0.115	0.411	0.232	-0.296	0.478	0.179
comp	wet	Leaf dissection index	-0.126	0.920	0.004	-0.343	0.566	0.18	0.132	0.618	0.093	-2.122	0.125	0.765
comp	wet	Leaf mass area-1	-0.720	0.580	0.375	0.140	0.397	0.24	0.317	0.028	0.946	-0.313	NA	1.000
comp	wet	Leaf biomass (sqrt)	1.044	0.043	0.791	0.462	0.136	0.57	0.206	0.230	0.430	0.887	0.051	0.767
comp	wet	Leaf production rate	1.437	0.165	0.528	0.117	0.539	0.13	0.110	0.626	0.089	0.692	0.012	0.911
comp	wet	Leaf number (ln)	1.292	0.085	0.683	0.174	0.601	0.10	0.276	0.189	0.489	0.665	0.008	0.932
comp	wet	Diameter (ln)	1.155	0.492	0.169	0.063	0.472	0.18	-0.063	0.541	0.210	-0.465	0.688	0.061
comp	wet	Height (sqrt)	1.187	0.169	0.520	0.028	0.742	0.04	-0.542	0.104	0.973	-0.283	0.385	0.255
comp	wet	Projected area (ln)	0.495	0.380	0.260	-0.055	0.710	0.05	-0.160	0.269	0.379	-0.461	0.271	0.376
comp	wet	Time to flowering (sqrt)	-0.997	0.123	0.602	0.036	0.832	0.01	-0.029	0.932	0.003	0.615	0.600	0.102
comp	dry	Photosynthesis	-0.237	0.169	0.520	0.565	0.095	0.65	-0.046	0.680	0.065	0.054	0.579	0.114
comp	dry	Respiration	0.009	0.954	0.001	-0.402	0.17	0.505	0.180	0.067	0.724	0.089	0.147	0.558
comp	dry	Leaf dissection index	0.629	0.011	1.000	-0.989	0.539	0.21	0.283	0.483	0.175	-0.556	0.128	0.592
comp	dry	Leaf mass area^{-1}	0.317	0.109	0.629	-0.038	0.959	0.00	0.052	0.758	0.036	0.097	NA	1.000
comp	dry	Leaf biomass (sqrt)	0.501	0.105	0.637	0.542	0.864	0.04	0.118	0.562	0.124	0.297	0.103	0.641
comp	dry	Leaf production rate	0.196	0.580	0.113	0.946	0.417	0.22	0.672	0.149	0.554	0.200	0.437	0.211
comp	dry	Leaf number (ln)	0.340	0.232	0.427	0.023	0.975	0.00	0.432	0.334	0.306	0.119	0.577	0.115

Table S5.1.1 *cont*

Competition	**Water**	**Trait**	**native-wet**			**invasive-wet**			**native-dry**			**invasive-dry**		
			Coefficient	*Pr*	R^2_{adj}	*Coefficient*	*Pr*	R^2_{adj}	*Coefficient*	*Pr*	R^2_{adj}	*Coefficient*	*Pr*	R^2_{adj}
comp	dry	Diameter (ln)	-0.128	0.879	0.009	0.097	0.962	0.001	0.013	0.957	0.001	-0.076	0.685	0.062
comp	dry	Height (sqrt)	-0.170	0.322	0.318	1.141	0.265	0.384	0.039	0.757	0.037	-0.093	0.428	0.218
comp	dry	Projected area (ln)	-0.158	0.118	0.612	-0.458	0.583	0.111	0.030	0.748	0.040	0.099	0.680	0.065
comp	dry	Time to flowering (sqrt)	0.134	0.457	0.195	-0.177	0.681	0.064	-0.104	0.572	0.118	0.323	0.366	0.273

Chapter 6 Discussion

Phenotypic plasticity may play a critical role in plant invasions as well as facilitating adaption and survival under current climate change. In this context, this thesis aimed to further develop and apply various techniques for assessing the relative adaptive value of phenotypic plasticity and limits to this plasticity in different species and environmental situations. The thesis also shed light on the patterns of adaptive phenotypic plasticity in populations of a sympatric native and invasive species pair found in south-eastern Australia, with a particular focus on responses to water availability: an important environmental factor for plant distributions in semi-arid and temperature regions, particularly under future climate scenarios.

6.1 Summary of results

6.1.1 Chapter 2: "Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis".

This chapter explored the patterns of phenotypic plasticity in native and invasive species and their relationship with fitness using meta-analyses of the published literature. The analyses found significantly greater plasticity in invasive species overall. When traits were assessed separately plasticity was found to be significantly higher in the invasive species for six out of the 11 traits measured and was never significantly higher overall in the native species. I tested whether phylogenetic relatedness at the genus, or family level significantly altered results. I found no evidence for a taxonomic bias, indeed the mean difference between invasive and native species was highest when both species were from the same genus. Nor did I find evidence for significant effects of habitat in the invaded environment or growth form of the invasive species on the observed patterns of plasticity.

Regressions of plasticity against fitness responses revealed that when conditions changed from average to more favourable, plasticity of traits in the invasive species correlated with increased fitness approximately half of the time, thus plasticity is only sometimes associated with a fitness benefit. Invasive species expressed both higher plasticity in focal traits and higher increases in fitness than their native counterparts in response to an increase in resources more often than expected, supporting the theory that invasive species display a master-of-some phenotype more frequently than do native species. However the reverse was true for the jack-of-all-trades scenario, with native species more often displaying this pattern. In this scenario, higher average phenotypic plasticity is correlated with greater fitness homeostasis (lower reductions in fitness) in response to a reduction in resources (a change from average to more stressful conditions).

6.1.2 Chapter 3: "Patterns of local adaption and phenotypic plasticity in sympatric Erodium species along a rainfall gradient"

Chapter three looked at the plastic responses of an invasive and native *Erodium* species and wet and dry site populations of these *Erodium* species to four different frequencies of saturating watering events. I found very high levels of plasticity in both the native and invasive *Erodium* species in response to water treatment (significant effects in 22 out of 30 traits). Traits which did not display significant plasticity included: leaf dissection index, stomatal density (abaxial and adaxial) and phenological traits (time to flowering and seeding). There was little difference in either the number of traits displaying plasticity or the magnitude of this plasticity between the native and invasive species or the wet site and dry site populations.

Adaptive plasticity, that is plasticity resulting in higher average fitness, was more common overall in the native species compared to the invasive species. Adaptive plasticity and selection for plasticity were also more often observed in plants of the dry site populations compared to plants from the wet site populations for both fitness proxies. The differences between sites were not dependent on the point along the water treatment gradient at which plasticity was measured. That is, I found no differences in the number of traits displaying adaptive plasticity between very low - low water compared to between medium - high water. Although there was a very slight trend towards greater adaptive plasticity of the native species at lower water availability.

As expected, the native species displayed greater evidence of local adaptation (differentiation between dry and wet sites) than did the invasive species. Given the lack of differences in plasticity between the species my results reject the notion that there is a trade-off between local adaptation and plasticity.

6.1.3 Chapter 4: "Winners and losers: A comparison of populations of native and invasive Erodium species' fitness under different water availability scenarios"

In chapter four, I compared the adaptive value of plasticity in four populations of *Erodium species* (native-wet site, native-dry site, invasive-wet site and invasive-dry site) to changes in the total amount of water provided as well as changes in whether the water was provided in saturating or half-saturating doses.

The low water half saturating dose treatment had a more significant impact on both my fitness proxies: seed number and total biomass than did the low water saturating dose treatment. For seed number, dry site populations maintained higher fitness across all three treatments compared to wet site populations. In contrast, when total biomass was the fitness proxy, populations from the wet site achieved higher fitness and better maintained fitness when water amount or dose were decreased.

I assessed the effect on adaptive plasticity of varying the frequencies of the water treatments to provide a range from more very stressful scenarios (more favourable treatment weighted at 0.1) to very favourable scenarios (more favourable treatment weighted at 0.9) as well as several stages in between (favourable treatment weighted at 0.25, 0.50 and 0.75). I found qualitative differences in which traits displayed adaptive plasticity but no overall quantitative differences in the number of traits displaying adaptive plasticity at different frequencies of water treatments. Similarly treatment frequencies had no effect on the population fitness ranking when seed number was the fitness proxy. However, when total biomass was the fitness proxy, the invasive wet population outperformed the native dry population (despite the native species generally being a bigger species) in the more stressful scenarios but not the more favourable scenarios.

6.1.4 Chapter 5: "Adaptive responses to water availability and competition in native and invasive Erodiums - are two stresses one too many?"

In chapter five I quantified costs and limitations to adaptive phenotypic plasticity in response to a reduction in water availability, competition for light or a combination of the two stresses. Despite finding evidence for costs and limits to plasticity my data did not support the theory that costs and limits to plasticity are greater under more stressful conditions.

Plasticity to competition was more limited in high water rather than the low water treatments and there were no difference in the number of costs to plasticity detected between water treatments. Local costs to plasticity were slightly higher in the competition treatments compared to the no competition treatments. Costs were highest in the invasive wet site populations and lowest in the native dry site populations. The native dry populations and invasive wet population were equally able to maintain fitness under competition, thus patterns in costs of plasticity did not correlate with fitness responses.

Plasticity to water availability was slightly more limited in the competition treatment however costs to plasticity were highest in the no competition treatment. Furthermore, the highest number of local costs to plasticity were found in the no competition high water treatment – the most favourable treatment competition. The native-wet population displayed the greatest number of traits with adaptive plasticity, however the population also showed the greatest limits to plasticity under competition and highest number of costs to plasticity. In addition, the population had the poorest performance under dry conditions. This suggests that the costs and limits of plasticity to water may have outweighed the benefits gained from traits displaying adaptive plasticity for the native-wet population.

The fitness responses to the competition and water treatments could be largely explained by the ecological contexts and evolutionary history of the populations. Dry site populations which would have been subjected to water stress more frequently than wet site populations over the course of the species evolution, were better at maintaining seed number in the low water treatments. It was expected that wet site populations would maintain fitness better than dry site populations in response to competition for light. Although this was true for the invasive species the opposite occurred for the native species. I suggest that one possible reason for this is because *Erodium* species are annuals; and are therefore unlikely to successfully compete with the perennial grasses that occupy the wet sites. As such, native-wet site populations may have evolved to be competition-avoiders. However, in years of high rainfall, the native-dry population must compete with other annual weeds, and for this reason may have evolved greater adaptive plasticity and fitness homeostasis in response to competition than the native-wet population.

6.2 Key findings

In this section I outline my key findings making linkages across chapters two to five. The findings are separated into three main areas: 1) Patterns in phenotypic plasticity; 2) Assessing adaptive plasticity; and 3) Assessing costs and limits to plasticity.

6.2.1 Patterns of phenotypic plasticity

6.2.1.1 Plasticity and invasiveness

My meta-analysis of 75 species pairs found that across multiple traits, phenotypic plasticity is generally higher in invasive species compared to non-invasive species. However, I did not find these same results for my sympatric *Erodium* species pair. I suggest one reason for this is that I controlled for the geographic range of both the native and invasive species in my experiments. It is likely that in many comparisons of native and invasive species the native species occupies a much more restricted environmental range than does the invasive species, as such environmental range may be confounded with invasiveness in many studies (Davidson & Nicotra, 2012). Sultan (2001) found evidence that *Polygonum* species which expressed higher phenotypic plasticity had a wider ecological breadth than those with lower phenotypic plasticity. A related concept is that pioneering plants express higher levels of phenotypic plasticity compare to other plants (Bazzaz, 1979). Invasive species are usually pioneer species which thrive in disturbed environments. However not all pioneer plants are invasive, thus there is a need for further information in order to separate where plasticity is related to pioneering characteristics or invasiveness.

My meta-analysis suggested that native species, while expressing lower overall levels of plasticity, more often expressed adaptive plasticity to stressful conditions. Consistent with this result, my native *Erodium* species tended to express adaptive plasticity in a greater number of traits in the more stressful treatment combinations than the invasive species when water amount was changed (but not when dose was altered, Chapters 3 and 4)

My meta-analysis (Chapter 2) also suggested that invasive species are better "masters-of-some", that is they respond with increased plasticity and fitness to an increase in resources, than do native species. I did not find any evidence of such patterns in my *Erodium* species. Indeed greater phenotypic plasticity is not the only means by which an invasive species may outperform a co-occurring non-invasive species. In Davidson and Nicotra (2012) I highlight three mechanisms by which an invasive species may outcompete a native species. These include, greater plasticity, higher average mean trait values, steeper trait-fitness relationships or a combination of these (*Figure 6.1*).

6.2.1.2 Plasticity and environmental heterogeneity

Another common theory in the phenotypic plasticity literature is that species and populations occupying more heterogeneous sites will display higher plasticity than plants from more stable environments (Sultan & Spencer, 2002, van Tienderen, 1991). Consistent with this theory and findings of Baythavong (2011) who studied populations of *Erodium cicutarium* in the USA, I found greater levels of adaptive plasticity in populations of the *Erodium* species from the drier more heterogeneous environment compared to populations from the wetter more stable environment (Chapters 3 and 5). Gianoli (2004) also found greater levels of plasticity in

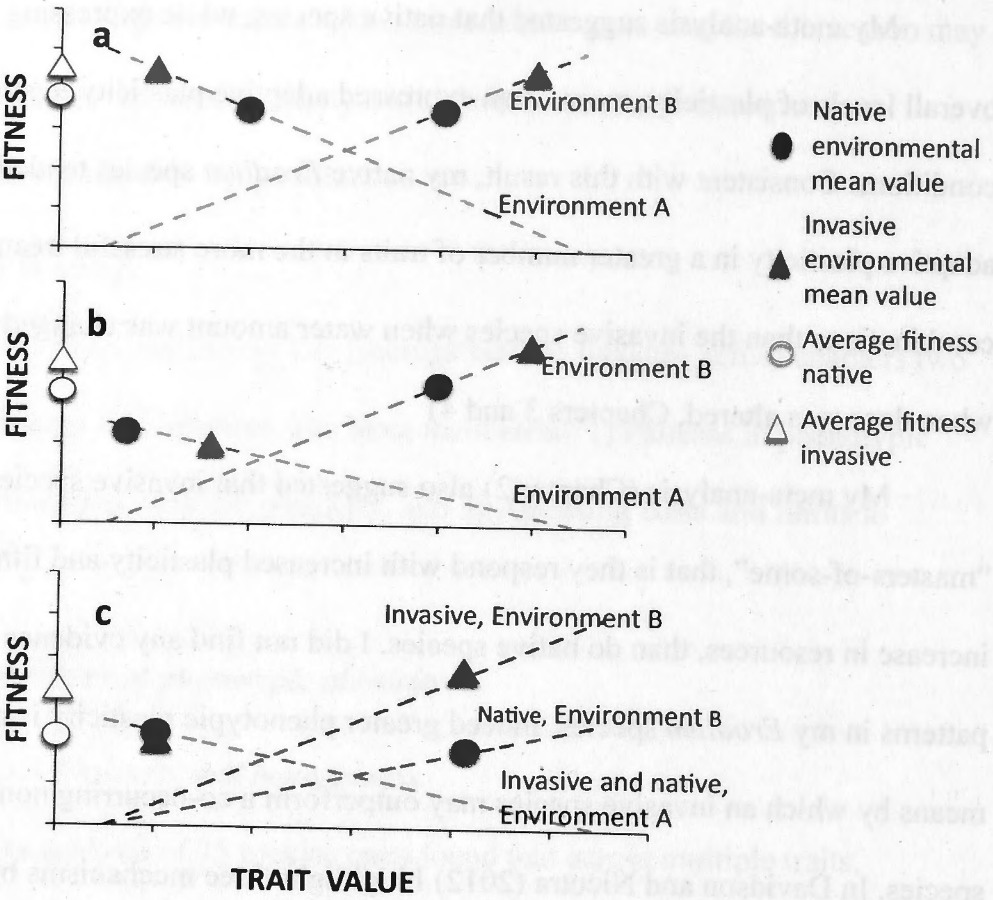

Figure 6.1 Theoretical trait-fitness relationships across two environments (A and B) for a native and invasive species demonstrating three mechanisms (a, b and c) by which the invasive species may achieve greater average fitness than a native species. Solid symbols denote mean trait values in each environment. Open symbols denote mean fitness for each species, across environments. In a) the invasive species displays greater plasticity than the native species (seen as a greater distance between the solid points) and higher average fitness. In b the invasive displays higher average mean trait values than the native species, which translates to higher average fitness despite identical levels of plasticity in both species. In c the invasive displays a steeper relationship between the focal trait and fitness in environment B and therefore exhibits higher average fitness despite having the same mean trait values and plasticity as the native species

populations from the more variable water availability site, however, Nicotra *et al.* (2007) and Heschel *et al.* (2004) did not.

Nicotra *et al.* (2007) did find significant differences in plasticity levels between populations. Weinig (2000) and Bell & Galloway (2008) found that the evolutionary history of a population influenced whether it was likely to respond with adaptive plasticity to a change in the environment or not. Thus, in the cases of Nicotra *et al.* (2007) and Heschel *et al.* (2004), it may have been that the more heterogeneous site was too unpredictable and therefore did not induce greater adaptive plasticity (Alpert & Simms, 2002). (Or alternatively that the plant perceived heterogeneity differently from the investigators.) Indeed, in my experiment native-wet populations, despite growing in temperate grasslands where competition for light is usually present, exhibited little plasticity to competition for light (Chapter 5). I suggest that plastic responses in annuals such as *Erodium* species aren't able to overcome competition from perennial grasses, given the grasses are fully established before the *Erodium* species even germinate. For this reason plastic responses to competition in these annual species, which would have no benefit but may incur some cost, have been selected out. Thus, plastic responses will only be selected for in variable environments where they can overcome the environmental conditions (Ghalambor *et al.*, 2007)

That different populations express different levels of plasticity and adaptive plasticity has important implications for predicting species responses to climate change. The results suggest that extrapolating the responses from one population of a species to all populations of that species, at least in the case of plasticity is likely to lead to erroneous results. If we can better understand when plasticity is likely to have evolved (i.e. when environmental heterogeneity is predictable enough and a plastic response can overcome the environmental constraints) and when it is not we may be able to help improve predictions of populations responses under climate change.

6.2.1.3 Comparing plasticity across environmental gradients

In Chapters 3 and 4, I compared the number of traits displaying adaptive plasticity in various water scenarios ranging from very stressful to quite favourable. There was no quantitative change in the number of traits expressing adaptive plasticity or maladaptive plasticity at the different scenarios. This demonstrates that plants with plastic phenotypes are able to respond adaptively to relatively novel conditions as well as more familiar resource level changes. This is important in the context of global change (Hulme, 2008) where the environments of the future may be very different from the environments of today.

Although there were no quantitative changes, I did find significant qualitative changes in the traits displaying adaptive plasticity under different conditions. In general the traits displaying adaptive plasticity under relatively favourable conditions did not express adaptive plasticity for that same population under stressful conditions. This suggests it is important to look at plasticity of a variety of traits and that extrapolation of the adaptive value of plasticity in a trait beyond the environmental conditions under which it was tested could lead to quite erroneous conclusions (Davidson & Nicotra, 2012).

6.2.1.4 Comparing plasticity across traits

I found significant plastic responses in a wide variety of physiological, morphological and growth traits, not only in my own experiments (Chapters, 3, 4 and 5) but also in a review of the literature comparing native and invasive species (Chapter 2). This suggests that plastic responses to environmental changes are relatively common. In my own experiments plasticity was less often significant in leaf traits (e.g. stomatal density (abaxial and adaxial) leaf shape (leaf dissection index, LDI) leaf water content and leaf mass per area (LMA)) than in physiological, size or growth traits. This is perhaps surprising given it is often thought that leaf traits should be particularly labile given the construction costs to produce new leaves are not overly high, especially in herbaceous annuals. The lack of consistent plasticity in LMA may be explained by the relatively low light levels in the greenhouse compared to in the field, which could have triggered plants to produce leaves with low LMA irrespective of water in order to capture light. Indeed several studies have reported that LMA (or its inverse specific leaf area) is highly plastic to light levels (e.g. Meziane & Shipley, 1999, Evans & Poorter, 2001, Poorter *et al.*, 2009) in order to maintain photosynthetic potential. Consistent with such plasticity in LMA to maximise photosynthesis I measured very high photosynthetic potential (as measured by carbon assimilation under saturating CO_2 and F_VF_M.) and these traits also displayed plasticity to water availability less often than other traits suggesting the *Erodium* species sought to maximise photosynthetic output, a characteristic consistent with many dessert annuals which complete their lifecycles before the summer drought hits (Arntz & Delph, 2001). Other leaf trait results, however can not be explained by light levels or mother plants effects, thus I propose that contrary to expectations leaf traits actually have reduced plasticity compared to physiological, growth and size traits. Of course, physiological traits must be underpinned by some changes in leaf characteristics but I propose these may relate more to behavior e.g. stomatal opening and sub cellular changes than to leaf morphology.

I also observed less consistent results for plasticity in phenology, specifically timing of flowering and seeding. Earlier flowering resulted in greater fitness under dry conditions in the selected *Erodium* species and this effect has also been reported for other dessert annuals (Aronson *et al.*, 1992) whereas delayed flowering is likely to maximize biomass and thus ultimate seed production in wet conditions (Lacey, 1986, Thompson *et al.*, 1991). However, gambling on rainfall may be risky, thus phenological traits may be genetically constrained (Aronson *et al.*, 1992) rather than relying solely on environmental variables to accurately cue plastic responses. Consistent with this hypothesis, time to seeding displayed a significant location effect (mantel tests revealed a significant difference between plants grown from seed collected at different locations within a site) for the wet site populations and a marginally significant effect for the dry site populations (Chapter 3, Appendix 2)

6.3 Assessing the adaptive value of plasticity

6.3.1 Quantifying plasticity and adaptive plasticity

I employed four main methods to assess patterns of plasticity and adaptive plasticity in my thesis: analysis of variance (ANOVA), partial-mantel tests, Plasticity Index (PI) and meta-analysis techniques. The following is a short discussion on the types of questions/studies the different methods could be applied to. As there is already information on the statistical assumptions of the techniques in the published literature, I concentrate on the ecological application rather than the technical details of the techniques.

ANOVAs can be used to identify whether a treatment effect and thus plasticity is significant as well as whether a treatment significantly effected fitness (Chapters 3, 4 and 5). Multivariate ANOVAs (sometimes termed MANOVA, or if a continuous covariate is used, ANCOVA/MANCOVA) allow variance to be partitioned into block, genetic/species and other effects in addition to the treatment effect thereby reducing type I errors and thus providing a more reliable test of treatment effects than a simple t-test (StatSoft, 2011). This is important in greenhouse and field studies where block or genetic effects may influence results. ANOVAs may also be used to compare the magnitude of the plastic response between species or populations by assessing interaction terms between treatments and species/populations (Chapter 3). However, ANOVAs provide information at a group but not an individual level and there is no output of an individual plasticity value for comparisons with fitness in adaptive plasticity analyses.

Where genotypes are not fully replicated across all treatments, partial mantel tests may provide a means for assessing the treatment effect while taking into account potential genetic influences. Mantel tests (and partial mantel tests) use dissimilarity matrices. These may be matrices of differences between treatment levels, genetics or other traits. Because I did not have information on the genetic sequences of my *Erodium* species I used the geographic distance between locations as a proxy for genetic differences (Chapter 3). Such a substitution may be valid in species such as those in the genus *Erodium* where seed dispersal is very limited and out-crossing relatively rare. However, distance as a proxy for genetic information should be employed with caution and genetic sequence information will always be preferable. Partial mantel tests enable assessment of one variable for example a treatment effect, while taking into account variation attributable to another variable (Goslee & Urban, 2007) e.g. genotype (or in my case geographic distance of seed collection locations). The strength of a treatment effect between two species, taking into account genetic influences, could thus be assessed by running separate partial mantel tests for each species and comparing the resulting mantel r value (a measure of the strength of the assessment) and probability associated with this value.

ANOVAs and partial mantel tests test whether a treatment effect is significant across a sample. They do not provide a measure of plasticity for each individual, thus they can't be used to assess adaptive plasticity. Testing the adaptive value of plasticity is important if we are to understand the ecological context of the plasticity and its role in invasions and adaption to different environments.

One way to understand whether selection of phenotypic plasticity exists is to regress traits values against fitness for each of the environmental treatments (Chapters 3 and 5), such regressions are based on analysis of selection differentials as described by Lande and Arnold (1983). If maximum fitness is achieved at different trait values in each of the environments then selection may favour phenotypic plasticity (e.g. see *Figure 6.2*), i.e. if the sign of the trait-fitness regression coefficient switches between environments there is potential selection for plasticity. Comparisons between species/populations of the number of traits displaying significant potential selection for adaptive phenotypic plasticity will provide information on which species/populations display greater trait lability and potential adaptive plasticity (Chapter 3, van Kleunen & Fischer, 2005).

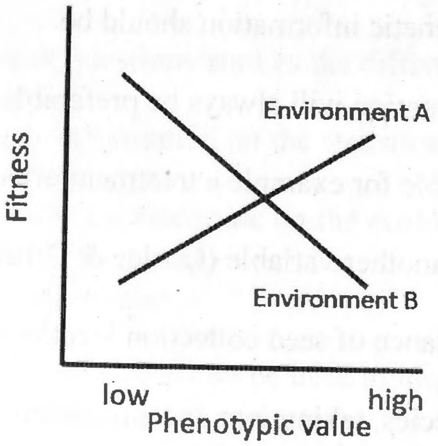

Figure 6.2 Hypothetical selection of trait values

Such analyses, should be coupled with other more direct assessments of adaptive plasticity, as costs and limits to plasticity, in particular phenotypic integration or difficulties in signal detection or lag times (DeWitt *et al.*, 1998), may actually mean that achieving optimal trait values is not possible via a plastic response and thus fixed rather than plastic responses are favoured (Alpert & Simms, 2002, Ghalambor *et al.*, 2007).

A common method of assessing plasticity is the plasticity index (PI). There are several variations for calculating PI, I chose to use the absolute difference between treatments normalized by the maximum trait value for the treatments. It is important to normalize the difference in trait values so that the relative plasticity of trait values are compared, to avoid positive bias of species/ populations with larger trait values and therefore larger gross differences between treatments (but not necessarily larger relative differences). Normalising plasticity also enables PI values to be compared across traits. PI is therefore effectively a measure of the effect size of treatments.

Another important consideration is whether to use absolute values or signed values. Van Kleunen and Fischer (2007) argue that when PI is used to assess costs, signed rather than absolute values of PI should be used to differentiate between adaptive and maladaptive responses. However, this requires firstly a knowledge of which direction is adaptive and secondly I argue that it depends on the question being asked. I suggest that in studies where researchers want to make comparisons between populations or species in their ability to respond adaptively to a certain treatment, and there is no ambiguity about which direction a trait change should occur in order to yield an adaptive response, then signed values are most appropriate. However, when a researcher wishes to investigate patterns of phenotypic plasticity to understand which populations have the greatest capacity to respond plasticity, or if maintaining a stable or labile trait in response to environmental change yields highest average fitness (even if the labile trait response is occasionally maladaptive), then using the absolute value of the trait difference between treatments is more appropriate. As such I chose to use the absolute value for my studies (*Equation 1, Chapters 3, 4 and 5*).

$$\frac{ABS(x1-x2)}{ABS(\max(x1,x2))}$$

Equation 1

where: x1, x2 = trait value in treatment 1 and treatment 2.

Another important decision to make is whether population means should be used in calculating PI or individual values. Again this depends on the situation and question being asked. For example, where it is suspected that there might be block effects and that these block effects may have impacted on the trait responses to one treatment more than another, it may be better to use individual values rather than treatment means. The resulting PI values can be later summarised using ANOVAs, which take into account both the block and species/population effects. Alternatively, if no block or other influences are suspected, then using the mean values provides a simple means of quantifying and thus comparing plasticity across species/populations and traits (although if individuals within a species/population are not full clones, the standard errors of such summary statistics should be calculated so that the variance surrounding the values of PI are accounted for).

Because the PI can provide a value of plasticity for each individual specimen/genotype within a population in an experiment, the PI values for the population can be regressed against average fitness across treatments to assess adaptive plasticity. Depending on the sample size, quadratic as well as linear relationships could be investigated. A significant positive linear relationship between PI and average fitness would indicate that plastic genotypes yield higher average fitness than do fixed genotypes i.e. adaptive plasticity. A significant negative linear relationship would indicate maladaptive plasticity. A significant negative quadratic term would indicate that genotypes with intermediary levels of plasticity yield highest fitness and a significant positive quadratic that a fixed or extremely plastic genotype would yield highest fitness.

Regressing PI against average fitness is a relatively straightforward means for assessing the adaptive vale of plasticity. Although regression analyses assess correlations between plasticity and fitness rather than causations they are nonetheless preferable than, for example, assuming that if an invasive species has higher phenotypic plasticity then this is what confers its fitness advantage over native species.

PI is one measure of effect size of a treatment (i.e. a measure of plasticity), the meta-analysis literature offers many others which should be considered in light of the data available. In Chapter 2, I used Hedges' *d* (i.e. *J*-corrected Hedges' *g sensu* (Rosenberg *et al.*, 2000) to calculate plasticity from the means and standard deviations of native and invasive species in response to various treatments. When the same treatments are imposed on all the data, summary analyses can be run on the effect sizes to analyse differences between species/populations or plasticity to different treatment levels.

In Chapter 2, I used comparisons of native and invasive species from a variety of studies, as such, while the treatment conditions for each native-invasive pair where the same, the conditions differed between pairs. I therefore calculated an effect size of the signed difference between the effects sizes (plasticity) of the invasive and native for each pair using equations from Borenstein *et al.* (2009). A positive value for this meta-effect size indicated the invasive had higher plasticity and a negative value that the native had higher plasticity. Summary analyses were then run on the meta-effect sizes of differences in plasticity between native and invasive species to investigate the significance of the overall effect size as well as investigate the influence of various moderator variables such as treatment, trait, phylogenetic relatedness etc.

Meta-analysis techniques may also be applied to assess patterns in adaptive plasticity by using the correlation values of plasticity (e.g. PI) against fitness. The advantage of using meta-analyses to study the adaptive value of plasticity is that all of the regression coefficients can be used to produce the summary statistics rather than only counting the individually statistically significant coefficients. Thus meta-analyses of adaptive plasticity may be better suited than counts of significant adaptive/maladaptive responses to comparisons of species or treatment gradients where it is hypothesised that one species or treatment gradient should yield overall adaptive plasticity or less maladaptive plasticity.

Meta-analyses could be applied to other question in the plasticity literature such as differences in plasticity of populations from more or less heterogeneous sites etcetera; noting that meta-analyses generally require a considerable amount of data. Thus, they may be best suited to very large datasets can be gathered, e.g. through collaborative projects such as the specific leaf area study (Wright *et al.*, 2005). Furthermore, unlike ANOVAs the summary statistics in meta-analyses generally only assess the influence of one explanatory factor at a time.

In addition to the methods described above for analysing plasticity, path analyses may also prove valuable. Path analyses show particular promise in addressing how traits interact to achieve maximum fitness and whether these interactions and their values change under different conditions (e.g. see Scheiner *et al.*, 2000). However, path analyses require very high replication if a large number of covariates are to be used and their values quantified at different treatment levels (for this reason I did not employ path analyses in my studies). I suggest that future studies wishing to employ this technique may need to first identify the essential covariates and then conduct a second experiment measuring only these factors and thereby maximizing replication.

6.3.1.1 Considerations for assessing adaptive plasticity

Assessment of adaptive plasticity requires data on fitness. True fitness is difficult to measure and thus this is rarely done, rather biologists rely on fitness proxies. The choice of fitness proxy could greatly influence results. For example, I found often contrasting patterns of adaptive plasticity when I used biomass compared to when I used seed number (Chapter 4, see also *Box 1* of Chapter 2).

In assessments of adaptive plasticity fitness across environments must be considered. In general, the average between two environments is taken. However, two environments are unlikely to occur with equal frequency, as such fitness should be weighted according to expected environmental frequency. In assessments of the value of plasticity under climate change, it may be particularly valuable to vary the frequencies of environments to reflect potential climate change scenarios or to investigate the influence of greater variability (i.e. novel environmental scenarios) on the value of adaptive plasticity in different species/populations (e.g. Chapter 4).

6.3.2 Assessing constraints on plasticity

6.3.2.1 Quantifying constraints

In Chapter 5 I used several methods for assessing the constraints on plasticity. Assessment of adaptive and maladaptive plasticity using PI regressed against average fitness (section 2.2.1, Chapters 3, 4 and 5) was one method. Quantitative comparisons of numbers of traits displaying significant adaptive plasticity (or comparisons of meta-analysis summary statistics of the regressions between plasticity and fitness (see section 2.2.1 and chapter 3) between environments could provide information about when adaptive plasticity is inhibited (or alternatively when maladaptive plasticity is highest). This inhibition could be due to resource limitations, phenotypic integration or costs of plasticity. Further analyses would then be required to understand the causes, however such regressions provide a quick method of identifying which conditions constrain plasticity most (e.g. Chapter 5 compared treatment combinations representing more and less stressful conditions).

In situations where more than one environmental variable is manipulated, selection analyses (Lande & Arnold, 1983), such as described in section 2.2.1 may also be used to assess potential constraints to plasticity, with a few minor variations (van Tienderen, 1991). Prior to assessing trait-fitness regressions, all traits should be normalised and scaled so that they have a mean of 0 and standard deviation of 1. This enables comparisons of the selection coefficients across traits and treatments. The regression coefficients for trait-fitness analyses can then be compared under the different treatment combinations of the manipulated environmental variables. If there is potential selection for adaptive plasticity in trait_A in response to variable_B (as described in section 2.2.1) but when variable_C is introduced, plasticity in trait_A to variable_B is no longer under selection, this suggests that the presence of variable_C constrains plasticity in trait_A. The evidence for constraints is stronger, if when variable_C is introduced the regression coefficient under one or more of the states of variable_B is significantly close to zero i.e. the null hypothesis is supported (e.g. $P>0.95$) and the regression coefficient is very small e.g. <0.05.

Where genotypes are well replicated, constraints may be measured by regressing fitness against the mean trait value as well as plasticity in that trait *Equation 2*, (van Tienderen 1991).

$$W_{j,k} = \text{Constant}_k + \alpha_k X_{j,k} + \beta_k P_j \qquad \textit{Equation 2}$$

Where:

W = fitness

j = a genotype or population

k = focal environment or treatment combination

X = trait value

P = plasticity, calculated as the absolute difference in trait values between environments

A positive coefficient for plasticity indicates that plasticity benefits fitness, whereas a negative coefficient for plasticity may indicate there are costs of plasticity. *Equation 2* assesses these costs separately for each environment (rather than regressing plasticity against average fitness as is usually done in studies of adaptive plasticity), this is because costs of plasticity may be expressed locally, that is in one environment only. *Equation 2* separates the relationship between plasticity and fitness from the relationship between fitness and the mean trait value. Because the costs of plasticity are analysed separately for each environment, plasticity is calculated only as the absolute difference in trait values (rather than a normalized index such as PI, see section 2.2.1).

This method has the advantage of controlling for potential bias of genotypes with larger mean trait values displaying greater plasticity thus confounding selection on high plasticity with selection for greater mean trait value. However, care must be taken to assess whether the mean trait value and plasticity are correlated, as if the latter is true then the assumptions of the linear regression are violated (see Auld *et al.* 2010).

If a study has sufficient replication of genotypes and no significant correlation is detected between mean trait values and plasticity than *Equation 2* is an effective way of assessing for potential constraints on plasticity. In more complex situations where these assumptions are not met, however, the use of more simple regression of traits against fitness and comparison under different treatment conditions as well as assessments of adaptive/maladaptive plasticity using regression of PI against fitness can be very valuable in highlighting where plasticity is constrained.

6.3.2.2 Considerations in assessing constraints to plasticity

I found no evidence to support the hypothesis that constraints (costs and limits) on plasticity will be higher under more stressful conditions compared to more favourable ones (Chapter 5). However, more tests on a greater variety of species are required in order to properly test this theory. In testing this theory the following points should be kept in mind.

Previous authors have suggested that the majority of costly plastic responses will have been removed by selection (Weinig *et al.*, 2006). This hypothesis is supported by the fact that studies using recombinant inbred lines (RILs) have generally found higher rates of costs to plasticity than have studies using natural populations (Auld *et al.*, 2010). If costs are only removed when exposed to selection, then natural populations should also display costs to plasticity if they are exposed to novel conditions.

Exposing natural populations to novel conditions may provide more relevant information for predicting costs and limits to plasticity than studies of RILs, particularly in the context of the role of plasticity in climate change adaption. (In so saying, I also acknowledge the importance of RILs for understanding the evolution of adaptive plasticity and costs and limits to plasticity). In species which outcross at least occasionally, one out-crossing event can re-introduce unfavourable genetic linkages, thus costs of plasticity should appear in natural populations of out-crossing species. We therefore need to understand how prevalent such costs are and how common they may become under the new selective pressures created by rapid climate change. Such changes will most likely force trait values to their extreme and thus exacerbate existing constraints and introduce previously unnoticed costs associated with phenotypic integration, epitasis and pleiotropy (Schlichting, 2008). In order to quantify these potential constraints it is thus important that a representative sample of the genetic variation present in the field is used in experiments and that plants are exposed to complex environments representative of the novel conditions likely under climate change.

6.4 Future directions

In this section I highlight some logical next steps for my experiments with my case study sympatric *Erodium* species as well as outlining some broader hypotheses relevant to the study of phenotypic plasticity in plants.

6.4.1 Next steps

I did not have sufficient replication of mother plants to quantify the genetic components of variation in my assessments of adaptive plasticity and costs of plasticity. Results of the partial mantel tests were largely concordant with the results of the ANOVA, in addition mantel tests revealed few traits with significant seed location effects within sites, indicating genetic variation is unlikely to have had a significant impact on results. However, it would be interesting to quantify the genetic component of variation in the plasticity responses and costs and limits observed. Given both *Erodium* species are self-compatible (*personal observation*, full-sibling seeds were obtained but not used due to time constraints) future studies could use full-siblings to quantify the genetic component of variation in trait responses and costs associated with these. Furthermore, studies over multiple generations could assist in calculating heritability's of plasticity and its constraints. Such studies should ensure full representation of genetic diversity while ensuring genotypes are not over - represented (e.g. each of the mother plant lines collected at the dry and wet sites for the *Erodium* species used in this study were collected at five kilometre intervals). It is important to ensure the genotypes are representative of those found in the field and avoid any bias due to non-representative sampling of a subset of genetic lines if ecologically relevant conclusions are to be drawn (Richards *et al.*, 2006).

Testing the patterns of adaptive plasticity observed to water availability under more realistic conditions i.e. field conditions would have been desirable had time permitted. My results in Chapter 5 demonstrated that the traits displaying adaptive plasticity and costs associated with this plasticity can alter when multiple environmental variables are interacting and this is likely to be the norm rather than the exception (Valladares *et al.*, 2007). For example, field or common garden experiments which manipulated water availability to produce similar treatments to those imposed in Chapters 3 and 4 could provide information on the level to which the patterns of adaptive plasticity observed under controlled conditions are maintained in the field.

6.4.2 Hypotheses

The results of this PhD have, as is common in science, raised more questions than answers. I propose the following hypotheses and methods for testing these, which may be addressed in future analyses.

1. *Plasticity is not a factor of invasiveness rather plasticity is higher in pioneers compared to non-pioneer species.*

   This hypothesis could be tested using meta-analysis techniques i.e. test the relative importance of invasiveness (invasive versus non-invasive or other categories based on for example rankings on national/provincial weed lists) and pioneering ability (need to develop a method for quantifying this e.g. categories of dependence on disturbed areas for colonisation). Alternatively, studies could employ a measure of range size as a covariate in explaining differences in patterns of adaptive plasticity. A measure or range size that relates to variation in an environmental factor rather than just geographic range would be particularly desirable in explaining differences in plasticity. This has been partially addressed by Dawson *et al.* (2012) however see Davidson & Nicotra, (2012) for a review of logical next steps following from Dawson and colleagues work for progressing this hypothesis.

2. *Plasticity is higher in populations from more heterogeneous habitats* (where environmental heterogeneity is relevant to the treatment being imposed).

   Although this hypothesis has been tested many times in one or two populations, we have yet to reach a consensus (Davidson & Nicotra, 2012). I suggest there is now sufficient data to run a meta-analysis to address this question. It would also be valuable to identify reasons why populations in heterogeneous environments do not always respond with higher plasticity. To this end it may be useful to partition the plasticity to adaptive, neutral and maladaptive plasticity (or quantify as a continuous variable using regression coefficients and their associated error from plasticity - fitness regressions). In addition, studies may wish to quantify the time scale of environmental fluctuations relative to the plants' generation times or quantify the magnitude of the environmental fluctuations at the different sites e.g. in terms of standard deviation of the mean (Bureau *et al.*, 2011).

3. *Non-invasive native species display adaptive plasticity more often than invasive species when conditions are stressful.*

Although this hypothesis was tested in Chapter 2 and supported by the meta-analyses, the studies used were not specifically designed to address this question. Collection of more empirical data on the plasticity of invasive and native species under a wide range of conditions including both stressful and more favorable conditions would enable more complete summary analyses to be undertaken (Davidson & Nicotra, 2012).

4. *Plastic changes in leaf morphology in responses to water availability are less significant than plasticity in physiological, growth or size traits.*

   This should be tested in multiple species including annuals versus perennials, ANOVAs would probably be sufficient to explain variation. Ideally genetic effects should be controlled for.

5. *Plasticity to water availability in key physiological, size and growth traits is relatively more important for maintaining seed production than maximising biomass for annual species in resource limited environments.*

   This could be tested using analyses of adaptive plasticity (e.g. regression of PI against average fitness) in multiple species using both seed number and biomass as fitness proxies.

6.5 Conclusion

In this thesis I investigated several widely held theories regarding patterns of phenotypic plasticity. I also examined several techniques for assessing the role and limitations of phenotypic plasticity in plant invasions and adaptation climate change. I found many incidents of significant adaptive and maladaptive plasticity and evidence of constraints on plasticity across my populations of a native and invasive *Erodium* species. However there were no clear patterns regarding which traits or scenarios yielded greater levels of adaptive plasticity or greater constraints on plasticity.

While I did find some support for greater levels of phenotypic plasticity in invasive compared to non-invasive species, I suggest that this plasticity is related to pioneering ability rather than invasiveness. Results from case studies and my meta-analysis also suggest that native species exhibit greater characteristics of jack-of-all-trades phenotypes. That is, they are more likely to respond to stressful conditions with adaptive phenotypic plasticity so as to achieve relative fitness homeostasis when resources decline.

I found evidence that the ecological context of a population is likely to dictate how readily that population will respond with adaptive plasticity to a change in a particular environmental factor. For example, plants from the drier more heterogeneous site display greater adaptive plasticity to changes in water availability. My results also showed slightly higher levels of maladaptive plasticity in invasive populations suggesting that, although often more plastic, the reduced residency time may mean that inappropriate responses have not always been selected out of introduced populations.

Under rapid anthropogenic and climate change, neutral or maladaptive plasticity may become adaptive, thus it is important that studies assess responses across the full environmental gradient. Future studies must also be careful to assess populations appropriately, given the strong difference I found in the adaptive responses of dry and wet populations of an invasive and native *Erodium* species.

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Plant phenotypic plasticity in a changing climate

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Climate change is altering the availability of resources and the conditions that are crucial to plant performance. One way plants will respond to these changes is through environmentally induced shifts in phenotype (phenotypic plasticity). Understanding plastic responses is crucial for predicting and managing the effects of climate change on native species as well as crop plants. Here, we provide a toolbox with definitions of key theoretical elements and a synthesis of the current understanding of the molecular and genetic mechanisms underlying plasticity relevant to climate change. By bringing ecological, evolutionary, physiological and molecular perspectives together, we hope to provide clear directives for future research and stimulate cross-disciplinary dialogue on the relevance of phenotypic plasticity under climate change.

Climate change and plant adaption

Climate change is altering the environments in which all organisms develop. Plant species can adjust to these novel conditions through phenotypic plasticity (see Glossary), adapt through natural selection or migrate to follow conditions to which they are adapted; these options are not mutually exclusive. For any given plant species or population, determining responses to environmental changes will require an understanding of the environmentally induced variation in the phenotype of individual plants. Once regarded as noise, phenotypic plasticity is now understood to be genetically controlled, heritable and of potential importance to species' evolution [1,2]. With mounting evidence from molecular and developmental biology, we are now at the threshold of gaining a sophisticated understanding of the mechanisms of plasticity, which will be crucial for predicting changes in species distributions, community composition and crop productivity under climate change [3,4].

Some authors have argued that plastic responses to rapid climate change are less important than adaptation

Glossary

Adaptive plasticity: Phenotypic plasticity that increases the global fitness of a genotype (Figure 2).

Environmental sensing loci: Genes or gene regions that encode sensors, or receptors, for environmental signals, e.g. genes encoding photoreceptors or receptors detecting microbial signals.

Epialleles: Different forms or alleles of a gene that are identical in DNA sequence but differ in epigenetic markers. These epigenetic differences are usually associated with differing expressions of the epialleles. The causes of their formation are as yet poorly understood.

Epigenetic: Includes the mechanisms of gene regulation that lead to heritable, but potentially reversible, changes in gene expression without changing the DNA sequence of the gene (Box 1).

Fitness: The fitness of an individual is taken as the relative abundance and success of its genes (often measured as the number of surviving offspring) over multiple generations. In many cases, especially with large or long-lived species, direct estimates of fitness are not feasible and total biomass, seed number or biomass, survivorship or growth rates of a single generation are used as proxies.

Genome plasticity: A change in genome structure or organization associated with environmental signals, leading to the evolution of new phenotypes, might result from mutational hotspots, genome expansion, transposable elements or somatic recombination.

Genotype: When we refer to a genotype we do so in a population genetic sense, not in reference to a molecular sequence of a single gene, but to the complete genome.

Phenotype: The appearance or characteristics of an organism resulting from both genetic and environmental influences. In our terms, all organisms have a phenotype not just those expressing a mutation in a given gene of interest.

Phenotypic plasticity: The range of phenotypes a single genotype can express as a function of its environment.

Plant functional traits: Quantitative traits related to the fitness and success of individuals in a given environment, they provide good indicators about species' ecologies (e.g. what growth rates they are likely to exhibit, what recruitment strategy they rely on) and are often related to competitive status, commonness/rarity or dominance in the community (Box 2).

Plant functional types: Categorical assessments enabling plant species to be grouped according to functional position in a community or ecosystem. For example, classifications can be based on growth form (e.g. herb, grass, shrub), nitrogen fixing status, photosynthetic pathway or leaf longevity.

Post-transcriptional and post-translational modifications: Chemical modifications to mRNA or proteins that are made after an mRNA or protein is transcribed or translated, respectively (e.g. the phosphorylation of proteins).

Regulatory gene transcription: The process of making mRNA of a regulatory gene. The RNA is subsequently translated to form a protein, the product of the gene.

Signaling cascades: These are cascades of events that mediate cellular responses to external signals, for example the cascades of protein phosphorylation and second messenger generation following the perception of a signal by a receptor kinase.

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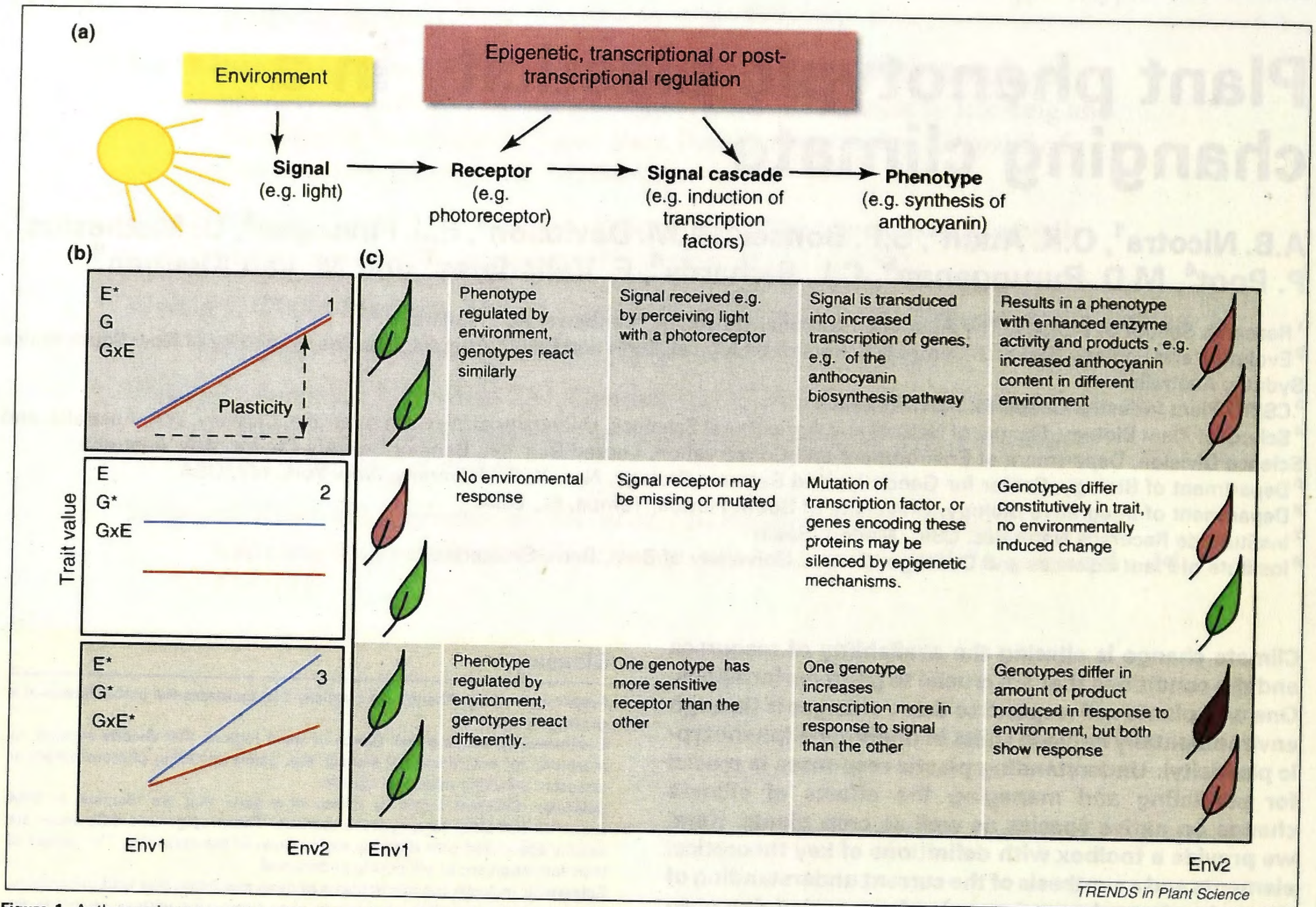

Phenotype regulated by environment, genotypes react similarly	Signal received e.g. by perceiving light with a photoreceptor	Signal is transduced into increased transcription of genes, e.g. `of the anthocyanin biosynthesis pathway	Results in a phenotype with enhanced enzyme activity and products , e.g. increased anthocyanin content in different environment
No environmental response	Signal receptor may be missing or mutated	Mutation of transcription factor, or genes encoding these proteins may be silenced by epigenetic mechanisms.	Genotypes differ constitutively in trait, no environmentally induced change
Phenotype regulated by environment, genotypes react differently.	One genotype has more sensitive receptor than the other	One genotype increases transcription more in response to signal than the other	Genotypes differ in amount of product produced in response to environment, but both show response

Figure 1. Anthocyanins are produced in leaves in response to excess light and temperature and osmotic extremes, and serve as a reversible plastic mechanism for the protection of photosynthetic machinery [86–88]. Here, we use an anthocyanin example to illustrate **(a)** the points in the molecular machinery, which translate an environmental signal (excess light in this case) into a phenotype. **(b)** In the evolutionary and ecological literature, these responses are commonly presented as reaction norms. Here, the blue and red lines indicate the reaction norms of two different genotypes responding to a change from a low light environment (Env1) to a high light one (Env2). The extent of phenotypic change in response to a signal is its phenotypic plasticity. Asterisks in the panels denote whether there is a significant effect of environment (E) or genotype (G), and whether there is a significant genotype by environment interaction (G × E). **(c)** Likely examples of the mechanisms underlying the cases depicted in panels 1–3 are given separately for each point in the signal pathway. The leaves on the left and right represent the phenotypes in Env1 and Env2, respectively.

or shifts in the geographic range of distribution [5,6]. These studies argue that the failure to expand beyond current limits demonstrates that a species' adaptive potential has been largely exhausted, or argue that plasticity will be an unimportant factor because the cues that signaled the plastic responses in the first place might no longer be 'reliable' in changed climates [7]. However, as we show below, plastic changes in seed longevity, phenology, leaf lifespan and the temperature responses of metabolic processes are all well documented in response to elevated CO_2 and climate change factors.

There is general acceptance that high levels of genetic variation within natural populations improve the potential to withstand and adapt to novel biotic and abiotic environmental changes including the tolerance of climatic change [8]. A portion of this genetic variation determines the ability of plants to sense changes in the environment and produce a plastic response. For example, genetic variation in genes encoding temperature sensors and transcription factors regulating vernalization (see below) could help plant populations adapt to changes in temperature. Plasticity, therefore, can both provide a buffer against rapid climate changes and assist rapid adaption [2,9]. Thus, we argue that, in the context of rapid climate change, phenotypic plasticity can be a crucial determinant of plant responses, both short- and long-term.

Here, we provide a conceptual toolbox with definitions of the key theoretical elements and a synthesis of the current understanding of the molecular and genetic mechanisms underlying phenotypic plasticity, as relevant to climate change. We discuss how new developments in our understanding of signaling cascades and epigenetics in particular hold promise for interdisciplinary approaches to understanding the evolution of plasticity and for predicting how plasticity will influence the responses of native plants and agricultural systems to climate change. We aim to provide background on the ecological and evolutionary literature on phenotypic plasticity and outline emerging techniques in molecular biology. By bringing these perspectives together, we hope to stimulate crucial cross-disciplinary dialogues on the topic of plasticity and plant responses to climate change [2,9] (Box 1).

Molecular basis of plastic responses in key traits

The ability of an organism to express plasticity in a given trait must be mediated at the molecular level [10] (See

Box 1. Outstanding questions

Modern techniques and the potential for cross-disciplinary approaches mean that we are now in a position to address the following questions effectively.

Q1: Molecular basis of plasticity:

- What is the genetic control of plasticity and how is it linked to epigenetics?
- Can we identify 'plasticity genes'?
- Does identifying such plasticity genes improve our ability to predict the longer term responses of traits and species to climate change?

Q2: Adaptive plasticity:

- What traits are likely to show adaptive plasticity?
- Will species with differing ecologies (i.e. differing functional types) exhibit adaptive plasticity in different traits?
- Will the incidence of adaptive plasticity vary among types of traits (e.g. those related to anatomy versus allocation versus physiology)?

Q3: Functional traits:

- Are the traits most commonly identified as plant functional traits also those that show adaptive plasticity?
- Is plasticity in functional traits important in determining response to climate change under future climates, regardless of current adaptive value?

Q4: Plasticity and evolution:

- How has plasticity contributed to the diversification of lineages and can the evidence of this contribution be found by comparing the distribution of adaptive plasticity or relevant plasticity genes with population or species phylogenies?
- How will plasticity contribute to rapid evolution in response to climate change?
- How much variation is there for plasticity and how does it respond to selection?

Q5: Plasticity in crop species:

- Has breeding led to reductions in adaptive plasticity in contemporary crop varieties relative to older ones or wild ancestors?
- Can we breed for plasticity in key traits in agricultural systems to improve yield stability under climate change?

Figure 1 and Figure I in Box 2). For example, developmental transformations have been shown to be controlled by environmental signaling pathways that sense abiotic cues such as light and nitrogen [11] and drought [12], as well as biotic signals such as Nod factors that cause nodulation in legumes under low nitrogen conditions [13]. For many other environmentally induced phenotypic responses, the mechanisms of how environmental signals are sensed and processed are still largely unknown [e.g. 14,15]. An improved understanding of the molecular basis of environmentally induced changes in plant traits will yield insight into possible ecological and evolutionary responses in wild species and will be useful for engineering plasticity in crop species (Box 1, Q1).

Flowering time is a good example of a crucial trait that has been shown to be both under genetic control and plastic (see below). Under climate change, the temperature cues triggering the chain of events leading to flowering might cease to be reliable if they occur at the wrong time with respect to the lifecycle and ecology of the species. Such changes in cue, signal or response schemes might thereby elicit maladaptive responses [7]. Alternatively, they can lead to the expression of phenotypic responses that are currently hidden [16]. Current techniques in molecular biology and genetics allow for studies of plastic trait responses that scale from a description of molecular mechanism to the assessment of adaptive value under current or simulated future climates [17]. Thus far the genetic basis of plasticity has been examined in greatest depth in model and crop species. As new tools become available, the extension of these studies to more non-model species becomes increasingly possible and will help us determine the extent to which there are genetic homologs in other species (Box 2).

Plasticity in key plant functional traits in response to climate change

Plasticity is a characteristic of a given trait in response to a given environmental stimulus, rather than a characteristic of an organism as a whole. Likewise, some responses are examples of adaptive plasticity, providing a fitness benefit, whereas others are inevitable responses to physical processes or resource limitations [18,19] (Figure 2). Both adaptive and non-adaptive plasticity will play a role in the context of plant responses to climate change. Differentiating between the two is important to our understanding of both the current value and the evolution of plasticity (Box 1, Q2). The consensus from the theoretical literature is that adaptive phenotypic plasticity should evolve in heterogeneous environments where signals of environmental conditions are reliable [19,20]. Hypotheses about what sort of species will be most plastic also abound in the literature [21–26], yet our ability to predict patterns of plasticity in key traits in response to climate change remains limited.

Given that it is not feasible to assess plastic responses to current or future environments on all species, it is important to identify which traits are likely to show important plastic responses to particular changing environmental conditions and to develop predictors to enable us to generalize about the sorts of species likely to exhibit these plastic responses [9]. Those traits can then be examined in current or projected climate conditions to determine the extent of plasticity and assess the extent to which the underlying molecular and genetic pathways are shared (Box 2).

Plasticity in plant functional traits

In recent years, ecologists have categorized species according to plant functional types and have also identified several continuous plant functional traits that vary in predictable ways along environmental gradients. Functional types are widely used in global climate models to group species according to their function in the ecosystem or community (e.g. C3 or C4 grasses, herbs, shrubs, deciduous trees, N-fixing legumes, etc.). Functional traits are those that help describe the ecology of species using a few, easily quantified variables (e.g. seed size, plant height, leaf lifespan, leaf mass per area, etc.) [27]. Functional traits are relevant to both global climate models and mechanistic models of plant distributions (see below). Considering their probable importance, we advocate that plant functional traits should have priority for the investigation of (adaptive) phenotypic plasticity and identification of molecular and genetic mechanisms across species (Box 3).

Adaptive plasticity in functional traits is likely to assist rapid adaptation to new conditions. Thus, a natural ques-

Box 2. Bridging evolution, ecology and molecular biology

Plastic molecular responses to environmental signals can occur in many ways. An external stimulus must first be perceived at the cell surface by a receptor (Figure I, 1) that then initiates a signaling cascade. Responses to the environmental challenge (lightning bolts) can include the post-translational modifications of the components of signaling pathways [69] (2). Alternatively, regulatory gene transcription can occur in many ways and in response to a broad range of stimuli (3,4,6,7). Epigenetic processes, including DNA methylation (4,5), histone modification (4) and transposable element activation (5,6), can also alter gene expression (5,6) and thereby mediate plasticity [70]. Changes to the population of small RNAs can lead to post-transcriptional control (RNAi) as well as changes in chromatin modification (4,8). Lastly, the expansion of short repeat sequences can affect gene expression (7).

New developments in our understanding of signaling cascades and epigenetics in particular hold promise for understanding the evolution of plasticity in natural systems and for predicting how plasticity will influence the responses of native plants and agricultural systems to climate change. For example, mutant or gene expression studies are useful for discovering genes underlying specific responses [12,71,72]. Techniques, such as quantitative trait loci [72,73] and linkage disequilibrium mapping [71,74], have also been used to identify natural variants in plasticity genes [19]. Plasticity genes might also evolve by the diversification of gene families in which the promoters of different family members perceive specific environmental cues. Once specific genes that lead to genetic variation in phenotypic plasticity have been isolated, one can employ molecular population genetic analyses of natural 'plasticity alleles' to infer the evolutionary histories of plastic phenotypes and the evolutionary forces that shape variation in these key loci. On a genome-wide scale, approaches to characterizing gene expression and epigenetic changes, including high-throughput sequencing, microarrays and proteomic approaches, offer the possibility to characterize patterns of plasticity at the scale of the genome rather than gene by gene [75,76].

This discussion demonstrates how phenotypic responses to environmental signals can be correlated with molecular signals at single genes and across the entire genome. Further investigation to identify the genome architecture that confers the responsiveness of key traits to particular stimuli might enable the prediction of plastic responses to novel environments posed by climate change.

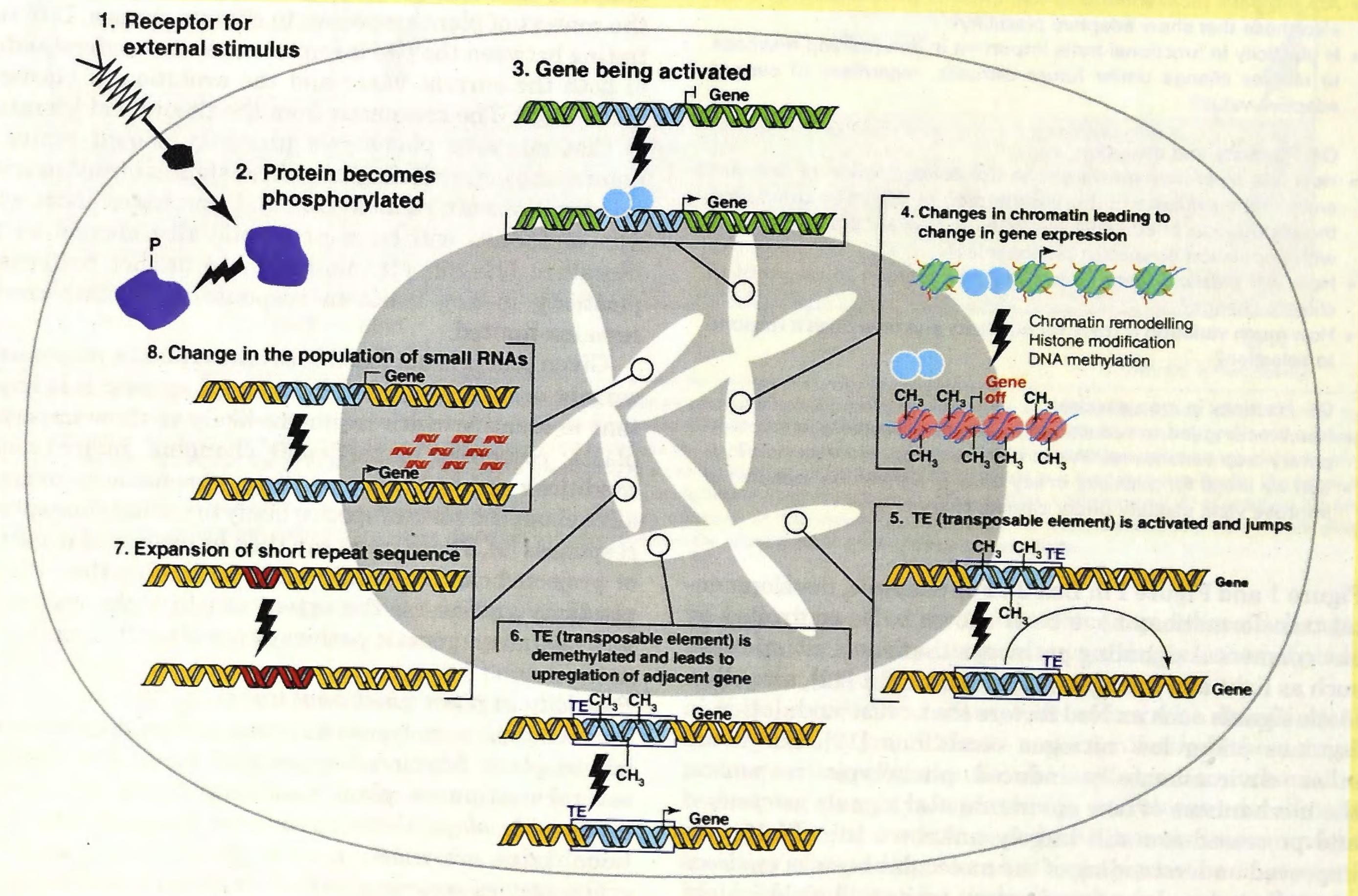

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Figure I. A variety of signaling cascades can be triggered in response to environmental signals. The subsequent genetic and epigenetic changes can occur in different cells/tissues, but are here presented in a single cell.

tion to ask is whether we can predict patterns of plasticity in functional traits based on the means of those traits themselves or based on other aspects of a species' ecology (Box 1, Q3). Although many studies have compared patterns of phenotypic plasticity in small numbers of species of contrasting ecologies, little consensus has emerged. As such, this question might be best addressed using a meta-analysis approach [28].

Plasticity in leaf phenology, flowering time and seed or seedling traits

Some of the best-documented effects of climate change have been shifts in leaf phenology [29] and flowering time [30]. Among plant species included in a meta-analysis, 87% show shifts in phenology to earlier spring times [31]. These changes might reflect both genetic (i.e. rapid evolution) and plastic changes [30].

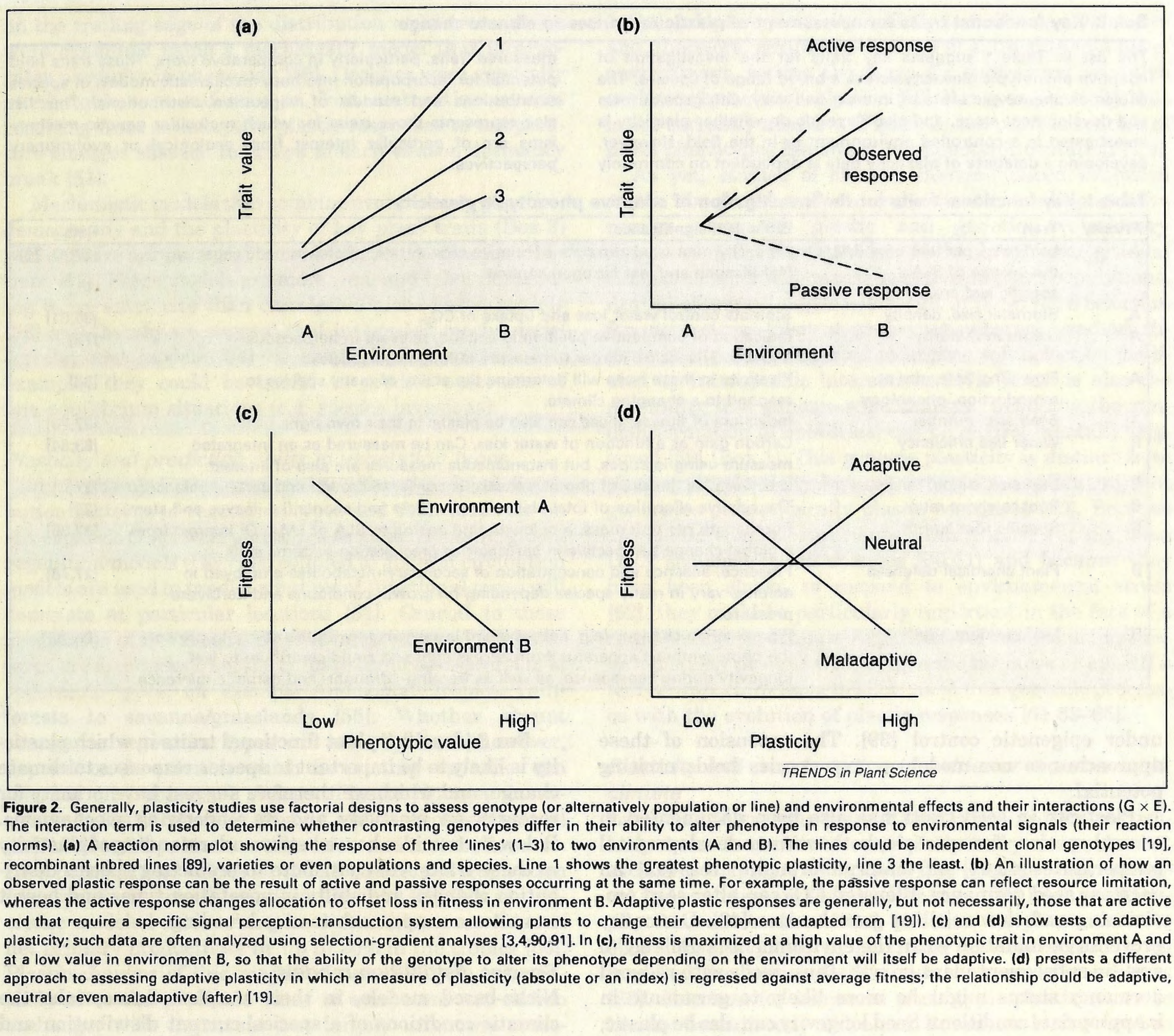

Figure 2. Generally, plasticity studies use factorial designs to assess genotype (or alternatively population or line) and environmental effects and their interactions (G × E). The interaction term is used to determine whether contrasting genotypes differ in their ability to alter phenotype in response to environmental signals (their reaction norms). **(a)** A reaction norm plot showing the response of three 'lines' (1–3) to two environments (A and B). The lines could be independent clonal genotypes [19], recombinant inbred lines [89], varieties or even populations and species. Line 1 shows the greatest phenotypic plasticity, line 3 the least. **(b)** An illustration of how an observed plastic response can be the result of active and passive responses occurring at the same time. For example, the passive response can reflect resource limitation, whereas the active response changes allocation to offset loss in fitness in environment B. Adaptive plastic responses are generally, but not necessarily, those that are active and that require a specific signal perception-transduction system allowing plants to change their development (adapted from [19]). **(c)** and **(d)** show tests of adaptive plasticity; such data are often analyzed using selection-gradient analyses [3,4,90,91]. In **(c)**, fitness is maximized at a high value of the phenotypic trait in environment A and at a low value in environment B, so that the ability of the genotype to alter its phenotype depending on the environment will itself be adaptive. **(d)** presents a different approach to assessing adaptive plasticity in which a measure of plasticity (absolute or an index) is regressed against average fitness; the relationship could be adaptive, neutral or even maladaptive (after) [19].

One example of an environmentally induced mechanism of regulating flowering time has been studied in detail in *Arabidopsis thaliana*. Flowering in *Arabidopsis* depends in part on the plastic downregulation of the transcription factor gene *FLC* (*FLOWERING LOCUS C*), which is regulated by epigenetic changes in histone modification in response to vernalization (prolonged exposure to cold) [30]. Here, the vernalization treatment acts via the transcriptional induction of a gene (*VIN3*), which then controls the recruitment or activity of protein complexes that modify chromatin and thereby silence the *FLC* locus [30]. Because FLC acts as a repressor of several flowering genes, its epigenetic silencing allows flowering to occur. The epigenetic silencing of *FLC* can only be reversed in the next generation. This pathway has been largely conserved in Brassicaceae, but with some variation [32]. The most extreme example being in *Arabis alpina* where *PERPETUAL FLOWERING* 1, an *FLC* ortholog, regulates flowering in response to vernalization and conditions a perennial growth habit [33].

The modifications of these regulatory pathways have been observed in several other plant species as well. For example, putative *FLC* homologs have been identified from different eudicot taxa including chicory (*Cichorium intybus*) [34] and sugar beet (*Beta vulgaris*) [35]. The sugar beet *FLC*-like gene is transiently downregulated by cold and delays flowering when expressed in *Arabidopsis* [35]. The response of *FLC* homologs in other species to cold and their roles in regulating flowering have not been elucidated. Phylogenetic analyses in four legumes found no close *FLC* homolog, but identified several homologs of genes that regulate FLC expression, e.g. VIN3 [36].

Despite the similarity in the physiology of the vernalization response between species, it is clear that this response has evolved independently in dicots and monocots. Cereals including rice, wheat and barley do not encode any homologs of *FLC*, but an unrelated transcription factor VRN2 plays a similar role as a flowering repressor as FLC [37,38], and a positive regulator of flowering VRN1, which is activated by cold temperatures to repress VRN2, is

Box 3. Key functional traits for assessment of plastic responses to climate change

The list in Table I suggests key traits for the investigation of adaptive phenotypic plasticity across a broad range of species. The choice of phenotypic traits of interest will vary with growth form and development stage, and also depends on whether plasticity is investigated in a controlled environment or in the field. However, developing a database of plasticity data is dependent on commonly measured traits, particularly in comparative work. These traits hold potential for incorporation into both mechanistic models of species distributions and models of vegetation distributions. This list also represents those traits for which molecular genetic mechanisms are of particular interest from ecological or evolutionary perspectives.

Table I. Key functional traits for the investigation of adaptive phenotypic plasticity

Priority	Trait	Biological significance	Refs
A	Leaf mass per unit area (LMA, the inverse of SLA, specific leaf area)	An easily measured correlate of relative growth rate, photosynthetic capacity, leaf lifespan and leaf nitrogen content.	[27,77–79]
A	Stomatal size, density	Stomata control water loss and uptake of CO_2.	[80,81]
A	Height at maturity	Indication of competitive position in a stand, relevant in herbaceous and woody species, harder to measure in long-lived species.	[78]
A	Flowering time, size at reproduction, phenology	Plasticity in these traits will determine the ability of many species to respond to a changing climate.	[82]
A	Seed size, number	Indicators of fitness; these can also be plastic in their own right.	[27,78]
B	Water use efficiency	Carbon gain as a function of water loss. Can be measured as an integrated measure using isotopes, but instantaneous measures are also of interest.	[83,84]
B	Leaf size, shape, thickness	Leaf form, as the site of photosynthesis, is crucial to growth and carbon balance.	[27]
B	Root-to-shoot ratio	The relative allocation of total plant mass to roots and shoots (i.e. leaves and stem)	[27]
B	Specific root length	Root length per unit mass, a belowground analog to SLA or LMA. Of interest from a global change perspective in particular as precipitation patterns shift.	[27,85]
B	Plant chemical defenses	Presence, absence and concentration of secondary metabolites employed in defense vary in many species depending on growth conditions and herbivore pressure.	[27,78]
B	Leaf pigmentation	Pigmentation changes (e.g. anthocyanin) is associated with the ability to protect the photosynthetic apparatus from excess light and could contribute to leaf longevity during senescence, as well as freezing-, drought- and osmotic-tolerance.	[86–88]

under epigenetic control [39]. The extension of these approaches to non-model or crop species holds exciting potential.

Plasticity in seed traits has also been documented in several species. For example, both warmer developmental temperatures [40,41] and maternal drought stress [42] can decrease seed dormancy. Elevated CO_2 can alter seed provisioning and slow seedling growth rates [43]. Dormancy prevents germination when the environment is unlikely to sustain subsequent plant growth; thus, seeds with reduced dormancy status might be more likely to germinate in inappropriate conditions. Seed longevity can also be plastic; for example, changes in temperature and rainfall experienced during seed development have the potential to halve seed longevity [44]. These examples demonstrate that the effects of climate change on plastic regeneration traits could be substantial. As yet, the mechanisms underlying these plastic responses and whether they could be adaptive under current conditions remain unknown.

Plasticity and shifts in the distribution of species and vegetation types under climate change

Future changes in climate could result in extinctions, range shifts, changes in major vegetation types and alterations in feedbacks between vegetation and the atmosphere. Indeed, the distribution of many plant species has already altered in response to climate change; some species have shown up to 6 km pole-ward migration each year over the past 16–132 years [31]. Recent years have seen tremendous progress in species distribution and vegetation models but as yet most of these models do not consider the phenotypic plasticity of existing genotypes or the evolution of either traits or plasticity itself [9].

Box 3 identifies plant functional traits in which plasticity is likely to be important to species responses to climate change, and which we therefore suggest have priority for research on plasticity and its underlying mechanisms. Below we consider how a better understanding of plasticity in these traits will contribute to predicting species distribution changes and shifts in vegetation types and how it can alter our approach to crop breeding.

Species distribution models

Niche-based models, in their simplest forms, take the climatic conditions of a species' current distribution and use modeled future climatic scenarios to project future distributions [45]. They generally assume that distributions reveal the ecological potential of the current gene pool and that the niche does not change over time [46]. However, the environmental conditions currently occupied by a species can fail to reveal the full extent of its potential range (fundamental niche) for reasons such as dispersal limitation, the effects of species interactions and the likelihood that portions of that potential niche are currently unexpressed because they do not correspond to any contemporary environment [46].

Phenotypic plasticity will be particularly important in predicting dynamics at population boundaries. At the trailing edge, plasticity can buffer population declines and influence the potential of the species to adapt to novel conditions [47]. At the leading edge, shifting species interactions might lead to unanticipated plastic responses. Recently, mechanistic models that incorporate physiological knowledge about variation within a species in response to environment have offered an alternative to purely correlative models [48,49]. For example, population declines on the trailing edge of the distribution of 16 tree species were examined using a mechanistic model that incorporates plasticity in phenology [50]. Declines were generally attributable to a reduction in fruit maturation success resulting from maladaptive plastic responses to temperature changes that led to delays in early-season dormancy break [51].

Mechanistic models that combine evolutionary genetics, demography and the plasticity of key plant traits (Box 3) will improve our potential to model future species distributions [52]. These models are more time and labor demanding to parameterize than correlative niche-based models [53] and thereby we suggest that integrated mechanistic/correlational models [54] be strategically directed. For example, they could be useful to predict outcomes of non-equilibrium situations (e.g. species invasions).

Plasticity and predicting shifts in vegetation types

Climate change is also predicted to affect the global distribution patterns of vegetation types and their feedback on atmospheric CO_2 levels and temperatures. Dynamic global vegetation models (DGVMs) coupled to general circulation models are used to predict what plant functional types will dominate at particular locations [51]. Crucial to these predictions of increased CO_2 concentrations and temperatures are feedbacks from the climate-induced conversion of vegetation types, for example Amazonian tropical rainforests to savanna/grasslands [55]. Whether abrupt changes in vegetation types will actually occur, however, depends on the extent to which the existing vegetation can tolerate environmental change. Plastic changes in response to temperature or drought are commonly observed in leaf chemistry, biomass allocation and metabolic rates. Incorporating real values for the acclimation of respiration in response to growth temperature into DGVMs can decrease modeled rates of respiration and increase rates of net primary productivity by up to 20% in the tropics [56]. Plastic changes of this magnitude are likely to substantially alter the predicted rates of ecosystem net carbon exchange, with important but largely unknown consequences for future atmospheric CO_2 concentrations and global temperatures.

Some of the tools to incorporate phenotypic plasticity are already available for DGVMs [56,57]. Most of these models work on a functional type basis, where types might reflect growth form or photosynthetic pathway rather than incorporating detailed trait data for specific species. Thus, studies of whether species can be classified into functional types for plasticity are also needed to make use of these tools. Strategic data collection to answer these questions will require effective dialogue between modelers and biologists to identify both traits and key species or functional type definitions on which to focus efforts (Box 1, Q3).

Plasticity, phenotypic evolution and breeding in response to rapid changes

In the short-term, the plastic responses of existing genotypes will be of particular importance in determining plants' persistence under climate change. These plastic responses might, however, also have important consequences for longer term evolutionary pathways [58] (Box 1, Q4). Adaptive plasticity is likely to facilitate persistence and, therefore, reduce the chances of extinction in a novel environment, setting the stage for subsequent adaptive evolution by natural selection [58]. But even plasticity that is not currently adaptive (Figure 2) can provide sources of novel phenotypes important in phenotypic evolution [2,9].

As yet, studies of climate change-induced evolution under simulated and natural climatic conditions have rarely integrated plastic and genetic evolutionary responses [17]. Nevertheless, both abrupt and gradual climate changes will impose selection on plant populations. Abrupt climate changes will result in rapid hard selection for more stress-tolerant genotypes, whereas gradual climate changes are expected to impose soft selection mediated by intraspecific interactions [17]. There is also the possibility that genome-wide changes, including the random formation of epialleles, can be environmentally triggered [59] (Box 1). This genome plasticity is distinct from phenotypic plasticity but can provide a mechanism that generates phenotypically plastic responses [60]. Because epigenetic changes can happen much more rapidly than DNA sequence-based changes [60,61] and because they have been shown to respond to environmental stress [62], they could be particularly important in the face of a rapid change in climate. Experimental studies using classic plasticity designs with epigenetic markers or epi-RILs will be important tools to allow us to link genomic processes with the evolution of plastic responses [61,63–65].

Plasticity and crop breeding in a drier or more variable climate

Lastly, amid growing fears of food crises, we are particularly keen to motivate cross-disciplinary research that synthesizes applied research in crop systems with ecological and evolutionary theory. Crop scientists have traditionally focused on directional selection on plant traits to obtain higher yields in particular environments, or on breeding for homeostasis under a range of conditions [66]. Selection for increased phenotypic plasticity *per se* has not been directly addressed. Because selection is often conducted on trait values under a single productive condition, we suggest that it is currently unclear whether domestication and breeding have led to increased or decreased plasticity in traits indirectly associated with yield. Genetic lines selected for relative yield stability could have high phenotypic plasticity because relatively large morphological and physiological changes can underlie yield stability [66].

Breeding for phenotypic plasticity in traits other than yield will potentially afford resilience in an increasingly unpredictable environment [67]. For example, breeding for plasticity in water use traits could lead to better survival and higher average yields [68]. Likewise, novel approaches to identify key environmental sensing genes in crop and model systems can lead to an opportunity to breed for phenotypic plasticity to build resilience in an increasingly variable environment [68] (Box 1, Q5).

Concluding remarks

There is increasing evidence of the importance of plasticity in plants under climate change in both natural and agricultural systems. Our aim has been to discuss the potential roles of plasticity in determining plant response to and effects of climate change in a way that is accessible and relevant to ecologists, physiologists and molecular biologists alike. We see progress in this field as being very much dependent on multidisciplinary approaches and the application of emerging techniques. We have identified outstanding questions in the field as directions for future research (Box 1). Many of these are extensions of longstanding questions such as how common and important is adaptive plasticity, what is the molecular genetic basis of plasticity and what is the relevance of plasticity in determining species distributions and vegetation processes? Answers to these tantalizing questions are now relevant in an applied context and are closer to our grasp thanks to exciting new technical progress and the potential for integrative multidisciplinary approaches.

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Adaptive phenotypic plasticity and plant water use

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Abstract. The emergence of new techniques in plant science, including molecular and phenomic tools, presents a novel opportunity to re-evaluate the way we examine the phenotype. Our increasing capacity for phenotyping means that not only can we consider increasing numbers of species or varieties, but also that we can effectively quantify the phenotypes of these different genotypes under a range of environmental conditions. The phenotypic plasticity of a given genotype, or the range of phenotypes, that can be expressed dependent upon environment becomes something we can feasibly assess. Of particular importance is phenotypic variation that increases fitness or survival – adaptive phenotypic plasticity. Here, we examine the case of adaptive phenotypic plasticity in plant water use traits and consider how taking an ecological and evolutionary perspective on plasticity in these traits might have relevance for agriculture, horticulture and the management of native and invasive plant species in an era of rapid climate change.

Additional keywords: fitness, G × E interaction, phenomics, phenotyping, water use efficiency, WUE.

Introduction

A 'water-wise' perennial plant is not like a 'water-wise' dishwasher. While the appliance should conserve water at all times (and wash dishes well), a 'water-wise' plant should conserve water only when water is limiting. It will adjust growth to optimise fitness or maximise probability of survival under stress. When water is abundant, however, a 'water-wise' plant should capitalise on available water, maximising growth and flowering, because unused water will either be used by competitors or lost from the system as evaporation or runoff. This is a teleological view but the point holds: when assessing whether a plant is 'water-wise', we must consider not just efficient water use when water is limiting, but also the plant's ability to utilise water when it is available. Here, we consider how modern plant science and evolutionary ecology can work together to understand the adaptive and applied significance of environmentally induced variation in plant water use traits.

Mutation is traditionally seen as the source of variation in evolution. But selection acts on the products of gene expression – the phenotype – not only on the underlying genetic code. The phenotype, unlike the genotype, varies over the course of an organism's life and depends on the environment in which the organism develops. Phenotypic plasticity describes the range of phenotypes a single genotype can express as a function of its environment (Bradshaw 1965; Schlichting 1986). By genotype, we refer not to the sequence of a single gene, but to the complete genome of a single genetic individual. In this era of 'omics' – be it genomics, proteomics, or phenomics – understanding the causes and consequences of phenotypic variation is becoming more relevant and, most importantly, more feasible.

Over recent years, phenotypic plasticity has moved from being seen as a troublesome source of noise to being accepted as a characteristic that is itself under selection and of ecological and evolutionary significance (Via *et al.* 1995; Reymond *et al.* 2003; Schlichting 2004; de Jong 2005; Murren *et al.* 2005; West-Eberhard 2005; Forde 2009). It is now recognised that plasticity is heritable (Tucic *et al.* 2005; Weijschedé *et al.* 2006) and can be altered in artificial selection experiments (Garland and Kelly 2006; Teuschl *et al.* 2007).

It is easy to see that plasticity of key functional traits may determine an organism's ability to establish (Schlichting and Levin 1986). Further, if the plasticity increases that organism's fitness, it may enable the taxon to persist in highly variable environments or over broad niches. Through plasticity, novel phenotypes can be exposed to selection (Agrawal 2001; Pigliucci *et al.* 2006). If those phenotypes increase fitness, as some researchers have proposed, they may become fixed in the population via genetic assimilation (Waddington 1953; West-Eberhard 2005; Pigliucci *et al.* 2006). Thus, it has been posited that differing selection pressure on novel phenotypes could lead to local adaptation and speciation – or phenotypic evolution (Schlichting 2004; de Jong 2005; Pigliucci *et al.* 2006).

All organisms possess some degree of phenotypic plasticity, but for sessile organisms like plants, including food crop species, plasticity may be of particular importance. Traditionally, agricultural plant breeders have viewed plasticity as an unwanted complication (but see Johnson and Frey 1967), but perspectives on that are changing (Bradshaw 2006; Chapman 2008; Forde 2009; Sadras *et al.* 2009). If we can understand the genetic mechanisms underlying phenotypic plasticity (Schlichting and Smith 2002; Reymond *et al.* 2003; Forde 2009), we may well be able to breed for adaptive phenotypic plasticity that improves performance over a broad range of conditions.

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Here, we consider adaptive plasticity in plant water use traits from an ecological and physiological perspective. We review the literature on adaptive plasticity in water use traits – both evolutionary and agricultural. We also explore invasive and agricultural species as case studies in growing plants under novel environmental conditions. And lastly, we discuss how future research building on links between genetics, ecology and evolution might be directed to develop truly 'water-wise' plants.

The adaptive value of water use efficiency in dry environments

Water use efficiency (WUE) refers to carbon gained per unit water lost. This can be calculated at a stand level, at a whole-plant level or at the leaf level and over time scales ranging from instantaneous (a few minutes) to a season (Sinclair *et al.* 1984; Condon *et al.* 2004). Here, we focus our discussion on a physiological definition of WUE at the leaf level, rather than an agronomic definition (see Sinclair *et al.* (1984) and Condon *et al.* (2004) for further discussion of WUE measures and terminology). Our examples are drawn from measures of integrated WUE using isotopic indicators, δ^{13}C in particular (Farquhar *et al.* 1989), or instantaneous measure using gas exchange. Instantaneous measures are derived from A (carbon gain, μmol m^{-2} s^{-1}) and E (transpiration, mmol m^{-2} s^{-1}) or g_s (stomatal conductance to water, mmol m^{-2} s^{-1}) or even ET (evapotranspiration); A/E is often described as transpiration efficiency (TE; Condon *et al.* 2004). Measurements using δ^{13}C are often more reliable than instantaneous measures of WUE because the latter are sensitive to measurement conditions (see Seibt *et al.* (2008) and Cernusak *et al.* (2009) for a discussion of the utility of δ^{13}C measures). As with any ratio, WUE is a function of its component traits: carbon assimilation and water loss. Assimilation (A) is the product of stomatal conductance to CO_2 and the gradient of CO_2 concentration from inside to the outside of the leaf (Condon *et al.* 2004); however, recent work suggests that this physiological correlation is not necessarily accompanied by strong genetic correlations (Caruso *et al.* 2005). Further, water use patterns are determined by other traits not explicitly considered in the calculation of WUE at leaf level (WUE_L) (but implicit in measures of WUE at the whole plant or stand level). Traits that influence WUE include leaf level traits, for example leaf architecture and cuticle properties, leaf anatomy and mesophyll conductance as well as plant level traits such as root : shoot ratio, the turnover rate of fine roots and presence of root symbionts (e.g. Chaves *et al.* 2003; Picotte *et al.* 2007; Cattivelli *et al.* 2008; Forde 2009). There are also fundamental co-variances between physiological and structural traits (Reich *et al.* 1997) and these have consequences for WUE at all scales. For example, Knight *et al.* (2006) suggest that the higher LMA (leaf mass per unit area) of many drought-affected plants contributes to higher WUE. Higher LMA in drought-tolerant species is often due to specialised biophysical properties of the leaves to reduce heat load as well as a greater investment in leaf structure to prevent wilting under water stress (Wright and Cannon 2001).

It is almost implicit in the concept of *efficiency* that conservative resource use is good, and therefore adaptive, but this assumption is seldom tested and when tested, it is not consistently supported. Plants native to or grown in lower rainfall areas do generally have greater root : shoot ratios (Nicotra *et al.* 2002), lower stomatal conductance (Warren 2008) and higher WUE (Dudley and Schmitt 1996 and references therein; Picotte *et al.* 2007), though among arid-adapted species, there are a range of strategies for surviving the vagaries of life in a drought-prone environment (Jordan and Miller 1980; Schwinning and Ehleringer 2001). In any case, for more efficient water use in water-stressed environments to be adaptive, it must increase fitness. Studies directly examining fitness consequences of WUE show that in some cases, selection favouring individuals with high WUE is advantageous, while in other cases, favouring low WUE is advantageous. In other cases, there is no correlation at all between WUE and growth or survival (e.g. Condon *et al.* 2004).

In the context of domesticated species (especially fruit and grain crops) subject to artificial selection, yield or harvest index (the proportion of biomass that is marketable grain) become the 'agricultural fitness' indicators of interest. Cattivelli *et al.* (2008) provide some evidence to suggest that breeding for increased wheat (*Triticum aestivum* L.) yield in high resource conditions has indirectly resulted in increases in yield across all environments, even low-rainfall ones. Note that in some cases, increases in yield are a function of phenology or changes in flowering time (Sadras *et al.* 2009). Presumably, these increases must be accompanied by increased WUE at low water. In contrast, a strong negative relationship between WUE and yield has been demonstrated in other crops, for example, sunflower (*Helianthus annuus* (Douglas ex Lindl.); Virgona and Farquhar 1996) and groundnut (*Arachis hypogaea* (L.); Nautiyal *et al.* 2002).

Likewise, studies from natural ecosystems are inconsistent on the adaptive value of WUE. Although some studies report relatively high WUE being favoured in areas of lower rainfall, including desert (Ehleringer 1993*a*; Dudley 1996*a*, 1996*b*) and temperate species (e.g. Heschel and Riginos 2005; Knight *et al.* 2006), there are also several examples in the literature where higher WUE is not favoured in dry environments at all (e.g. Geber and Dawson 1990; Donovan and Ehleringer 1994; Geber and Dawson 1997; Pennington *et al.* 1999; Arntz and Delph 2001; Donovan *et al.* 2007).

In large part, these conflicting results reflect differences in patterns of water availability. A range of strategy schemes has been proposed to help explain these differences. One of these classifies plants as avoiding, or escaping dehydration, the other is tolerating drought (Kramer 1980). High WUE in dry environments is consistent with a dehydration avoidance strategy, which is often associated with slower overall growth rates but greater survival under low water availability. This ability to tolerate drought may be underpinned by a myriad of physiological and morphological adaptations associated with high WUE, some of which are likely to be plastic. In contrast, drought escape is common for many short-lived or annual dessert species for which periods of water availability are sporadic and highly variable. Such species generally have low WUE, high rates of carbon assimilation, fast growth rates and phenologies that enable them to reproduce before water limitation becomes severe. Arntz and Delph (2001) suggested that particularly for annual species, a drought escape-type strategy is likely to confer higher fitness when the growing season is short or disturbances are frequent; the opposite conditions would select for dehydration avoidance. Schwinning and Ehleringer (2001) explore tradeoffs in soil water use patterns that influence the evolution of architecture and allocation patterns in arid-adapted species. These authors identify four different phenotypes that each represent optima at particular conditions of pulsed rainfall events – depending on the frequency, size and duration of the pulses (Schwinning *et al.* 2004). The four phenotypes broadly encompass the range of arid zone growth forms found in nature. Each of these frameworks provides an explanation for species level differences in allocation strategies, but neither explicitly explores the role of phenotypic responses to environment at the organism level. Presumably, the strategies are characterised not only in mean differences in form, but also in the plasticity of these traits.

How well a plant tolerates limitations in water availability is also mediated by tradeoffs between water conservation and gain or tolerance of other resources or conditions. A classic example of this is the observation that resource availability may affect the adaptive value of WUE at a given water availability. For example, WUE is often lower when nitrogen is limiting. Leaf N is a major driver of photosynthetic capacity and is critical to determining high WUE when high WUE is due to high assimilation rate rather than low stomatal conductance (Donovan *et al.* 2007). For two desert sunflower species of hybrid origin, Donovan *et al.* (2007) demonstrated significant selection pressure for higher leaf N uptake. In *Helianthus anomulus* Blake, this selection for high N is accompanied by direct selection for low WUE whereas in *Helianthunus deserticola* Heiser, selection on WUE is indirect. Ludwig *et al.* (2004) also studied hybrid sunflowers and found that although the hybrids occupied drier environments than their parents and had lower leaf N and smaller leaves, selection was currently favouring phenotypes with larger leaves and higher leaf N; there was no direct selection for WUE itself. In the case of several cultivars, selection for higher yield has indirectly selected for stomatal characteristics that favour high conductance at the expense of WUE but that confer heat resistance (Radin *et al.* 1994; Fischer *et al.* 1998; Soar *et al.* 2009). This pattern of results may reflect that these species are colonisers where fast growth in order to acquire more resources is favoured over more conservative growth strategies. Ehleringer (1993*b*) found that in disturbed situations where competition was low, *Encelia farinose* Torr. & A.Gray, a desert shrub, adopts high carbon gain and low WUE to facilitate rapid establishment, whereas when competition for water limits growth it has a high WUE phenotype.

There is also evidence of changing selection pressure on WUE depending on developmental stage. Caruso *et al.* (2006) find that the direction of selection changes from favouring low to high WUE as *Lobelia* plants mature. In the borage *Cryptantha flava* (A. Nelson), selection pressure on WUE changes from significant and negative to less substantial but positive as the plants age (Fig. 1; Casper *et al.* 2005). Early survival is improved by low WUE, but subsequent size, and by extension fitness, is positively correlated with higher WUE (see Donovan and Ehleringer 1991; Cavender-Bares and Bazzaz 2000). These results suggest that higher WUE later in life may arise in part because of increased photosynthetic capacity rather than decreased stomatal conductance, perhaps driven by photosynthetic sink strength during flowering or fruiting. Alternatively, changes in root : shoot ratio or root depth with ontogeny may be responsible for the observed change in selection for WUE as root : shoot ratio often decreases with age (Gedroc *et al.* 1996). If changes in selection pressure on WUE over the course of an organism's life are common, we would likewise expect there to be widespread adaptive value for phenotypic plasticity in WUE.

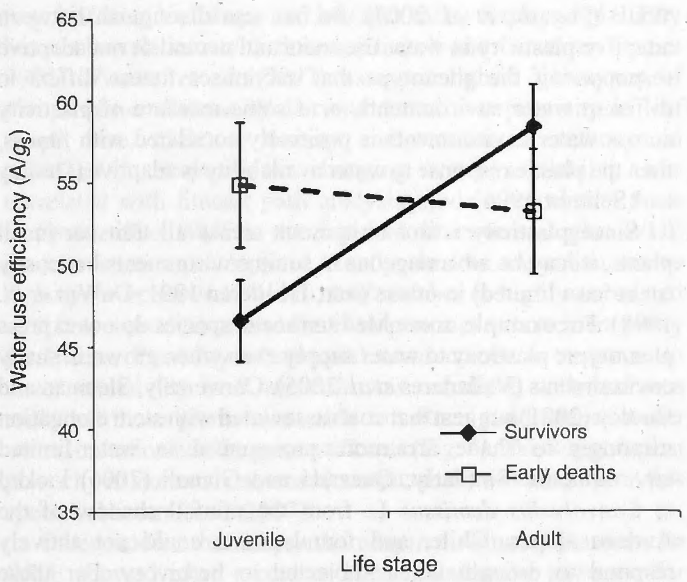

Fig. 1. Water use efficiency at leaf level (WUE$_L$, A/g_s) of the perennial sub-shrub *Cryptanthra flava* (Boraginaceae) as a function of plant size and whether the plants survived past adulthood or not (adapted from data in Casper *et al.* 2005). Those plants that survive to adulthood change from low WUE as juveniles to high WUE as adults.

Phenotypic plasticity in water use traits

Plant physiologists have long studied plastic responses of plants to different environments. All of these responses, be they reversible acclimatory responses of biochemical or membrane properties, or non-reversible alterations in growth and allocation over the course of a lifetime, are expressions of plasticity. Previously, the adaptive value of plasticity in traits was taken almost as a null hypothesis, but phenotypic plasticity in a given trait need not be adaptive – some plastic responses will be neutral, others even maladaptive (van Kleunen and Fischer 2005). Plasticity is also both trait- and environment-specific; e.g. patterns of plasticity in response to water limitation may be very distinct from response to N limitation and plasticity in photosynthetic rate or stomatal conductance may well underlie homeostasis in WUE. Variation in some traits arises passively when growth is slowed by resource limitation or as a result of genetic correlations with traits that are under selection (van Kleunen and Fischer 2005). Such variation in a trait can be described as passive plasticity and is not likely to be directly correlated with fitness (e.g. Caruso *et al.* 2006). In other cases, a plastic response can actually reduce fitness. For example, Scots pine (*Pinus sylvestris* L.) trees tend to adopt higher transpiration rates at drier/warmer sites resulting in a counter productive lower

WUE (Poyatos *et al.* 2007). So, we can distinguish between adaptive plasticity in water use traits and neutral or maladaptive responses: if the phenotype that maximises fitness differs in different water environments, or if some measure of plasticity across water environments is positively correlated with fitness, then the plastic response to water availability is adaptive (Dudley and Schmitt 1996).

Since plasticity is not ubiquitous across all traits or in all plants, it may be advantageous in some environments but costly (or at least limited) in others (van Tienderen 1991; DeWitt *et al.* 1998). For example, some Mediterranean species do not express phenotypic plasticity to water supply even when grown in shady environments (Valladares *et al.* 2005). Conversely, Sleeman and Dudley (2001) suggest that costs associated with stem elongation responses to shade are more pronounced in water-limited environments. Similarly, Quezada and Gianoli (2006) looked at *Convolvulus demissus* L. from the rainfall shadow of the Andean slopes, Chile, and found plants could not actively respond to drought when subjected to herbivory. For these reasons, it is often suggested that plastic responses are more costly when resources are limited, or that plasticity should be limited in consistently stressful environments (Valladares *et al.* 2005; but see Funk and Vitousek 2007). Finally, a further question is whether plasticity in fitness determinants itself could be adaptive (Weiner 2004). In a recent study, and one of few looking at plasticity *per se* in agricultural crops, Sadras *et al.* (2009) demonstrate how plasticity in yield (phenology) can be a potentially adaptive trait, if it enables a genotype to take advantage of environments that support high yield, while not compromising minimum yield in lower quality sites (Fig. 2).

Thus far, empirical examples of adaptive plasticity are still relatively rare. The best examples come not from studies on water use traits, but rather those involving induced defences to herbivory (Strauss *et al.* 2002) and stem elongation in response to crowding (Schmitt *et al.* 1999). For both of these traits, we have a fairly good understanding of mechanism – from gene to function. Water use traits and plasticity therein are of primary importance to plant growth and survival. Although we have a growing understanding of the genetic and molecular drivers of water use traits and WUE, adaptive plasticity in these has received relatively little attention.

The adaptive value of plasticity in WUE

Hundreds, maybe thousands of ecophysiological studies document plants' responses to drought and then infer an adaptive value to plasticity in water use traits. For example, a trade-off between stomatal size and number and the production of smaller stomata at low water is well documented, though the underlying determinants of stomatal patterning and plasticity therein are poorly understood (Croxdale 2000). It has also been known for a long time that many desert species maintain high stomatal conductance rates and therefore lower leaf temperatures as a result of accessing deep water when it is available (Smith 1978; Berry and Bjorkman 1980). These leaves also often have high LMA and high photosynthetic rates. Many arid zone species are ephemeral or facultatively deciduous and thus exhibit plasticity in phenology or leaf lifespan. More explicitly with respect to WUE, a study comparing native and exotic dandelions (*Taraxicum*) found that the native had high, but not plastic WUE_L, whereas the exotic was plastic (Brock and Galen 2005). Or, examining plasticity in water use traits in *Populus* species, Funk *et al.*

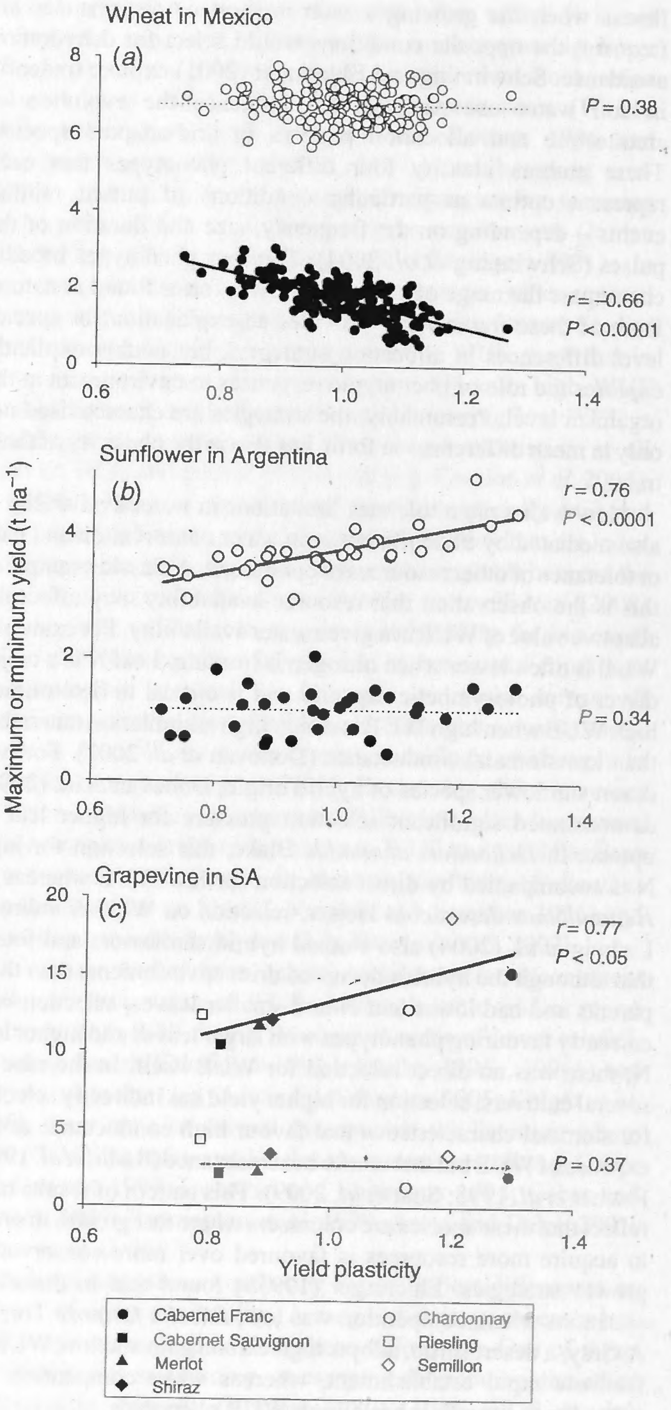

Fig. 2. Relationships between yield plasticity and maximum (open circles for wheat and sunflower) and minimum yield (closed symbols for wheat and sunflower) in wheat, sunflower and grapevine. (*a*) For these wheat lines, high yield plasticity is an undesirable traits because it is associated with low yield in low yielding environments and is unrelated to yield in the more productive environments. (*b*) For the sunflower and (*c*) grapevine lines, in contrast, high yield plasticity was associated with increased maximum yields in better environments and was largely independent of minimum yields in low yielding environments (reproduced from Sadras *et al.* 2009 with permission).

(2007) found that stem and leaf traits varied in plasticity in response to water and nutrient availability. While such studies demonstrate natural plasticity in WUE and related traits, markedly few studies have directly tested whether this measurable plasticity is adaptive.

One good example of adaptive plasticity in WUE comes from the work of Heschel *et al.* (2002). They showed that in inbred lines of *Impatiens capensis* Meerb., an increase in WUE in response to drier conditions was correlated with increased fitness under these conditions (Fig. 3). Lines sourced from the more heterogeneous rainfall area (the dry area) displayed more plasticity in WUE in response to water availability than lines sourced from wet areas, largely due to changes in stomatal conductance. This response resulted in higher fitness irrespective of light conditions despite selective pressure for higher photosynthetic rates (which may cause WUE to be reduced) in shade environments (Heschel and Riginos 2005). Heschel *et al.* (2004) also examined plasticity in WUE across multiple populations of *Polygonum persicaria* L. They found evidence of selection for increased mean photosynthetic capacity and WUE in *P. persicaria* plants from two variably dry sites and one wet site. All three populations also showed plasticity to water conditions although plants from the wet site had greatest plasticity to WUE and those from the dry sites showed relatively higher plasticity in root allocation. None of the populations showed a decline in achene number with drought stress, suggesting an ability to moderate fitness under drought stress; the fitness homeostasis was maintained despite decreased plant size.

In *Convolvulus chilensis* Pers., plasticity in trichome density in response to precipitation patterns was adaptive; other traits (e.g. leaf area, leaf shape and leaf area ratio) displayed plasticity but not correlations with fitness (Gianoli and Gonzalez-Teuber 2005). Picotte *et al.* (2007) assessed hybrid genotypes of *Piriqueta caroliniana* Walter and found that plasticity in leaf shape, size, trichome density and amount of anthocyanin produced in response to changing water availability was correlated with fitness; path analysis models reveal that these leaf traits affect WUE in the expected direction so higher WUE in drier sites was adaptive. Finally, in the Australian native *Pelargonium australe* Willd., leaf number is highly correlated with flower production and therefore provides a convenient proxy for fitness. Plasticity of stomatal conductance in response to water availability in *P. australe* was correlated with leaf area such that those plants most able to reduce conductance under water limitation had the greatest fitness (Fig. 4). This suggests that plasticity in stomatal response (conductance) was adaptive in this species (Nicotra *et al.* 2007).

Other studies that assess the adaptive value of plasticity in WUE showed more mixed or even maladaptive patterns. For *Cakile edentula* Bigelow grown in wet and dry environments, selection analyses (see below) suggested that WUE was adaptive in low but not in high water, indicating that plasticity should be under selection (Dudley 1996*a*, 1996*b*). Likewise, analyses showed selection gradients for intermediate leaf size at low water and no selection on leaf size at high water. Leaf size and WUE were under correlational selection, with a higher leaf size being selected in plants that had high WUE in arid environments. Oddly however, the plants grown under dry conditions had higher A and lower WUE than those grown at high water. So, selection favours higher WUE under dry conditions and adaptive plasticity

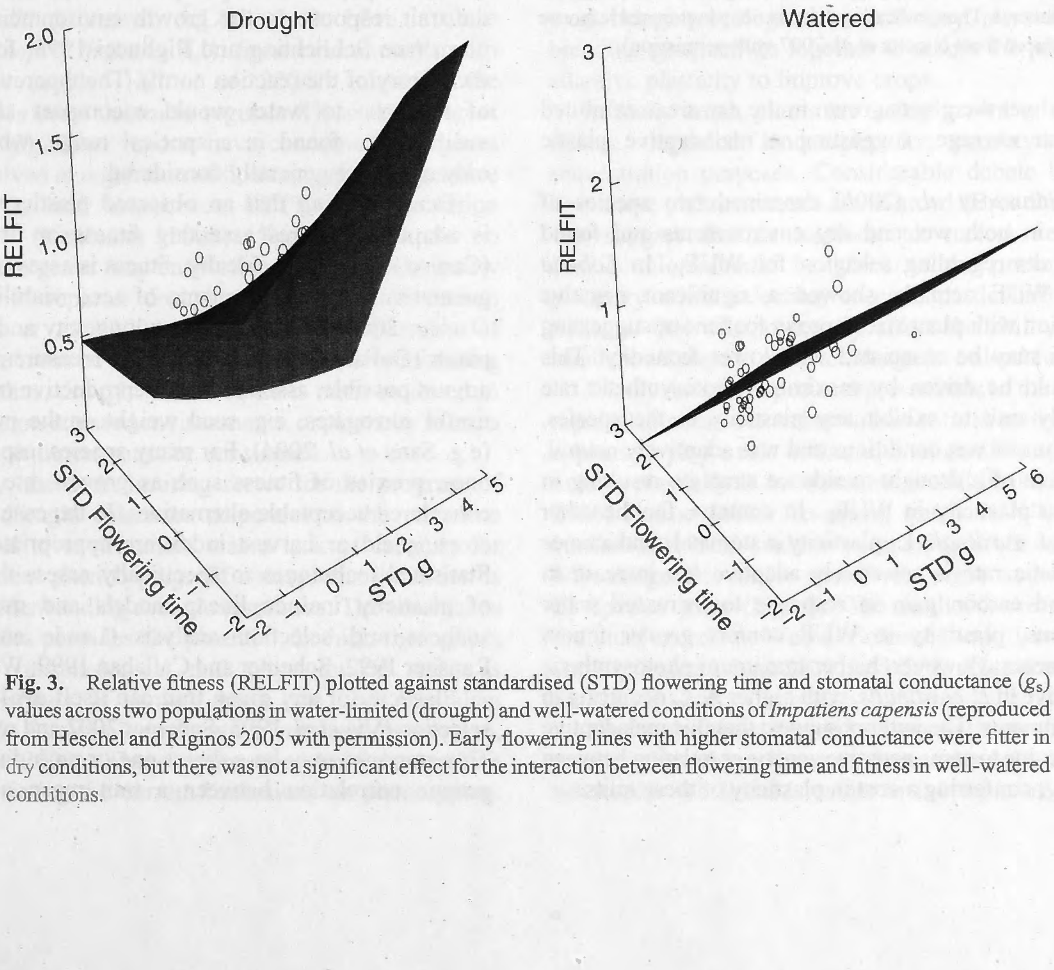

Fig. 3. Relative fitness (RELFIT) plotted against standardised (STD) flowering time and stomatal conductance (g_s) values across two populations in water-limited (drought) and well-watered conditions of *Impatiens capensis* (reproduced from Heschel and Riginos 2005 with permission). Early flowering lines with higher stomatal conductance were fitter in dry conditions, but there was not a significant effect for the interaction between flowering time and fitness in well-watered conditions.

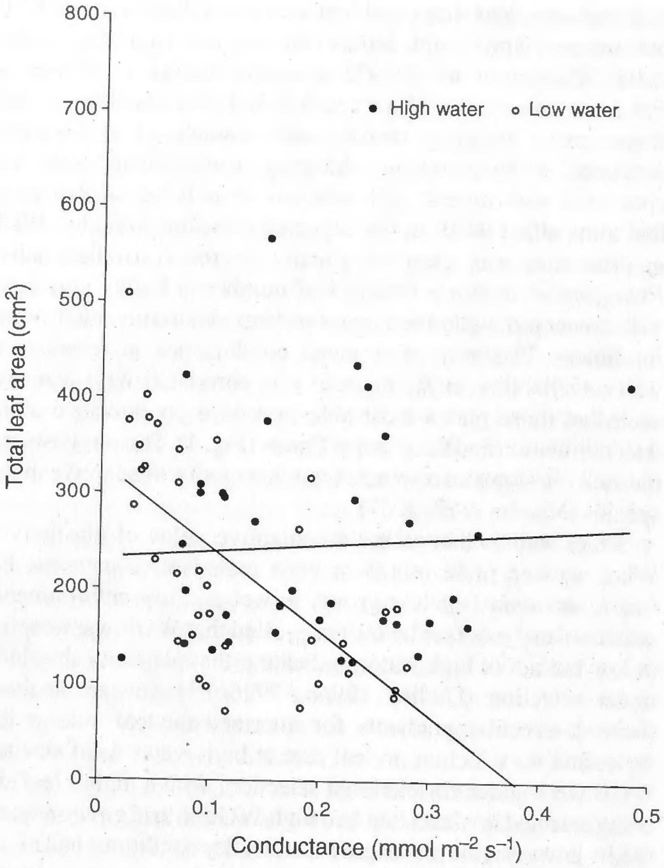

Fig. 4. Regressions of total leaf area (a proxy for fitness) of the Australian native herb *Pelargonium australe* against stomatal conductance. The linear model included terms for growth water level (low and high) and stomatal conductance. In this analysis, a significant water treatment by conductance interaction (d.f. = 1; $P \leq 0.009$) indicates that the regression slopes differ between water treatments. Thus, indicating that plasticity in stomatal response is adaptive (reproduced from Nicotra *et al.* 2007 with permission).

is indicated, and yet the plants grown in the dry areas exhibited lower WUE on average, suggesting a maladaptive plastic response.

Likewise, Caruso *et al.* (2006) examined two species of *Lobelia* grown in both wet and dry environments and found unexpected results regarding selection for $\mathrm{WUE_L}$. In *Lobelia siphilitica* L., WUE actually showed a significant negative genetic correlation with plant size, a proxy for fitness, suggesting that high WUE may be associated with lower fecundity. This relationship could be driven by maximum photosynthetic rate (A_{max}), the only trait to exhibit any plasticity in the species. A_{max} increased under wet conditions and was adaptively neutral, perhaps indicative of a drought avoidance strategy, resulting in selection against plasticity in $\mathrm{WUE_L}$. In contrast, for the other *Lobelia* species, *L. cardinalis* L., plasticity in stomatal conductance and photosynthetic rate is selectively adaptive (an increase in conductance and carbon gain in response to increased water availability); thus, plasticity in WUE confers greater fitness across environments. However, higher maximum photosynthetic rate is maladaptive in *L. cardinalis*, with higher A_{max} occurring in the drier environments. The authors suggest that this maladaptive response may be due to some negative genetic correlation between A_{max} and A or g_s conferring a cost in plasticity of these traits.

The above results suggest that there is adaptive plasticity in particular water use traits in some species, but we have yet to understand what underlies the variation among species – is it species ecology, evolutionary history or experimental artefact? Further, moving from natural systems to production systems, do our crop plants have adaptive plasticity for water use traits? Do their ancestors? And, could we increase these traits through breeding?

Researching adaptive plasticity in water use traits

To answer the questions above, studies must quantify the effects of environment, genotype and their interaction (G × E interaction) on the expression of a trait. The plasticity is reflected in a significant environment effect and variation between genotypes in plasticity is exhibited by a significant G × E interaction. To assess plasticity in response to water availability, it is therefore necessary to grow genotypes under a range of water supply conditions; plasticity cannot be measured on only a single plant (Scheiner 2002). The water supply conditions used should be as realistic in terms of amount and timing of application, and yet must differ adequately to elicit plastic responses. When aiming to predict plastic responses to novel conditions, it may be valuable to work outside the range of water availability currently experienced – to reveal the 'hidden reaction norms' of plasticity (Schlichting 2008).

There are several methods for assessing the plasticity of a trait. These include the significance of the environment effect and G × E interaction terms in a linear model, the co-efficient of variation (CV, s.d./mean × 100) across a set of growth environments, deriving a normalised index for the trait across the environmental range, for example (max – min)/(max + min; for further discussion see Valladares *et al.* 2007) or the slope of the trait response to the growth environments – the reaction norm (see Schlichting and Pigliucci 1998; for a discussion of the history of the reaction norm). The theoretical reaction norm of response to water would encompass all possible water availabilities found in a species' range, whereas in practice only a few are generally considered.

Demonstrating that an observed plastic response to water is adaptive requires assessing fitness or fitness components (Caruso *et al.* 2006). Ideally, fitness is assessed across multiple generations, e.g. assessments of seed viability (Goergen and Daehler 2001) and incorporates longevity and survival of adult plants (DeFalco *et al.* 2003). Where measurements on offspring are not possible, assessment of reproductive output can provide useful surrogates, e.g. seed weight or the number of flowers (e.g. Sans *et al.* 2004). For many species, especially long-lived ones, proxies of fitness such as growth rate and biomass are considered acceptable alternatives. In the case of fruit and grain crops, yield or harvest index are appropriate fitness proxies. Statistical techniques to specifically assess the adaptive value of plasticity include linear models and multiple regression analysis and selection analysis (Lande and Arnold 1983; Rausher 1992; Scheiner and Callahan 1999; Weinig *et al.* 2006).

Plasticity of any given trait can itself evolve in response to selection (Via *et al.* 1995; Scheiner 2002 and references therein). This response may be a direct one or an indirect outcome of a genetic correlation between a trait mean and its plasticity;

likewise, selection on a trait's plasticity may have indirect effects on the trait mean (Callahan and Pigliucci 2005). Further, artificial selection experiments have proven informative in examining the underlying genetic architecture and in quantifying the selection potential for plasticity (Callahan 2005; Kurashige and Callahan 2007); but to the best of our knowledge, the selection potential of plasticity itself has not been explicitly assessed on water use traits.

There is a growing strength in our understanding of the molecular and genetic mechanisms underlying phenotypic plasticity (Schlichting and Smith 2002; Reymond *et al.* 2003; Forde 2009). Likewise, there is a breadth of research on the genetics of WUE. Modern cultivars of wheat have higher WUE than older ones, so clearly WUE can be improved with breeding (Cao *et al.* 2007). A wide range of QTL (quantitative trait loci) has been identified that are associated with plant response to water stress (Reymond *et al.* 2003; Cattivelli *et al.* 2008 and references therein; Collins *et al.* 2008 has a comprehensive list of QTLs for drought stress). These include QTLs that are associated with plasticity itself, as in the case of leaf elongation rate in response to water stress in maize (*Zea mays* L.) (Reymond *et al.* 2003). Thus far, improvements to drought tolerance via QTLs and marker assisted selection (MAS) have been small, but breeding to make use of naturally occurring variation has led to significant improvements of molecular genetic methods do not always create realistic environments or stresses (Collins *et al.* 2008). New approaches that incorporate QTL × environment interactions hold promise in addressing this gap (Malosetti *et al.* 2006; cited in Collins *et al.* 2008).

Candidate gene approaches are also proving promising, particularly in model species. For example, Knight *et al.* (2006) identify candidate genes that respond plastically to water stress, and are differentially expressed in populations of *Boechera holboellii* (Hornem.) Á.Löve & D. Löve, a close relative of *Arabidopsis thaliana* (L.), from environments with contrasting water supply (Hill *et al.* 2006). Likewise, the ERECTA gene has been shown to regulate TE in *Arabidopsis* (Masle *et al.* 2005). Other reports have concerned key genes and proteins involved in regulation of flowering (FT), vegetative growth (DELLA), leaf senescence (IPT) and desiccation tolerance (LEA) (Neumann 2008). Further research on promoters for these genes may enable breeders to produce new crop varieties with superior drought performance (Neumann 2008). Cattivelli *et al.* (2008) stress that improvement of drought tolerance must be sought with attention to minimising yield reduction; thus, drought tolerance traits must be tested in both stressed and non-stressed environments, or plasticity in traits and their effects on fitness must be considered.

Until recently, the most limiting factor for the progress of studies linking drought tolerance responses at the phenome and genome level to environment was a lack of capacity for precise and efficient phenotyping. Of course, this limitation is not specific to understanding drought responses. The emergence of plant phenomics provides the potential for high throughput phenotyping of morphological and physiological traits as well as measurements of growth rates (e.g. the Australian Plant Phenomics Facility; http://www.plantphenomics.org.au, accessed 3 June 2009). These approaches hold tremendous promise for alleviating the current limitations on phenotyping. As the capacity for high-throughput phenotyping grows, the opportunity to assess phenotypic plasticity – its importance and its potential – likewise, increases.

Applied plasticity research

Re-evaluating plasticity in water use traits from an evolutionary perspective has relevance in several applications – agriculture, horticulture and understanding invasive species in particular. In the following section, we consider why each of these is important, then discuss methodological considerations around researching plasticity in these areas.

Traditionally in crop breeding, G × E interactions are seen as hampering selection for grain yield under water-stressed conditions (e.g. Rebetzke *et al.* 2006; Cattivelli *et al.* 2008). Results from ecological studies, however, demonstrate that plasticity (the environment effect component of the G × E interaction) in WUE can be adaptive and this is supported for some agricultural species as well (see Sadras *et al.* 2009). However, there has been only limited assessment of the relationships between phenotypic plasticity in WUE and crop yield.

Sadras *et al.* (2009) advocate breeding for plasticity in phenological development to improve yield across environments. Other authors suggest that we shift our attention from WUE or transpiration efficiencies to component traits or other determinants of water use and performance under drought (Araus *et al.* 2002; Blum 2009). If, as climate models predict, frequency of unpredictable drought will increase, plasticity in water use may result in higher average fitness than consistently high WUE. Such plasticity could conceivably be determined by any number of traits at the leaf, root or biochemical level. We suggest that there are exciting opportunities for interdisciplinary work bringing ecological, evolutionary, physiological and crop breeding perspectives together to assess the potential for using adaptive plasticity to improve crops.

In many areas, plants are being grown or re-introduced to restore degraded landscapes for conservation or carbon sequestration purposes. Considerable debate has occurred on how these plants or seeds are sourced (Broadhurst *et al.* 2008). We suggest that in addition to current selection criteria, consideration should be given to the presence of adaptive plasticity when selecting species, particularly for water use traits, as this will potentially affect the ability of the restored stands to respond to climate change (see also Murray *et al.* 2002).

Finally, invasive species represent one of the best examples of rapid adaptation to novel environments. In many cases, plasticity appears to play a role in this adaptation although empirical data linking plasticity to invasiveness is still limited (Richards *et al.* 2006; Hulme 2008). To assess trends in WUE plasticity, we conducted a meta-analysis comparing WUE in invasive plants with congeneric native species for eight studies comprising of 21 species pairs (Fig. 5). The studies were selected using a literature search on Web of Science (http://isiwebofknowledge.com; accessed 1 June 2009) and CAB Abstracts (http://cabi.org.default.aspx?site=170&page=1016&pid=125; accessed 1 June 2009) for the terms [invas* or nonnat* or alien* or weed or nonindig*] and [nat* or indig* or endemic] where stars denote wildcards. We limited results to the topic of plant science or

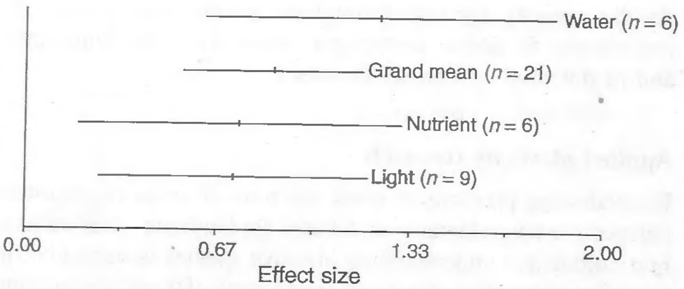

Fig. 5. Meta-analysis comparing the plasticity in water use efficiency of invasive species and co-occurring native species by resource treatment. Positive effect size values on the *x*-axis indicate that invasive species are significantly more plastic than their co-occurring native species, an *x*-value of zero indicates no significant difference between the plasticity in WUE of congeneric native and invasive species. The horizontal lines represent 95% confidence intervals around the effect sizes. Confidence intervals that do not overlap the *y*-axis are significant at $P \leq 0.05$. Number of species pairs is indicated as (n). A full reference list and more detailed description of method are available from authors on request.

weeds, respectively. The analysis demonstrated that invasive plants had significantly higher plasticity in WUE overall compared with native plants ($P \leq 0.001$; Fig. 5). Among the three studies (six pairs of species) that compared the WUE of invasive and native plants across a water gradient (as opposed to light or nutrients), invasive species were again significantly more plastic in WUE in response to water. In some cases, high plasticity in WUE provided invasive species with a fitness advantage when water availability increased from average conditions (e.g. Brock and Galen 2005) while for other species, higher plasticity in WUE enabled invasive plants to better maintain fitness when water availability was reduced (e.g. Hill *et al.* 2006). Though based on relatively few studies, we take this as indirect evidence that plasticity in WUE is a shared characteristic of these exotic species that may well contribute to their invasiveness.

Conclusions

The phenomics/genomics era presents exciting opportunities for cross-disciplinary exploration of the role of phenotypic variation in plant evolution and in determining performance in cultivation, management and natural conditions. We have argued that one key question that remains unanswered is how often is phenotypic plasticity in water use traits adaptive? We encourage further consideration of whether adaptive plasticity is likely to arise in integrated measures of WUE (i.e. carbon isotope signatures), in the instantaneous determinants of WUE (gas exchange traits), or in the underlying anatomical and morphological traits. By determining which traits confer a fitness advantage when responding to drought or variable water supply, and assessing how those traits vary among species or functional types, we will gain critical insight into the evolution of drought responses.

As molecular and genetic mechanisms underlying not just the traits, but their plastic responses, become better understood, and as we assess the extent to which adaptive plasticity for water use traits is heritable, we may be able to actually breed for 'water-wise' plasticity. Invasive species provide an interesting opportunity to examine the importance of adaptive plasticity; for example, whether adaptive plasticity is a key to successful invasion. Further, invasive species provide a case study to examine how and how rapidly adaptive plasticity changes following invasion. This may provide clues to the processes driving adaptive plasticity in other species, and may help predict the impacts of invasive species and shifts in plant community assemblages more generally under changing climates.

Amid growing concern about how plants will respond to climate change, the question of what makes a plant 'water-wise' has great relevance. Current advances in our capacity to quantify phenotypic traits, be they anatomical, morphological or physiological, mean that we have an unrivalled opportunity to test for and examine the role of adaptive phenotypic plasticity in a great range of traits.

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Commentary

Beware: alien invasion. Where to next for an understanding of weed ecology?

In this issue of *New Phytologist*, Dawson *et al.* (pp. 859–867) take an important step in assessing the role of plant traits, and plasticity therein, in determining invasiveness. They ask whether alien plant species that have *larger* invasion ranges also have *greater* plasticity in three functionally important traits: biomass, root/shoot ratio (*R:S*) and specific leaf area (SLA). This approach is novel in that it effectively treats invasiveness as a continuum, rather than a categorical (invasive/noninvasive) state. In addition it examines plasticity in ecologically significant plant functional traits, which is of interest given the association of these traits with global species distributions (see Wright *et al.*, 2004; Nicotra *et al.*, 2010).

> *'This approach is novel in that it effectively treats invasiveness as a continuum, rather than a categorical (invasive/noninvasive) state.'*

The ability of a species to respond to changes to environmental conditions, particularly increased resource availability, is often proposed to facilitate invasions (Baker, 1965; Davis *et al.*, 2000). 'Phenotypic plasticity' describes an organism's morphological, anatomical and developmental response to the environment (Schlitching, 1986). Analyses of the broad literature tend to support the concept that invasive plants display higher plasticity (Daehler, 2003; Davidson *et al.*, 2011; but see Palacio-López & Gianoli, 2011).

When asking a question about movement and potential diversification of species, the characteristics of species' lineages, and the shared evolutionary history of the species in these lineages, is important. Dawson *et al.* explicitly incorporate phylogeny into their meta-analysis and find differing degrees of phylogenetic structure in the traits they consider (little in biomass and SLA but quite a lot in *R:S* ratio). Overall their analyses shows that widespread invasive species have greater plasticity in biomass, but plasticity in both *R:S* and SLA is not correlated with number of regions invaded.

Dawson *et al.* conclude that focusing on the 'endpoint of trait plasticity' – the performance or fitness of the plant under different environmental conditions – may be a more fruitful approach than continued studies of plasticity in functional traits themselves. They recommend detailed field experiments on multiple co-occurring native and alien species. We agree that the endpoint of plasticity is critical, but as we discuss, we are reticent to discourage further consideration of plasticity in underlying functional traits.

When is plasticity important to the invasion process?

An invasion can be divided into two stages; (1) introduction and naturalization and (2) expansion of species (Williamson, 1996). Phenotypic plasticity may be involved directly in either or both stages. Dawson *et al.* analysed whether plasticity in functional traits is associated with stage two. It is possible that plasticity is more important for stage one, establishment in novel environments, than for spread or competiveness (Palacio-López & Gianoli, 2011). Such a role for plasticity is consistent with suggestions that pioneer species may be more plastic than non-pioneer species (Bazzaz, 1979). Although invasive plants are generally pioneering species, in that they often establish in novel environments with very different climates from their home range (Gallagher *et al.*, 2010), not all pioneer plants are invasive. As such, there is a need for further investigation to separate whether plasticity is associated with pioneering characteristics and/or plays a more direct role in providing a competitive advantage of invasive species over the native flora.

With regard to stage two, it is likely that plasticity in functional traits would be related not simply to expansion of geographic range, but to increasing environmental range. This is a subtle but important difference. Dawson *et al.* assessed whether the number of regions invaded was correlated with levels of plasticity. This approach could be extended to address the more complicated issue of whether plasticity in functional traits is associated with the number of biomes/habitat types an invasive covers. The question then becomes one of how much larger an environmental range an invasive species can occupy and whether plasticity in functional traits facilitates this expansion.

It has also been proposed that instead of being a characteristic that increases the likelihood that a species will become invasive, plasticity may be a by-product of selection on changes to mean values of traits during invasion (rapid evolution of plasticity post-colonization; Agrawal, 2001). However, there is only limited support for the latter hypothesis: Colautti *et al.* (2009) found no consistent evidence of evolution for changes in mean trait values between native and invasive populations in a meta-analysis of 28 species. Likewise, glasshouse experiments paired with genetic analyses of invasive *Senecio inaequidens* plants in Europe revealed the native population that was most closely related to the invasive populations also had the greatest phenotypic plasticity (Bossdorf *et al.*, 2008). Thus, it seems that high trait plasticity is a pre-existing characteristic of invasive species.

Patterns of plasticity in functional traits may differ under stressful vs favourable conditions

In evaluating the relative response of invasive and noninvasive species it is important to consider the environmental context. As described in Richards *et al.* (2006) and further discussed in Davidson *et al.* (2011) plasticity in underlying traits would be adaptive in an invasive species if it enabled a genotype to maximize fitness under optimal conditions ('master-of-some' response to increased resources) or maintain homeostatic fitness under poor conditions ('jack-of-all-trades' response to decreased resources) or both (jack-and-master). The master-of-some response provides a mechanism by which higher plasticity of invasive species could enable invasive species to out-compete native species and thus facilitate the invasion process.

Dawson *et al.* examined plasticity in response to an increase in resources; however, the range of conditions examined inevitably varied across studies within the meta-analyses. Especially in the context of climate change (e.g. increased frequency of drought events in many areas) it remains topical to differentiate between responses to a reduction in a resource below average conditions, and responses to an increase in that resource. For example, Davidson *et al.* (2011) found that, in response to a decrease in resources from average to deficient, native species were better able to maintain fitness homeostasis than co-occurring invasive species.

To put this in a slightly different context, Poorter *et al.* (2012) advocate the use of dose–response curves because measures of plasticity depend strongly on the conditions under which the plastic response (or reaction norm) is assessed. Rather than quantifying plasticity at a discrete interval (noting that Dawson *et al.* did include magnitude of resource level increase as a covariate for the chosen interval), a dose–response curve integrates over a wide range of conditions and investigates changes in the response across the conditions (Fig. 1; Poorter *et al.*, 2012). Thus, the question of whether invasive or noninvasive species differ in their ability to respond adaptively to *decreased* resource availability remains somewhat open.

The importance, and the challenge, of assessing relative performance

The adaptive value of a plastic response depends on whether it increases average net fitness (ideally taken as multigenerational fitness measures). Measuring fitness, however, is not a simple matter and relies on proxies, which must be selected with care (see Box 1 in Davidson *et al.*, 2011). Measurements of adaptive plasticity should also consider the representation of different conditions in the environment and assess the impacts of altering the frequencies/likelihoods of encountering these different resource conditions. Furthermore, one can consider adaptive plasticity at different time scales: plasticity which was adaptive under past conditions and may be of neutral or maladaptive importance now; plasticity which is currently adaptive; and plasticity which may now be neutral or maladaptive but that could represent hidden adaptive potential under novel environments. Each of these is potentially important: the first for understanding the history of diversification of lineages, the second for understanding current selective pressures and the last for determining responses to future environments. The latter two are therefore relevant for understanding invasion biology and predicting responses to climate change.

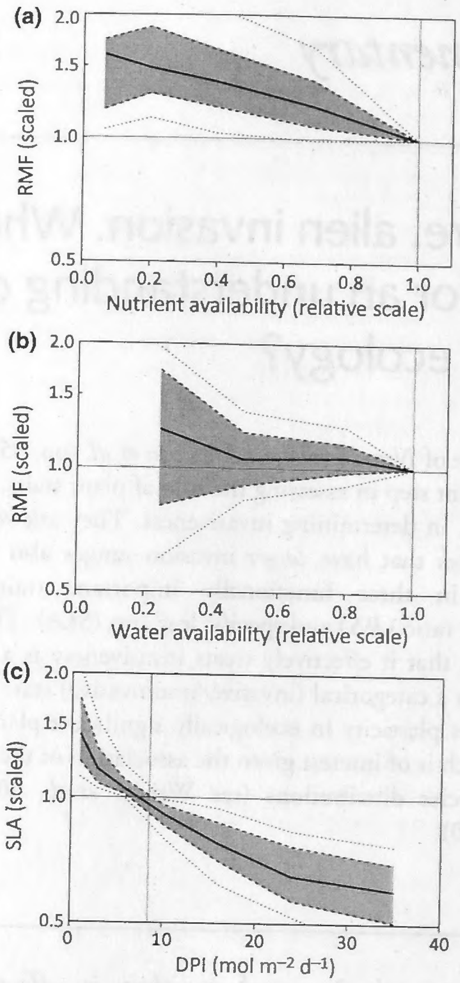

Fig. 1 Dose–response curves of the absolute response of the fraction of 10 whole plant mass represented by roots (RMF, analogous to the scaled root/shoot ratio (*R:S*) in Dawson *et al.*, this issue pp. 859–867) to (a) nutrient 8 availability, (b) water availability and (c) of the response of specific leaf area (SLA) to light availability. Data are a compilation based on many species. For each environmental factor, a reference condition was chosen (indicated by a vertical line), and data for each species in each experiment were subtracted from the allocation values observed or interpolated for that reference level. The shaded area indicates the interquartile range (between 25th and 75th percentile) of the observed ratios in that part of the response curve. The dotted lines indicate the 10th and 90th percentiles. The bold continuous line within the shaded area indicates the median value. Both traits show plasticity, but in the case of the response of RMF to water availability in particular, the position of sampling points along the response curve will dramatically affect the estimate of plasticity. Figures modified with permission from http://www.metaphenomics.org; see Poorter *et al.* (2009, 2012) for further information.

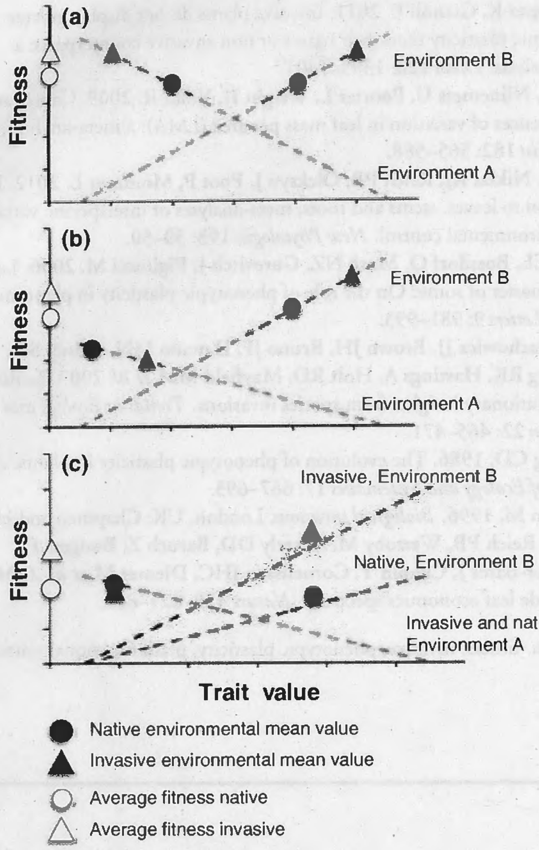

Fig. 2 Theoretical trait–fitness relationships across two environments (A and B) for a native/non-invasive and invasive species demonstrating three mechanisms (a–c) by which the invasive species may achieve greater average fitness than a native species. Closed symbols, mean trait values in each environment; open symbols, mean fitness for each species, across environments. (a) The invasive species displays greater plasticity than the native species (seen as a greater distance between the closed points) and higher average fitness. (b) The invasive displays higher average mean trait values than the native species which translates to higher average fitness despite identical levels of plasticity in both species. (c) The invasive displays a steeper relationship between the focal trait and fitness in environment B and therefore exhibits higher average fitness despite having the same mean trait values and plasticity as the native species.

In the context of invasions, however, the important question is not simply whether plasticity in a given trait is adaptive, but how the performance of invasive compared to native plants differs under the same conditions. Superior performance may be underpinned by greater plasticity, higher average mean trait values, steeper trait–fitness relationships or a combination of these (e.g. van Kleunen *et al.*, 2010; Godoy *et al.*, 2011; Fig. 2). For example, van Kleunen *et al.* (2010) found greater mean trait values for several functional traits in invasive compared to noninvasive species.

Conclusions and future directions

We agree with Dawson *et al.*'s recommendations: to understand the role of plasticity in the spread of species (be it now, historically or in the future) will depend on detailed field experiments on multiple co-occurring native and alien species that include direct fitness measurements (including mutigenerational fitness where possible). Such multi-species studies, when conducted across a broad environmental range, also provide an ideal opportunity to examine under what circumstances plasticity in functional traits is important to fitness. So doing will enable us to examine the relationship between traits and their plasticity, and to identify when plasticity in one trait provides for homeostasis in another.

Understanding the role of plasticity and detecting patterns in adaptive plasticity of key functional traits and species types is important not only for managing invasions but also for managing populations under climate change (Sax *et al.*, 2007) and for improving modelling of species/community responses to climate change (Ghalambor *et al.*, 2007). Dawson *et al.* have shown that plasticity in biomass in response to increases in resources may be important for spread of invasive species, however many more questions remain. For example, what effect does position on the dosage response curve have? Does the response vary dramatically among species of different ecological, as well as evolutionary history? What effect does varying the likelihood of encountering different conditions have on the adaptive value of plasticity? Hypothesis driven meta-analyses, such as conducted by Dawson *et al.*, are useful to establish broad patterns regarding the likely role of plasticity in the invasion process or in responding to novel environments. These analyses provide a more informed starting point for essential empirical enquiries.

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