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.

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, $\mu mol\,m^{-2}\,s^{-1}$) 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
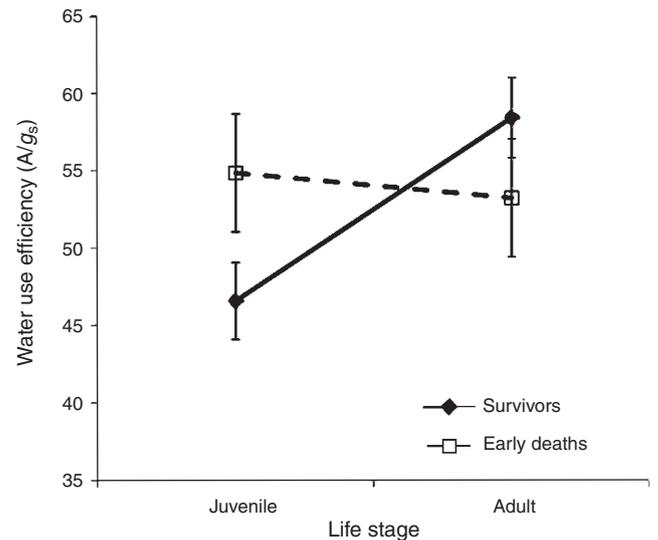

Fig. 1. Water use efficiency at leaf level (WUE_L, A/g_s) of the perennial sub-shrub *Cryptanthra flava* (Boraginaceae) as a function of plant size and whether the plants survived past adulthood or not (adapted from data in Casper *et al*. 2005). Those plants that survive to adulthood change from low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

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.

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
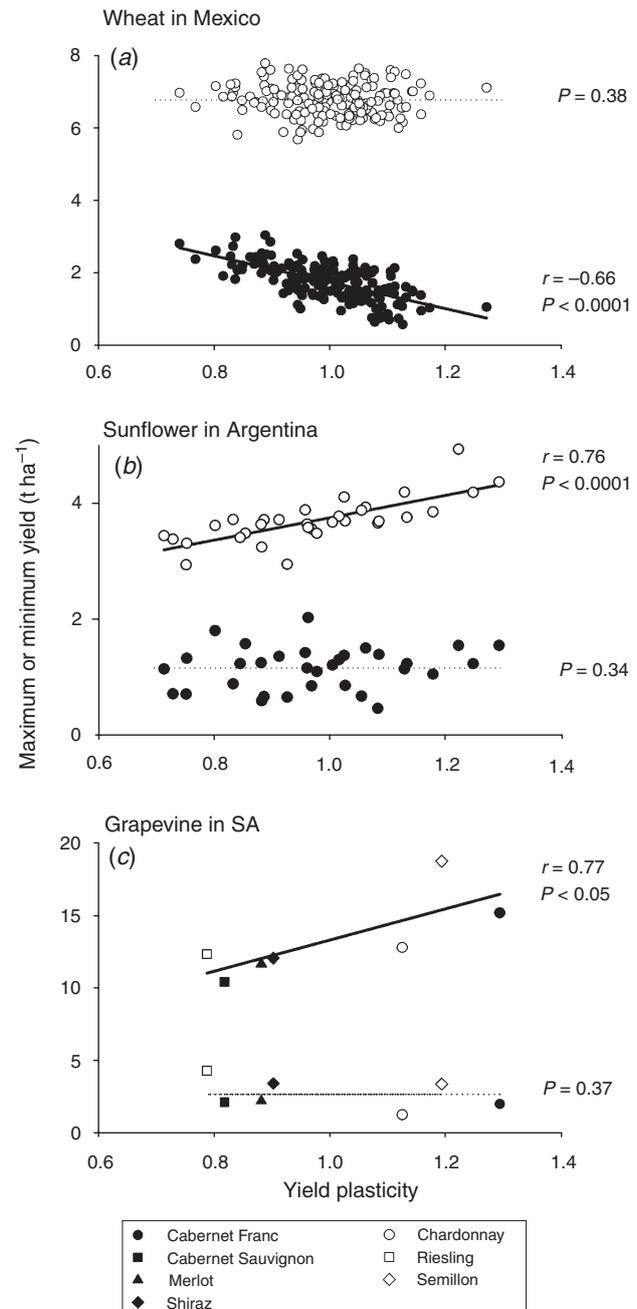

Fig. 2. Relationships between yield plasticity and maximum (open circles for wheat and sunflower) and minimum yield (closed symbols for wheat and sunflower) in wheat, sunflower and grapevine. (*a*) For these wheat lines, high yield plasticity is an undesirable traits because it is associated with low 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).

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*.

(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
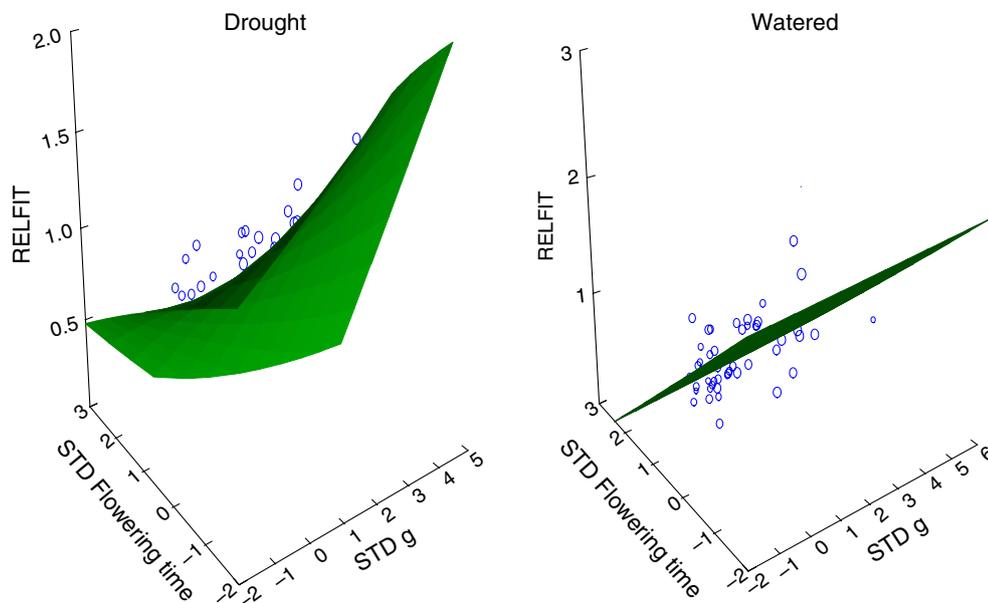

Fig. 3. Relative fitness (RELFIT) plotted against standardised (STD) flowering time and stomatal conductance (g_s) values across two populations in water-limited (drought) and well-watered conditions of *Impatiens capensis* (reproduced from Heschel and Riginos 2005 with permission). Early flowering lines 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.
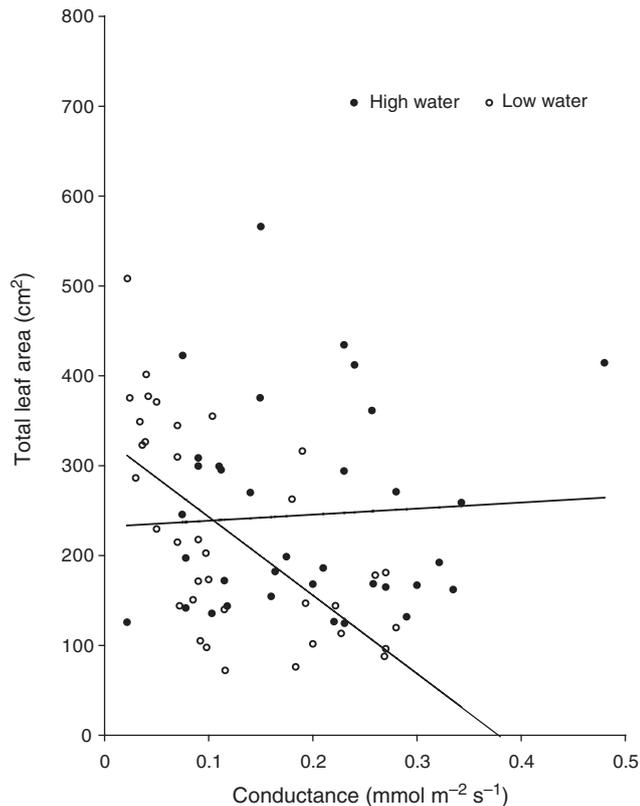
Fig. 4.   Regressions of total leaf area (a proxy for fitness) of the Australian native herb *Pelargonium australe* against stomatal conductance. The linear model included terms for growth water level (low and high) and stomatal conductance. In this analysis, a significant water treatment by conductance 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 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 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
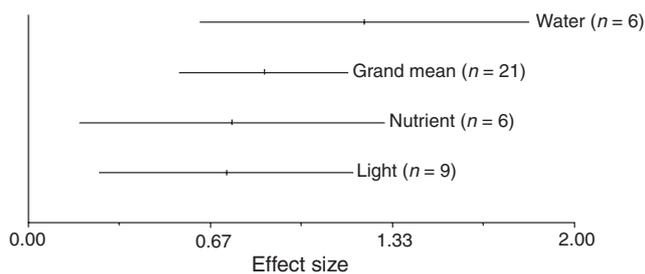
Fig. 5. Meta-analysis comparing the plasticity in water use efficiency of invasive species and co-occurring native species by resource treatment. Positive effect size values on the *x*-axis indicate that invasive species are significantly more plastic than their co-occurring native species, an *x*-value 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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