

Geographic variation and plasticity to water and nutrients in *Pelargonium australe*

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Summary

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• Here, patterns of phenotypic plasticity and trait integration of leaf characteristics in six geographically discrete populations of the perennial herb *Pelargonium australe* were compared. It was hypothesized that populations would show local adaptation in trait means, but similar patterns of plasticity and trait integration. Further, it was questioned whether phenotypic plasticity was positively correlated with environmental heterogeneity and whether plasticity for water-use traits in particular was adaptive.

• Seedlings were grown in a glasshouse at six combinations of water and nutrient availability. Leaf anatomical, morphological and gas exchange traits were measured.

• High amounts of plasticity in leaf traits were found in response to changes in growth conditions and there was evidence of local adaptation among the populations. While there were significant correlations between plasticity and environmental heterogeneity, not all were positive. Notably, patterns of plasticity and trait integration varied significantly among populations. Despite that variation, some of the observed plasticity was adaptive: fitness was correlated with conservative water use when water was limiting.

• *Pelargonium* arrived in Australia ~5 million yr ago. It is concluded here that high amounts of plasticity, in some cases adaptive, and weak integration among traits may be key to the spread and success of this species.

Key words: environmental heterogeneity, leaf traits, local adaptation, *Pelargonium australe*, phenotypic integration, phenotypic plasticity, water use.

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Introduction

'Phenotypic plasticity' refers to the range of phenotypes a genotype can express as a function of the environment (Scheiner, 1993). In recent years, it has moved from being seen as a troublesome source of noise to being accepted as a characteristic that itself is under selection, evolves, and is of ecological consequence (Miner *et al.*, 2005; Bradshaw, 2006). Although plastic responses now seem to be expected to have an adaptive value, trait plasticities are likely to exhibit the same range of relationships with fitness as other traits: some will be neutral, some passive or detrimental, and others adaptive (van Kleunen & Fischer, 2005). Differences in plasticity may

reflect differences in the ability of plants to establish in a given environment (Schlichting & Levin, 1986; Poorter & Nagel, 2000; Stenstrom *et al.*, 2002; Griffith & Sultan, 2005). Within the range of a species, differing selection pressures on plasticity may contribute to the evolution of local adaptation.

Plastic responses in plants result from both biotic and abiotic cues and can be seen in morphological, anatomical and physiological traits. There is a long history of research into leaf trait plasticity in particular, because leaves are the principal organs of energy gain in plants (Stephens, 1945; Bradshaw, 1965; Boardman, 1977; Sultan, 1987; Ackerly *et al.*, 2000). Leaf morphological, anatomical and physiological traits are often found to vary in concert (Niinemets *et al.*, 1998; Green

& Kruger, 2001; Marengo *et al.*, 2001). For example, leaves grown under low water or nutrient conditions are smaller and have lower specific leaf area (SLA; a measure of the light-capturing leaf area deployed per unit dry mass allocated), lower photosynthetic capacity and more conservative patterns of water and nutrient use than conspecifics grown at high water or nutrient supply.

The plasticity of a trait in response to a given cue is generally predicted to increase as the heterogeneity of that cue or the niche breadth of a species increases (Sultan, 1987; Sultan *et al.*, 1998a,b; Valladares *et al.*, 2000a, 2002a; Balaguer *et al.*, 2001; Grant *et al.*, 2005; Lehmann & Rebele, 2005; Rozendaal *et al.*, 2006), but evidence in favour of these hypotheses is mixed (e.g. Volis *et al.*, 2002). It is difficult to resolve clear links between heterogeneity and plasticity, first, because under natural conditions organisms receive and respond to multiple cues simultaneously and, secondly, because heterogeneity that is perceived by the researcher may not be evident to the study organism (Gomez *et al.*, 2004). The effects of interacting abiotic conditions and heterogeneity on plasticity, especially along gradients (Miner *et al.*, 2005), have received relatively little attention in the plasticity literature, despite general acknowledgement that resources have interactive effects and responses to these effects are not always predictable.

Studies of resource effects on leaf shape and display in particular remain relatively rare in the literature (Vogel, 1968; Givnish, 1987b; Gurevitch, 1992a,b; McDonald *et al.*, 2003). Leaves are predicted to become more dissected under conditions of water stress, where the decrease in effective leaf size is predicted to reduce boundary layer thickness and improve convective heat exchange (Vogel, 1968). Similarly, one would predict that, under conditions of water and nutrient limitation, leaf projection efficiency would decline to reduce water loss and direct light interception. However, leaf traits need not vary together as, for example, physiological plasticity may allay the need for morphological plasticity (Griffith & Sultan, 2006). Likewise, interacting abiotic conditions can actually alter the patterns of correlations among plastic traits, indicating that trait integration itself is variable (Schlichting, 1989a,b; Sardans *et al.*, 2006). In general, we have little basis for expectations of whether patterns of plasticity should differ among these leaf traits or groups of traits and whether interactive effects of abiotic resources can have an impact on the patterns of plasticity of single and correlated suites of traits.

Here, variation in leaf morphology (including shape and size), anatomy and physiology was assessed among six geographically discrete populations of the herbaceous *Pelargonium australe* in response to simultaneous changes in water and nutrient availability. *Pelargonium* species probably arrived in Australia through recent (at latest Pliocene) long-range dispersal from South Africa (Bakker *et al.*, 1998). *Pelargonium australe* (Geraniaceae) is an herbaceous perennial that grows in all Australian states but the Northern Territory and is most abundant in the south-east. Its distribution

therefore encompasses broad ranges of rainfall. Within its range, *P. australe* is frequently found on nearly soil-free rock faces, on beachesides, in grasslands, in the understory of open *Eucalyptus* forests and in subalpine areas. Populations exhibit significant intraspecific leaf morphological variation, including variation in leaf size, shape and patterns of display.

The first objective was to determine whether these geographically discrete populations of a geologically recent species differ in mean leaf traits or in plasticity of leaf traits in response to variations in water or nutrient availability. If so, we were interested in whether trait means are correlated with mean climatic variation across the geographic range and whether plasticity correlates with environmental heterogeneity. Given visible differences in leaf traits among populations, we predicted that trait means would reflect local adaptation to habitat differences. We hypothesized that under resource limitation all populations would increase resource use efficiency but would decrease growth and specific leaf area. The populations differ in water availability and heterogeneity, but the species has a propensity to grow on nutrient-poor soils and rock wherever it occurs. We therefore hypothesized that populations from habitats with greater heterogeneity in rainfall would show greater plasticity in leaf traits in response to changes in water availability than populations from more homogeneous rainfall environments, but that populations would not differ in plasticity of traits in response to changes in nutrient availability.

The next objective was to assess whether patterns of correlation among traits, both within and among trait types (morphological, anatomical and physiological), differed among populations. We hypothesized that similarity of genetic architecture underlying plasticity would constrain populations within this single species to have similar potential for plastic response. We therefore predicted similar patterns of integration within a given set of traits (e.g. within morphological traits) or among sets of traits (e.g. between morphological and physiological traits) across populations. Alternatively, differing selection pressures in these environments may disentangle such correlations so that populations differ not only in mean and plasticity but in correlations among plastic traits. Finally, we assessed relationships between plasticity and whole-plant total leaf area, a proxy for fitness, as an indication of the potential for selection on plasticity among these geographically discrete populations of this widespread species.

Materials and Methods

Study species

Six populations of *Pelargonium australe* Knuth in Pflanzern (including coastal, inland and grassland sites) were selected from the eastern range of the species (Fig. 1, Table 1). Annual rainfall, average monthly temperatures and open-pan evaporation were determined from climate records (1968–2003;

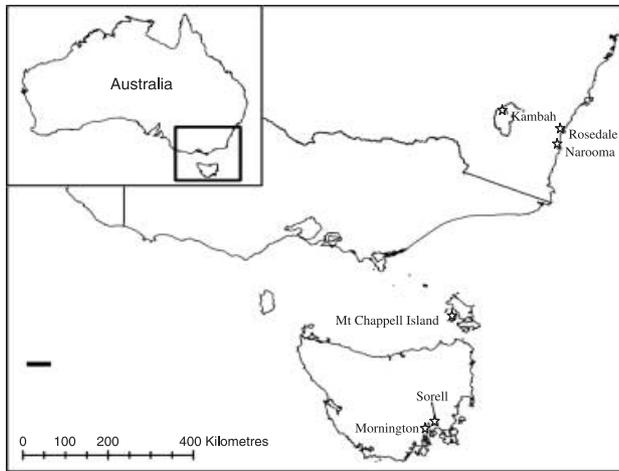


Fig. 1 Location of *Pelargonium australe* populations from which seeds were obtained.

Australian Greenhouse Office, 2005). Seeds were collected from the field (Kambah, Narooma and Rosedale populations in March and April 2002) or from plants growing at the Australian National Botanic Gardens, Canberra (Mt Chappell Island). Field-collected seeds for Mornington and Sorrell populations were obtained from Wildseed Tasmania (Hobart, Australia).

Experimental design

In May 2002, seeds were germinated in a glasshouse (50 : 50 sand:vermiculite mix) under uniform conditions. Seeds of all populations germinated and reached the two-leaf stage at the same time, suggesting that maternal habitat effects were minimal (Donohue, 2002). At the two-leaf stage, seedlings were potted into 15-cm-diameter pots containing 50 : 50 sand:sandy loam potting mix, allowed 2–3 wk for establishment, and randomly assigned to treatments.

We manipulated water and soil nutrient levels using two gradients of nutrient supply, one at a lower and one at a higher water supply. Plants were grown in a randomized complete block design (6 populations \times 6 treatments \times 6 blocks = 216 plants) and fertilized at each watering event, twice per week for the first 16 wk and weekly thereafter. Low-water plants received 100 ml of fertilizer per event and high-water plants 200 ml. The high water level saturated soil with minimal leakage. The low water level resulted in soil drying and reduced growth without causing wilting; it was a continuous but moderate stress. At each water level there were three nutrient supply rates: 0.06, 0.25 and 0.5 mg Aquasol fertilizer (Hortico Pty Ltd., Melbourne, Australia) per week and 0.125, 0.5 and 1 mg Aquasol fertilizer per week for low and high water levels, respectively. While gradients overlapped substantially, they were not factorial: nutrient supply rate was matched only at

Table 1 Descriptions of sites, local climate and climatic variability

Population	Habitat description	Latitude, longitude and altitude	Rainfall (mm) (Mean \pm SD; CV of avg)	Temperature ($^{\circ}$ C) (Max and min avg monthly temp; CV of avg monthly temp)	Annual open-pan evaporation (mm) (Mean \pm SE; CV of avg monthly pan evaporation)
New South Wales					
Kambah (K)	Grows on rocky outcrop; exposed	149 00'34"E 35 23'49"S 540 m	$X = 757 \pm 181$ CV = 0.24	$X_{\max} = 20.8$ $X_{\min} = 5.4$ CV = 0.40	$X = 1362 \pm 127$ CV = 0.58
Rosedale (R)	Coastal; grows on rocky outcrop close to beach; exposed	150 13'30"E 35 49'38"S 5 m	$X = 1050 \pm 320$ CV = 0.30	$X_{\max} = 21.1$ $X_{\min} = 11.1$ CV = 0.21	$X = 1500 \pm 112$ CV = 0.43
Narooma (N)	Coastal; grows on rocky outcrop close to beach	150 8'25"E 36 13'14"S 5 m	$X = 972 \pm 289$ CV = 0.30	$X_{\max} = 20.8$ $X_{\min} = 11.1$ CV = 0.20	$X = 1454 \pm 102$ CV = 0.44
Tasmania					
Mt Chappell Island (MCI)	Moderate north-facing slope; sandy soil; grassland	147 55'44"E 40 16'02"S 30 m	$X = 738 \pm 126$ CV = 0.17	$X_{\max} = 18.2$ $X_{\min} = 9.5$ CV = 0.21	$X = 1160 \pm 86$ CV = 0.49
Sorrell (ST)	South-east Tasmania; savanna grassland	147 33'17"E 42 47'39"S 15 m	$X = 574 \pm 132$ CV = 0.23	$X_{\max} = 17.6$ $X_{\min} = 8.2$ CV = 0.24	$X = 1073 \pm 94$ CV = 0.53
Mornington (MT)	South-east Tasmania; Open eucalypt forest	147 23'11"E 42 50'07"S 135 m	$X = 745 \pm 149$ CV = 0.20	$X_{\max} = 16.2$ $X_{\min} = 6.5$ CV = 0.29	$X = 978 \pm 98$ CV = 0.56

avg, average; CV, coefficient of variation of means; SD, standard deviation.

0.5 mg wk⁻¹. At the 0.5 mg wk⁻¹ supply rate, for example, the low-water plants received half the volume of nutrient solution but at double concentration. There was no indication of toxicity or salt build-up in the soil of the low-water plants.

Whole-plant and leaf morphology

In February 2003, the youngest fully expanded leaf was removed from each plant and stored overnight with its petiole sunk in wet floral foam in a dark cool room (~5°C) for 24 h and then weighed. Each leaf was scanned on a Hewlett Packard scanner, unflattened to determine projected area, and then flattened. Leaves were dried at 80°C for 72 h and weighed. The individual area and perimeter of both the projected and flattened scanned images were determined using Delta-T Scan (Delta-T Devices Ltd, Cambridge, UK). The specific leaf area (SLA = area (mm²)/dry weight (mg)), potential hydration ((leaf saturated wet weight (g) – leaf dry weight (g))/leaf saturated wet weight (g)), projection efficiency (PE = projected leaf area (mm²)/flat leaf area (mm²)), and leaf shape (quantified as a dissection index, DI = leaf perimeter (mm)/square root of flat leaf area (mm²), where higher indices reflect more highly dissected leaves; cf. McLellan & Endler, 1998) were measured for each leaf.

Leaf number and fitness

We estimated the total leaf area of each plant (total number of leaves × area of youngest fully expanded leaf) as an index of plant fitness. We evaluated the fitness index on field-grown plants in January 2007 at the Rosedale (*n* = 19 plants) and Kambah (*n* = 20) sites. Total leaf area (single leaf area × total number of current-season leaves) was a significant predictor of the number of inflorescences at both sites (*r*² = 0.78 and 0.72, respectively).

Leaf anatomy and physiology

We selected the three populations exhibiting the most distinct leaf forms (Kambah, Rosedale and Mt Chappell Island) for anatomical and physiological study. Plants were taken from two of the three nutrient treatments at each water level (0.06 and 0.5 mg wk⁻¹ for low and 0.125 and 0.5 mg wk⁻¹ for high). Gas exchange measurements were made on the youngest fully expanded leaf on each plant in spring (October 2003) using a LI-6400 Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA). Initial light response curves on plants of each population demonstrated that a light intensity of 600 μmol m⁻² s⁻¹ was saturating and all subsequent measurements were taken at this light intensity, a reference CO₂ concentration of 400 ppm, relative humidity between 34 and 62% and temperatures between 21 and 24°C, between sunrise and midday.

Photosynthetic capacity and stomatal conductance were determined on both area ($P_{\max a}$, μmol m⁻² s⁻¹) and mass bases

($P_{\max g}$, μmol g⁻¹ s⁻¹) using mean SLA data for entire leaves. Leaf nitrogen content was determined using a dynamic flash method with a Carlo Erba 1110 elemental analyzer (CE instruments, Milan, Italy) and VG Isochrom-EA mass spectrometer (Micromass UK, Ltd., Manchester, UK) for leaves from blocks 1–3. Water use efficiency (WUE = $P_{\max a}$ /transpiration) and photosynthetic nitrogen use efficiency (PNUE = $P_{\max g}$ /leaf nitrogen content (g m⁻²)) at photosynthetic capacity were calculated for the leaves from blocks 1–3. (As a result of a data handling error, transpiration data were lost from blocks 4–6.)

Digital scanning electron microscope (Hitachi S 2250 NSEM; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) images were made using preserved leaf sections (2 × 2 cm), taken from the centres of the two youngest fully expanded leaves on each plant in May 2003. Sections were snap-frozen in liquid nitrogen and then 'broken' using a cold razor blade and placed in the vacuum-sealed scanning electron microscope (SEM) chamber. The depths of upper and lower epidermal, palisade and spongy mesophyll tissue layers were measured on two digital images of each leaf section using IMAGEJ (Rasband, 2005).

Statistical analysis

The responses to the treatments were analysed using nested analysis of variance (SAS PROC GLM; SAS Systems Inc., Cary, NC, USA). Population and water treatment were fixed effects, and nutrient supply rate was nested within water level. This allowed analysis of interactions between population and water treatment and between population and nested nutrient supply rate, where these interactions serve as indicators of population differences in plasticity. Water contrasts were examined at a constant nutrient supply rate of 0.5 mg wk⁻¹. Leaf area, SLA, and dissection index were log-transformed to meet the assumptions of normality.

Correlations between climatic traits and mean values of leaf traits were calculated for all traits for which there were not significant population by water or nutrient interactions. The coefficient of variation of means (CV; standard deviation/mean) of each trait across all treatments was calculated to compare the extent of plasticity among traits. Other indices of plasticity (e.g. difference between minimum and maximum trait values in a given population relative to the grand mean) were also investigated and were found to be tightly correlated with CV (data not shown). Correlations between trait CVs and variance in rainfall, temperature and open-pan evaporation were used to assess relationships between amount of plasticity and habitat heterogeneity.

To assess plasticity in trait integration, we calculated correlation coefficients for trait relationships within treatments for each population, focusing on the provenances and treatments for which the complete set of measurements were taken. Because there was substantial plasticity of correlations

Table 2 Significance levels from analysis of variance of whole-plant and leaf morphological traits for plants from six populations at six treatment levels; and anatomical and gas exchange traits for three populations grown at four treatment combinations

	Pop	Water	NSR (water)	Pop × water	Pop × NSR (water)	Model R ²
Whole plant						
d.f.	5	1	4	5	20	
Leaf number	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.933
Total leaf area	0.055	< 0.0001	< 0.0001	0.88	0.029	0.77
Morphology						
d.f.	5	1	4	5	20	
Flat leaf area	< 0.0001	0.032	< 0.0001	0.71	0.047	0.641
Projection efficiency	< 0.0001	0.50	0.004	0.05	0.22	0.539
Potential hydration	< 0.0001	0.30	< 0.0001	0.25	0.004	0.77
SLA	< 0.0001	0.0009	< 0.0001	0.84	0.004	0.743
Dissection index	< 0.0001	0.20	< 0.0001	0.41	0.31	0.445
Anatomy						
d.f.	2	1	2	2	4	
Leaf thickness	0.68	0.44	0.043	0.72	0.37	0.368
UE depth	0.07	0.24	< 0.0001	0.38	0.06	0.501
Palisade depth	< 0.0001	0.27	0.12	0.59	0.21	0.647
Spongy depth	< 0.0001	0.03	0.04	0.39	0.45	0.444
LE depth	0.04	0.74	0.002	1.00	0.36	0.235
Gas exchange						
d.f.	2	1	2	2	4	
P_{max_a}	0.0003	< 0.0001	0.047	0.23	0.86	0.521
P_{max_g}	< 0.0001	0.22	0.008	0.15	0.89	0.556
Conductance	< 0.0001	0.0001	0.0002	0.20	0.59	0.608
WUE	< 0.0001	0.006	0.004	0.55	0.39	0.676
Nitrogen (g g ⁻¹)	0.72	0.012	< 0.0001	0.63	0.65	0.836
Nitrogen (area)	0.002	0.81	0.0008	0.60	0.044	0.715
PNUE	0.0001	0.002	0.0001	0.40	0.10	0.804

Values in bold are significant at $P = 0.05$.

d.f., degrees of freedom; LE, lower epidermis; NSR, nutrient supply rate; PNUE, photosynthetic nitrogen use efficiency; Pop, population; SLA, specific leaf area; UE, upper epidermis; WUE, water use efficiency; photosynthetic rates on area (P_{max_a}) and mass (P_{max_g}) bases.

across treatments, we calculated the pairwise deviations between treatments as the absolute value of the difference between correlation coefficients (treatment X – treatment Y). Inter-trait correlations were grouped by character type (correlations within morphological (M), anatomical (A), and physiological (P) trait groups, and the correlations between these groups: M:A, M:P and A:P), and the means for each group were determined, producing a 6×6 matrix for each population. Two Friedman's rank sum tests were performed on each matrix (SAS PROC FREQ, using the Cochran–Mantel–Haenszel, CMH, statistic). One tested for significant differences in the magnitude of correlation changes between trait groupings, and the other tested whether particular environmental transitions elicited more or less change in correlation values.

We examined the effect of population and water treatment on the relationship between total leaf area (our index of fitness) and the traits for which there was a significant water effect or water by population interaction (see Table 2). When variables were intercorrelated ($r^2 > 0.5$) only one of the intercorrelated variables was included. Our analysis included total leaf area as the response variable; population, water treatment and either conductance, potential hydration, dissection index or spongy

mesophyll thickness were included as factors. The full model was fitted and nonsignificant terms were dropped. Nonsignificant main effects were included if they were present in significant interactions. In this analysis a water treatment by dependent variable interaction indicates a significant difference in slopes between water treatments.

Results

Plasticity in response to changes in nutrients and water

Our indicators of whole-plant performance varied significantly among populations and in response to differences in water and nutrient supply (Table 2a). The total number of leaves and total leaf area increased with increasing nutrient supply and, at a constant nutrient supply rate (0.5 mg wk⁻¹), low-water plants had more leaves than high-water plants (Fig. 2a,b). Populations differed in their leaf number plasticity to water (i.e. a significant population-by-water interaction; Table 2a). In response to differences in nutrients, populations differed in the plasticity of both leaf number and total leaf area (Fig. 2a,b).

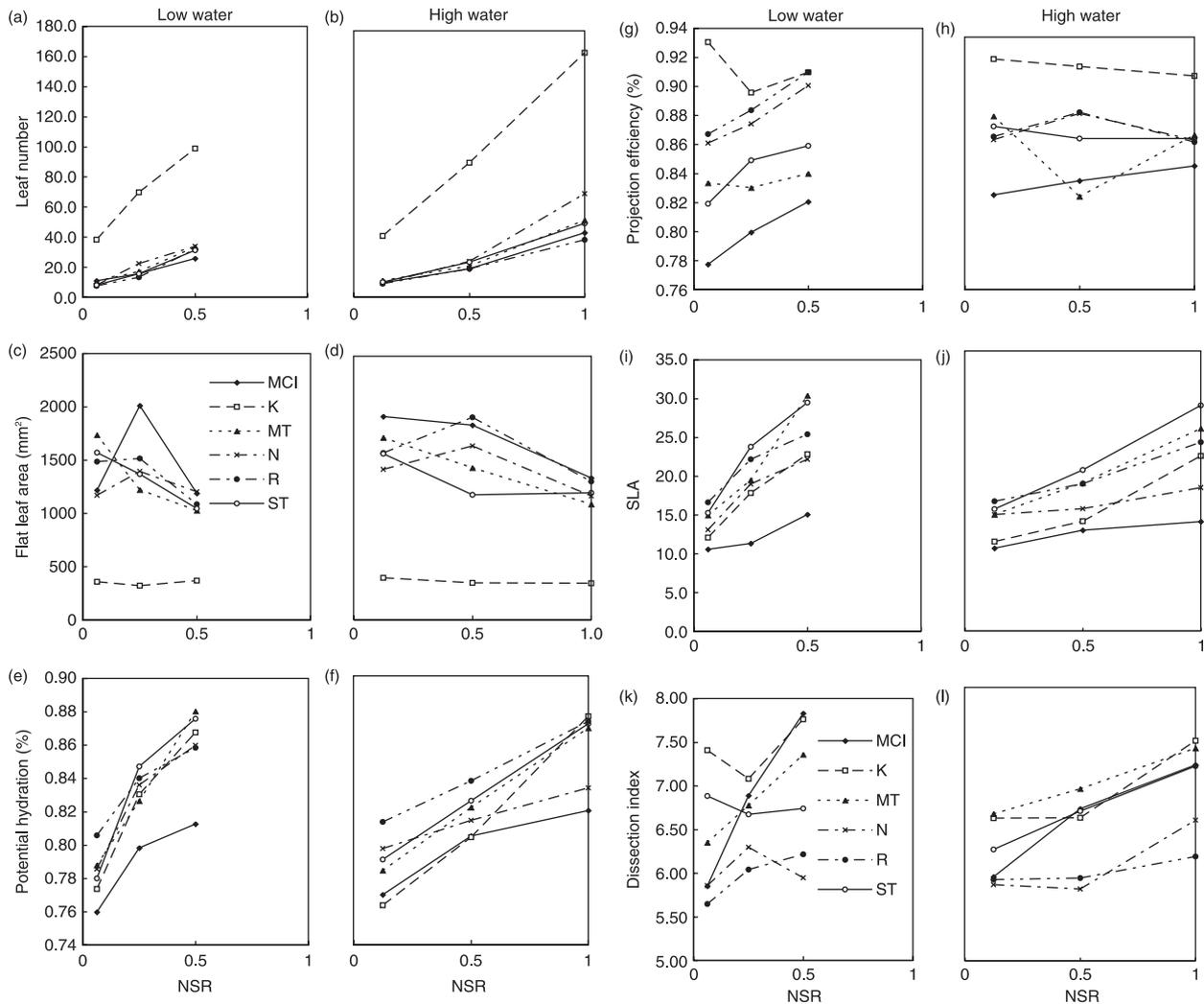


Fig. 2 Mean values of traits in six experimental regimes. (a, b) *Pelargonium australe* leaf number, (c, d) flat leaf area, (e, f) potential hydration, (g, h) projection efficiency, (i, j) specific leaf area (SLA; $\text{mm}^2 \text{mg}^{-1}$), and (k, l) dissection index. NSR, nutrient supply rate. Values for leaf number, leaf area and SLA are back-transformed expected means of log data. Abbreviations for population names are from Table 1.

Nutrient supply rate strongly affected all morphological traits (Table 2b). Individual leaf area tended to decline with increasing nutrient supply rate in both water treatments and a general trade-off between leaf size and number was evident across all populations (Fig. 2a–d). Leaf potential hydration, projection efficiency, SLA (area per unit mass) and leaf dissection also increased significantly at higher nutrient supply rates (Fig. 2e–l). Overall leaf thickness, and the thickness of all tissue layers except for the palisade layer were significantly lower at higher nutrient supply rates (Table 2c). All physiological traits were affected by nutrient supply rate (Table 2d). At high nutrient supply rate photosynthetic capacity and conductance increased, as did nitrogen concentration (N g g^{-1}), although not proportionately, resulting in significantly lower PNUE at higher nutrient supply rate.

We expected nutrient supply rate to affect leaf traits as it did, but because the species has a propensity to grow on nutrient-

poor soils and rock wherever it occurs, we did not expect population differences in plasticity in response to variations in nutrient supply rate. Contrary to our predictions, the amount and direction of plastic responses to nutrient supply rate differed significantly among populations (significant population \times nutrient supply rate interactions) for several traits: total leaf area and leaf number, individual leaf area, potential hydration, SLA, and nitrogen on an area basis (Table 2).

Lower water levels resulted in a decrease in individual leaf area but, contrary to expectations, SLA was higher at lower water levels (Fig. 2c,d,i,j). Effects on leaf anatomy were minor, with high-water leaves having greater mean spongy mesophyll thickness; there were no other significant water effects on anatomical traits. Among the physiological traits, P_{maxa} , conductance, nitrogen concentration and PNUE were lower at the low water levels, while WUE was higher. When water effects were compared only at a constant nutrient supply rate

Table 3 Significant values from analysis of variance of effects of water and population on leaf traits at the nutrient supply rate of 0.5 mg wk⁻¹ where the two gradients matched (this comparison was made to enable better assessment of the water effect)

	Pop	Water	Pop × water
Morphology			
d.f.	5	1	5
Flat leaf area	< 0.0001	0.0011	0.35
Leaf number	< 0.0001	0.0004	0.97
Total leaf area	0.85	0.37	0.52
Projection efficiency	< 0.0001	0.62	0.11
Potential hydration	< 0.0001	< 0.0001	0.010
SLA	< 0.0001	< 0.0001	0.046
Dissection index	< 0.0001	0.0034	0.33
Anatomy			
d.f.	2	1	2
Leaf thickness	0.08	0.85	0.41
UE depth	0.30	0.74	0.96
Palisade depth	< 0.0001	0.38	0.62
Spongy depth	< 0.0001	0.018	0.13
LE depth	0.32	0.15	0.28
Gas exchange			
d.f.	2	1	2
P_{maxa}	0.033	0.0003	0.33
P_{maxg}	0.004	0.40	0.25
Conductance	0.020	< 0.0001	0.20
WUE	0.014	0.0003	0.68
Nitrogen (g g ⁻¹)	0.931	0.014	0.58
Nitrogen (area)	0.013	0.62	0.43
PNUE	0.036	0.0005	0.045

d.f., degrees of freedom; LE, lower epidermis; PNUE, photosynthetic nitrogen-use efficiency; SLA, specific leaf area; UE, upper epidermis; WUE, water-use efficiency; photosynthetic rates on area (P_{maxa}) and mass (P_{maxg}) bases.

(0.5 mg), low-water leaves were also found to be significantly more dissected, and had higher potential hydration values. Because of the opposite plastic responses of leaf area and number, total leaf area was buffered against reductions at low water levels (Table 3).

We predicted that the plasticity of leaf traits in response to variations in water supply would differ among populations, given known differences in the amount and heterogeneity of water supply in their native environments. Surprisingly, population-by-water interactions were less frequent than population-by-nutrient interactions, being significant only for leaf number across all treatments (Table 2) and for potential hydration, SLA and PNUE when compared at a nutrient supply rate of 0.5 mg wk⁻¹ (Table 3).

Population differences

As already stated, our analyses demonstrated population differences for almost all traits investigated, and significant population-by-water or population-by-nutrient supply interactions for many of these as well (Table 2). This very plasticity

within and among populations made it difficult to detect patterns of local differentiation in trait means. For the seven traits that did not have significant interaction terms (dissection index, palisade and spongy mesophyll and lower epidermal cell thicknesses, photosynthetic capacity on a mass basis, conductance and instantaneous water use efficiency), however, we did find indications of correlations between climatic variables in the source populations and population mean leaf traits. Averaged across all treatments, leaf dissection was lower in sites with higher minimum temperatures ($n = 6$ populations, $R = -0.96$, $P < 0.01$) or greater average rainfall ($n = 6$, $R = -0.79$, $P = 0.06$). Among the physiological traits, photosynthetic capacity (mass basis) was higher for populations from higher rainfall sites ($n = 3$, $R = 0.99$, $P = 0.1$). Together these relationships provide evidence of local adaptation.

We further examined whether the amount of plasticity in leaf traits increased with increasing heterogeneity in climate variables. Not surprisingly, there were no significant correlations between population-level plasticity and climate variables when they were assessed for all traits simultaneously or for each of the three groups of traits (morphological, anatomical and physiological) separately. However, when traits were considered singly we found significant correlations between plasticity and habitat heterogeneity; these correlations were not consistently positive (Table 4). For example, among morphological traits, plasticity in leaf number increased and plasticity in dissection index decreased with increasing inter-annual variation in rainfall. For the anatomical and physiological data sets we had only three populations to compare; nonetheless, we found the same sort of variation. Increased variation in open-pan evaporation was associated with decreased plasticity in gas exchange traits, whereas increased temperature variation was associated with increased plasticity in palisade and spongy mesophyll depth. We found no differences between mean CVs when we compared populations from rocky outcrops with those from grassland or forest.

We also compared CVs to assess whether populations (independent of geography) or classes of traits differed in the amount of plasticity. There were no overall differences in amount of plasticity among populations; instead, the relative ranking of the populations differed among traits. For example, Narooma was among the most plastic populations for leaf number, but showed the lowest plasticity in dissection index (Fig. 3). Comparing across trait classes, leaf anatomical traits were least plastic on average and physiological traits generally intermediate, but there was considerable variation among trait groups and among populations within trait groups. Leaf number was the most plastic trait measured, and leaf projection efficiency was the least plastic trait considered (Fig. 3).

This variability in plasticity among traits and among populations was also seen in correlations among traits. There were no overall differences among populations but individual correlation coefficients between traits differed on average by ≈ 0.55 between treatments (Table 5). These results suggest very

Table 4 Correlations between climate variability and plasticity (coefficient of variation of means (CV) for each leaf trait for each population)

Plasticity of:	Rainfall CV		Temperature range		Temperature CV		Open-pan evaporation CV	
	R	P	R	P	R	P	R	P
Morphology (<i>n</i> = 6)								
Projection efficiency			-0.89	0.02				
Potential hydration					0.79	0.06	0.90	0.02
Dissection index	-0.82	0.05						
Flat leaf area			-0.93	0.01	-0.78	0.07		
SLA					0.73	0.10	0.82	0.05
Leaf number	0.81	0.05						
Anatomy (<i>n</i> = 3)								
Leaf thickness							0.99	0.08
UE depth								
Palisade			0.99	0.08	1.00	0.04		
Spongy mesophyll			1.00	0.06	0.96	0.17		
LE depth	-0.98	0.13						
Gas exchange (<i>n</i> = 3)								
$P_{\max a}$							-0.96	0.17
$P_{\max g}$							1.00	0.04
Conductance			-0.99	0.09	-0.95	0.20		
WUE							-1.00	0.00
Nitrogen (g g^{-1})								
Nitrogen (area)			-1.00	0.06	-0.96	0.17		
PNUE					-0.96	0.18	-0.99	0.08

Rainfall CV represents interannual variation in rainfall (*n* = 36 yr), temperature range is the average difference between the average daily temperature of the warmest and coldest months (*n* = 36 yr), and temperature and open-pan evaporation CVs are based on monthly averages (*n* = 423 months). Bold values are significant at *P* < 0.05 for morphological or *P* < 0.10 for other traits. Negative values of *R* denote negative correlations between trait plasticity and environmental heterogeneity. Blank cells did not approach significance (*R* < 0.70 and *R* < 0.95 for morphological and other traits, respectively).

LE, lower epidermis; PNUE, photosynthetic nitrogen-use efficiency; SLA, specific leaf area; UE, upper epidermis; WUE, water-use efficiency; photosynthetic rates on area ($P_{\max a}$) and mass ($P_{\max g}$) bases.

low degrees of integration in plasticity among traits. Populations showed different patterns of plasticity of integration: Mt Chappell Island and Rosedale had reduced plasticity of correlations among anatomical traits, whereas Kambah had reduced plasticity of correlations among morphological traits. When the data for trait groups were combined, there were pronounced differences among the trait groups (Friedman's rank sums: CMH statistic = 45.24, *P* < 0.0001). The most plastic correlations were those among anatomical and physiological traits (i.e. A:P). There were no significant differences in the magnitude of correlation change among the six possible treatment transitions for either individual provenances or for all data pooled.

Plasticity and fitness

We investigated potential relationships between plasticity in response to variations in water supply and fitness by assessing the relationship between the total leaf area (our index of fitness) and traits that showed significant plasticity to water; correlations were assessed within each water level. A significant water treatment-by-leaf trait variable interaction indicates

that the slopes of the relationship of the trait with fitness differ significantly between water treatments. There was no significant relationship between spongy mesophyll tissue layer depth and total leaf area, and there was no water treatment interaction for dissection index. Potential hydration yielded a significant water treatment interaction, but the slope of the relationship between potential hydration and leaf area was positive at both low and high water ($y = 1288.4x - 827.6$, $r^2 = 0.022$ and $y = 2643.7x - 1813.9$, $r^2 = 0.21$ at low and high water, respectively).

Patterns of water use, by contrast, suggest a fitness benefit to efficient water use when water is limiting. Total leaf area (fitness) was greater among plants that had low rates of conductance at low water levels ($y = -870.7x + 330$, $r^2 = 0.40$), but showed no relationship with conductance at high water levels ($y = 67.4x + 232$, $r^2 = 0.004$; Fig. 4, Table 6). Population effects and interaction effects on total leaf area were not significant in this model. The data do not show a corresponding cost to conservative water use when water is not limiting, but at high water plants had higher conductance on average. This result is indicative of an adaptive value of plasticity in stomatal conductance rates in *P. australe*.

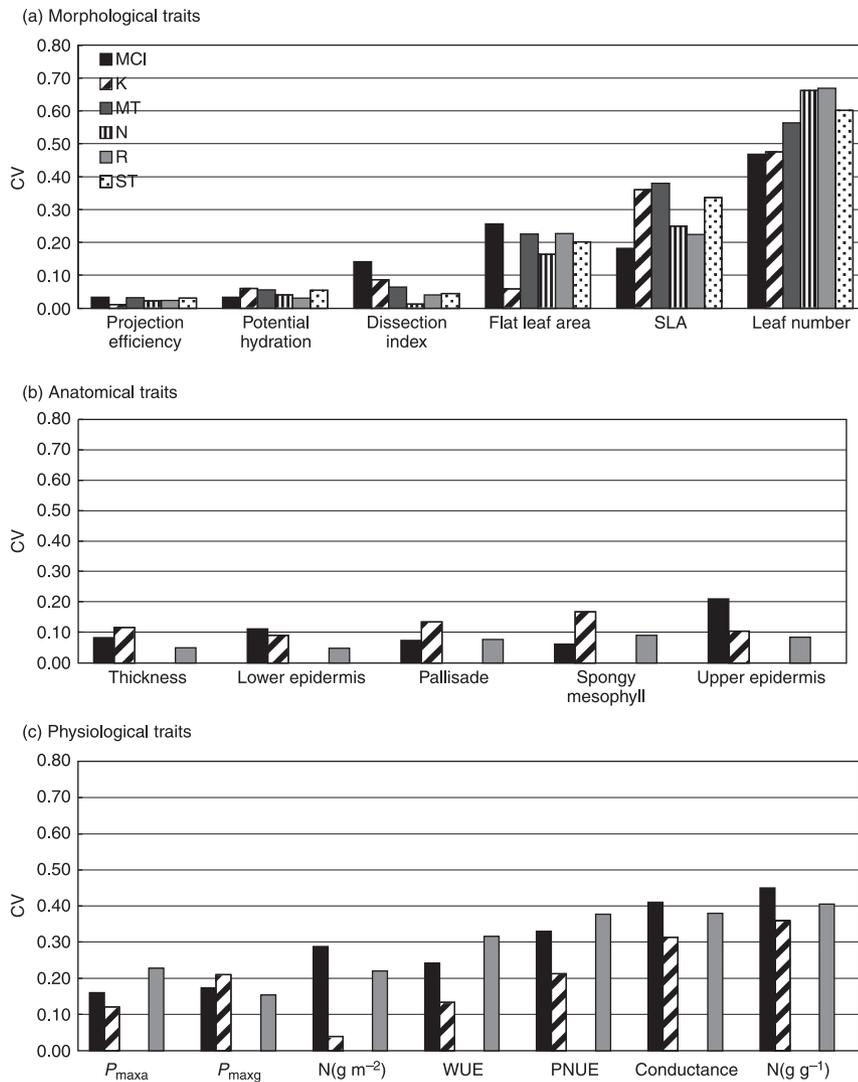


Fig. 3 Comparison of plasticity of traits of *Pelargonium australe* as indicated by coefficients of variation (CV; standard deviation divided by mean) for population \times treatment means. Traits are sorted by increasing mean CV across populations from left to right across the x-axis. Asterisks denote significant population \times treatment interactions (Table 2). (a) Morphological traits (projection efficiency, potential hydration, dissection index, single (flat) leaf area, specific leaf area (SLA), and total leaf number); (b) anatomical traits (leaf thickness and thickness of lower epidermis, palisade, spongy mesophyll and upper epidermal layers); (c) physiological traits (photosynthetic rates on area (P_{maxa}) and mass (P_{maxg}) bases, nitrogen on area ($N(g\ m^{-2})$) and mass ($N(g\ g^{-1})$) bases, water use efficiency (WUE), photosynthetic nitrogen use efficiency (PNUE) and conductance).

Discussion

Populations of *P. australe* showed significant differentiation in most of the leaf traits examined. Significant correlations between leaf dissection and both minimum temperature and average rainfall and between photosynthetic capacity and rainfall provide support for local adaptation (i.e. ecotypic differentiation). Most traits and most populations showed significant plasticity. The patterns of these plastic responses defy simple explanations based on habitat or climate of origin. Plasticity in response to variations in water and nutrient supply did not reliably increase with mean rainfall or heterogeneity in source climate; in fact in some cases it decreased. Nor did populations have any consistent ranking in plasticity to water or nutrient supply. Also, patterns of integration among plastic traits were themselves highly flexible across treatments. What, if anything, is the significance of this plasticity and flexibility in integration patterns?

Plastic response to variations in water and nutrient supply

For the most part, patterns of plastic response follow expectations: total leaf area increased and leaves had higher photosynthetic capacity but lower nutrient use efficiency at higher nutrient supply rates. At low water, plants produced smaller leaves that had lower rates of photosynthesis and conductance but higher water use efficiency. Dissection index and projection efficiency generally increased with nutrient supply rate.

Across a broad range of species, leaves are often smaller and have lower SLA when water and nutrients are less abundant (Givnish, 1987; Cunningham *et al.*, 1999; McDonald *et al.*, 2003). We found that the leaves were thicker at low nutrient supply rate, and might therefore be expected to have lower SLA, especially at low water (Dudley, 1996; Reich *et al.*, 1997; Poorter & De Jong, 1999; Sultan, 2003). However, *P. australe* apparently does things differently: plants produced

Table 5 Table of average magnitudes of differences in correlation coefficients among traits within (morphology (M), anatomy (A) and physiology (P)) or between (M:A, M:P and A:P) trait categories

		1 vs 2	1 vs 4	1 vs 5	2 vs 4	2 vs 5	4 vs 5	Mean	Row rank sum
MCI	M	0.43	0.47	0.63	0.46	0.42	0.44	0.48	15
	A	0.39	0.46	0.50	0.32	0.34	0.24	0.38	7
	P	0.61	0.65	0.72	0.71	0.61	0.74	0.67	28
	M:A	0.54	0.43	0.57	0.40	0.43	0.49	0.48	14
	M:P	0.67	0.62	0.69	0.60	0.63	0.65	0.64	26
	A:P	0.70	0.78	0.80	0.94	0.87	0.80	0.82	36
	Mean	0.56	0.57	0.65	0.57	0.55	0.56	0.58	<i>P</i> = 0.07
	Column rank sum	18.5	19.5	33	18	16	22		
	<i>P</i> = 0.097	0.23	0.24	0.17	0.40	0.35	0.37		
K	M	0.47	0.31	0.27	0.44	0.38	0.37	0.37	7
	A	0.59	0.55	0.24	0.61	0.54	0.52	0.51	17
	P	0.63	0.58	0.49	0.60	0.98	0.63	0.65	26.5
	M:A	0.51	0.52	0.33	0.68	0.45	0.58	0.51	18
	M:P	0.65	0.58	0.70	0.55	0.60	0.64	0.62	28.5
	A:P	0.64	0.50	0.61	0.75	0.72	0.66	0.65	29
	Mean	0.58	0.51	0.44	0.61	0.61	0.57	0.55	<i>P</i> = 0.004
	Column rank sum	26.5	15	12	27	23	23		
	<i>P</i> = 0.106	0.13	0.20	0.43	0.17	0.35	0.19		
R	M	0.68	0.40	0.53	0.66	0.66	0.32	0.54	19
	A	0.20	0.16	0.25	0.12	0.12	0.16	0.17	6
	P	0.36	0.37	0.86	0.48	0.73	0.71	0.59	22
	M:A	0.58	0.59	0.55	0.83	0.75	0.68	0.66	28
	M:P	0.87	0.64	0.51	0.74	0.68	0.60	0.67	24
	A:P	0.37	0.76	0.83	0.59	0.82	0.67	0.67	27
	Mean	0.51	0.49	0.59	0.57	0.63	0.52	0.55	<i>P</i> = 0.009
	Column rank sum	21	17.5	23	22	25	18		
	<i>P</i> = 0.82	0.48	0.44	0.38	0.44	0.40	0.44		

Columns (e.g. 1 vs 2) represent the average change in correlation between pairs of treatments. Treatment 1 was low water, low nutrient supply rate (NSR; 0.0625), and treatment 5 was low water, high NSR (0.5). Treatment 2 was high water, medium NSR (0.125), and treatment 4 was high water, high NSR (0.5). *P*-values associated with rank sums are from Friedman's rank sums tests, with a test for differences among rows in the last column and a test for differences among columns in the second column. Bold values are significantly different at *P* < 0.05.

fewer, larger leaves when grown under reduced nutrient supply, and both SLA and potential hydration increased along with leaf thickness at low water. We explain this as a tendency toward semisucculence, an alternative response to water stress. The observed increase in potential hydration may be attributable to increased leaf tissue and cell elasticity (Bosabalidis & Kofidis, 2002). Increased mesophyll thickness at low water may aid water conservation, and would be correlated with the observed reduction in photosynthetic rate as a result of decreased absolute internal air space and reduced CO₂ diffusion (Chartzoulakis *et al.*, 2002). Overall, *P. australe* appears to be genetically geared towards a morphology that results in efficient resource use under low water so long as nutrient availability is adequate, strategies that appear to be functionally appropriate for a species occupying water-limited environments (Valladares *et al.*, 2000a, 2002a).

Plasticity and habitat heterogeneity

We predicted that, because populations differ in the amount (and heterogeneity) of rainfall, they would differ in plasticity

in response to changes in water availability, but, because this species occupies nearly soil-free environments throughout its range, we did not expect significant population differences in plasticity to nutrient supply. We found significant population-by-treatment interactions for both responses to water and nutrient supply changes. Contrary to our prediction, interactions with nutrient supply were as strong as with water level, providing no support for our proposed distinction between plasticity to water and nutrient availability. In most cases, populations differed in the magnitude of the response, not in its direction *per se*, so the shapes of the plastic responses were generally similar among populations. For example, potential hydration in all populations increased with increasing nutrient supply rate, but the Mount Chappell Island plants showed less response than those from the other populations. In other cases, both the amount and the direction – or pattern of response – differed among populations, for example for dissection index.

Much previous work has addressed issues of whether phenotypic plasticity is correlated with increasing resource heterogeneity or range size (Sultan, 1987; Sultan *et al.*, 1998a,b; Bell & Sultan, 1999; Valladares *et al.*, 2000a, 2002a; Balaguer

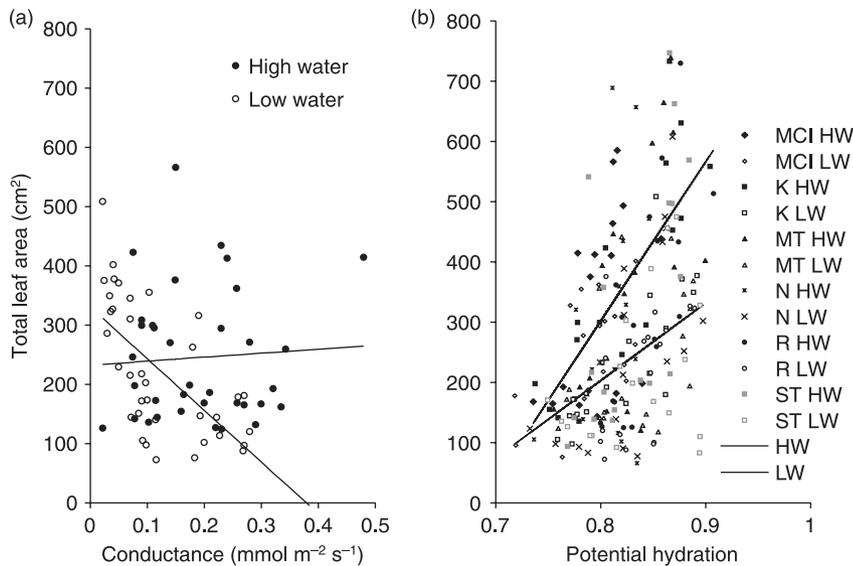


Fig. 4 Regressions of total leaf area (a proxy for fitness) of *Pelargonium australe* on (a) conductance and (b) potential hydration. In both cases, slopes for different water treatments are significantly different. (a) Population effects were not significant; (b) different symbols denote different populations (see Table 1 for abbreviations).

Source	<i>n</i>	d.f.	Sum of squares	<i>F</i> ratio	Probability > <i>F</i>
Potential hydration					
Population	5	5	469941.4	3.8381	0.0024
Water	1	1	895952.3	36.5869	< 0.0001
Potential hydration	1	1	1726676.2	70.5101	< 0.0001
Water × potential hydration	1	1	184249.0	7.5239	0.0066
Conductance					
Water	1	1	35921.71	3.4700	0.0669
Conductance	1	1	91457.05	8.8346	0.0041
Water × conductance	1	1	124699.28	12.0457	0.0009

d.f., degrees of freedom.

et al., 2001; Murray *et al.*, 2002; Grant *et al.*, 2005; Lehmann & Rebele, 2005; Pohlman *et al.*, 2005; Rozendaal *et al.*, 2006). For example, when *Quercus coccifera* populations from habitats differing in light heterogeneity are grown under two light treatments, light capture-related traits – leaf size, angle and LAR – show the greatest variation in extent of plasticity, and the population from the more heterogeneous habitat is the more plastic (Balaguer *et al.*, 2001). If increased plasticity represents a response to higher degrees of environmental heterogeneity, we would expect a relationship between the amounts of plasticity (CVs) of groups of traits (morphological, anatomical and physiological) and variation in rainfall, temperature and open-pan evaporation. We found no correlations across these groups; some traits were plastic and others were not (e.g. compare projection efficiency and leaf number; Fig. 2). Most strikingly, however, we found that, while some individual traits increased in plasticity with increasing environmental heterogeneity, others actually decreased. Thus, our data provide no clear link between habitat heterogeneity and plasticity in these *P. australe* populations. Although a small number of populations could be blamed for a lack of pattern for anatomical and physiological traits, some traits showed

positive and others negative relationships. The negative correlations we found may indicate that our measures of heterogeneity do not reflect the heterogeneity detected by the plants, or they may indicate passive plastic responses (van Kleunen & Fischer, 2005), or they may provide examples of environmental canalization – the lack or loss of plasticity in response to environmental variation that may result from stabilizing selection (Levin, 1988; Debat & David, 2001; Schlichting & Smith, 2002) – for specific traits varying among populations of a single species.

Variation in plasticity and integration

Previous studies have also shown differences in relative patterns of plasticity among morphological, anatomical and physiological traits (Valladares *et al.*, 2000b, 2002b; Gratani *et al.*, 2003). Lower plasticity for a physiological trait (Griffith & Sultan, 2006) may reflect canalization of that trait or may arise because small variations in key metabolic traits can have large functional ramifications. Regardless, we did not find strong patterns of difference in the extent of plasticity among trait types. The most and least plastic traits were both

morphological traits, and physiological traits were very variable in their extent of plasticity. The anatomical traits that we measured showed relatively little plasticity, although all showed significant effects of nutrient supply (Tables 2b, 5, Fig. 3).

There was substantial variation in the strength and even direction of correlations between traits across treatments; in some cases there were reversals from significantly positive to significantly negative. The populations did not differ from one another in their overall correlation plasticity. Taken together, these results suggest that populations of *P. australe* differ in trait means and plasticity, but that degrees of integration are low across all the populations. The traits we considered are unified by being leaf traits associated with carbon gain and water loss, but, compared with more tightly unified suites of traits (Parsons & Robinson, 2006), our traits comprise a varied collection. Within our more constrained trait groupings, correlations among leaf anatomical traits were least plastic – these correlations changed only by 0.17 for the Rosedale population (Table 5). The correlations between anatomical and physiological traits were generally the most plastic, suggesting that particular anatomical features are not tightly related to specific physiological outcomes in *P. australe*.

When species colonize novel environments, patterns of plasticity and integration among plastic traits are predicted to change (Price *et al.*, 2003; Monteiro *et al.*, 2005; Parsons & Robinson, 2006); this prediction has held among sunfish (*Lepomis gibbosus*), where colonization of a new habitat was associated with increased plasticity and integration of morphological traits. *Pelargonium australe* arrived in Australia at most 5 million yr ago, presumably via long-distance dispersal (Bakker *et al.*, 1998). We propose that the observed patterns of integration and plasticity may reflect the biogeography and history of these populations. Our study included one inland population (Kambah), one island population (MCI), and four 'mainland' coastal populations. At present, we cannot detect among-population differences in integration, and populations do not differ consistently in overall plasticity. Incorporating additional populations may provide an opportunity to map changes in plasticity and integration onto the ecological and evolutionary history of the species. High amounts of plasticity and low degrees of integration across a range of morphological, anatomical and gas exchange traits important to water loss and carbon gain may be critical to the ability of this species to spread across a wide range of habitats, and may also be associated with patterns of microevolution within the species. The alternative, of course, is the possibility that the interpopulation variation has arisen at random, without strong selection to limit it.

Adaptive plasticity in *P. australe*

To assess whether the plastic responses we have demonstrated are adaptive, one would have to compare the relationship between a plastic trait of interest and fitness or a proxy thereof

(Weinig *et al.*, 2004; Griffith & Sultan, 2005; van Kleunen & Fischer, 2005; Caruso *et al.*, 2006). The slope of this relationship is an indicator of the strength of selection, and if the slope of the relationship between fitness and a plastic trait differs between environments, or if fitness is correlated with measures of plasticity themselves, selection will favour plasticity (Weinig *et al.*, 2004; Caruso *et al.*, 2006). As we did not replicate genotypes or families within our populations, we were not able to apply a formal selection analysis to our data (Lande & Arnold, 1983; Rausher, 1992). Our analogous approach was to assess the interaction between a plastic trait of interest and water treatment in a regression on our fitness surrogate, total plant leaf area. This approach showed that efficient water use was correlated with fitness under conditions of water limitation, but not when water was in abundant supply. When water was in abundant supply, plants had higher water use on average. This accords with other recent studies that have shown adaptive plasticity in water use for other herbaceous species (Heschel *et al.*, 2002; Heschel *et al.*, 2004; Heschel & Riginos, 2005; Caruso *et al.*, 2006). These results indicate that some portion of the extensive plasticity demonstrated here is adaptive. It remains to be seen whether plasticity in other traits is adaptive in and of itself, results from integrated phenotypic responses, or is nonadaptive passive plasticity.

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