Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area and leaf size: congeneric species from desert and coastal environments

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Summary

• We examined whether increased high temperature photosynthetic thermal tolerance (PT), reduced specific leaf area (SLA) and reduced leaf size represent correlated and convergent adaptations for recently diverged *Encelia*, *Salvia*, *Atriplex* and *Eriogonum* congeneric species pairs from contrasting thermal and water environments (the Mojave Desert and coastal California). We also studied whether variation in PT is associated with inducible small heat shock protein expression (sHsp).

• Traits were measured in a common environment (CE) and in the field to partition effects of phenotypic plasticity and genetic divergence.

• We found little evidence for convergent adaptation of PT (CE measurements). Field measurements revealed significant plasticity for PT, which was also associated with increased sHsp expression. Compared to coastal congeners desert species had lower SLA in the CE. These differences were magnified in the field. There was a negative correlation between SLA and PT. Desert species also tended to have smaller leaves both in the CE and in the field.

• SLA and leaf size reductions represent repeated evolutionary divergences and are perhaps convergent adaptations for species radiating into the desert, while PT is highly plastic and shows little evidence for convergent adaptation in the congeneric species pairs we studied.

Key words: specific leaf area (SLA), heat shock protein, stress, plasticity, fluorescence (F_v/F_m), thermotolerance, convergent adaptation, phylogenetic independent contrasts.

Introduction

Temperature and water availability are prominent among the abiotic factors that limit the distribution and abundance of plants. Evergreen perennial plants cannot avoid these stresses and must tolerate great diurnal and seasonal fluctuations. In semiarid regions, temperature and precipitation are often negatively correlated, with lower rainfall in warmer environments. Remarkably, angiosperms possess a great capacity to adapt and tolerate far ranging differences in temperature and precipitation; they are common across the entire continuum of habitats, from those that experience just a few weeks of frost-free weather to cool, wet coastal environments and hot, dry deserts (Fig. 1). Many morphological and physiological traits contribute to this tremendous niche differentiation, including photosynthetic thermal tolerance and traits related to the energy balance of leaves (Gates, 1965; Berry & Bjorkman, 1980).

Here we examine whether increased photosynthetic thermal tolerance (PT, measured by F_v/F_m chlorophyll fluorescence), reduced specific leaf area (SLA) and reduced leaf size can be interpreted as convergent evolutionary responses for several lineages radiating across a temperature and precipitation gradient. We also study whether variation in these traits is associated with levels of inducible small heat shock protein expression (sHsp), both in a common garden and in the field.

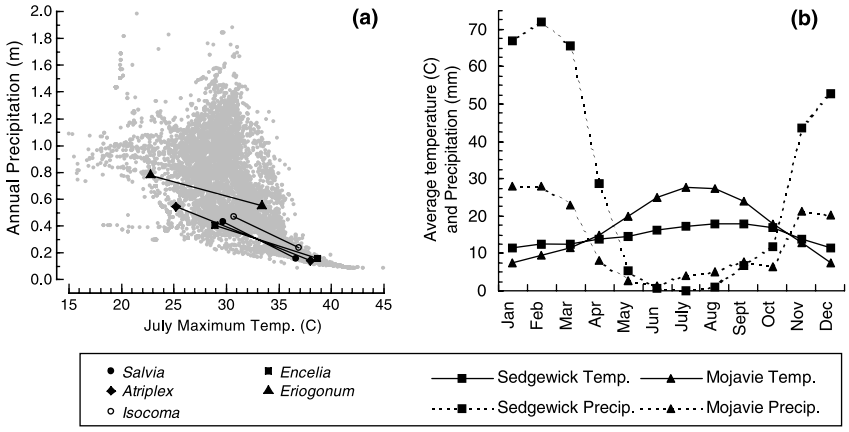

Fig. 1 (a) July maximum temperature and annual precipitation for each species in the California flora (each grey point represents a species). Lines connect the desert and coastal congeneric species chosen for this study. (b) Thirty-year average monthly temperature (solid lines) and precipitation (dashed lines) at our field sites in the Mojave Desert and on the southern coast of California (Sedgwick Ranch).

We used a set of phylogenetically independent species contrasts (PICs, in our case congeneric *Encelia*, *Salvia*, *Atriplex* and *Eriogonum* species) to test these hypotheses. Within each PIC there was one species from the hot, dry Mojave Desert and a congeneric species from the cooler coastal environments of southern California. Our PICs were arbitrarily chosen at the generic level for lack of better information concerning divergence times between species. We made measurements both in a common environment (CE) and in the field to help partition the effects of genetic divergence and phenotypic plasticity for observed trait variation.

The traits we studied (SLA, leaf size, PT and sHsp expression) vary in their responsiveness to environmental stimuli. SLA changes on the scale of the development and senescence of a leaf, but varies little within and between days – except during periods of rapid leaf expansion. PT and sHsp expression can vary rapidly on the scale of minutes to hours (Knight & Ackerly, 2002a,b, 2003). Therefore it is also interesting to test whether highly responsive traits (PT and sHsp expression) or more time-integrated traits (SLA) are more likely to exhibit convergent adaptation.

Like most other biochemical and physiological processes, photosynthesis is highly responsive to temperature. Photosynthetic thermal tolerance (PT) can be defined at several levels, from a change in the excitation capacity of photosystem II (PSII) to the rate of carbon assimilation or biomass accumulation. Within a range of temperatures the rate of photosynthesis responds rapidly and reversibly, with the functional integrity of the photosynthetic apparatus intact (Berry & Bjorkman, 1980). However, beyond extreme high and low critical temperatures, irreversible damage occurs, which can significantly affect short and long-term carbon gain. PSII has long been recognized as one of the most thermally labile components of photosynthesis (Weis & Berry, 1988; Havaux, 1993). Therefore we chose excitation capacity of PSII as our metric of comparison of PT between species.

Previous investigators have found considerable variation between species for PT, as well as pronounced acclimatory changes (Berry & Bjorkman, 1980; Weis & Berry, 1988, 1988; Knight & Ackerly, 2001, 2002a). However, most studies have involved only one or a couple of species with varying degrees of evolutionary relatedness. Therefore it is still not known whether the evolution of increased PT represents a repeated evolutionary response for independent lineages diverging across thermal gradients – although it is frequently assumed to be the case.

Accumulating evidence suggests that small heat shock proteins (sHsps) are important for the maintenance of photosynthetic and respiratory electron transport during and after heat stress (Downs & Heckathorn, 1998; Heckathorn *et al.*, 1999). Small Hsps dominate protein synthesis during and after high temperature stress and under some conditions can rapidly accumulate to greater than 1% of total leaf protein (Vierling, 1991; O'Connell, 1994). While most eukaryotes have just a few sHsps, in plants the protein class has duplicated and diversified to include 20–50 nuclear encoded genes. In general it is thought that Hsps prevent irreversible aggregation of denatured proteins, thereby facilitating protein refolding following high temperature stress (Jakob *et al.*, 1993; Lee *et al.*, 1997). Variation between species for expression levels of the chloroplast sHsp following heat stress is positively correlated with the maintenance of PSII electron transport (Preczewski *et al.*, 2000; Knight & Ackerly, 2001, 2003). Several sHsps (including the chloroplast sHsp) are not constitutively expressed. Therefore, induced sHsp expression is a useful indicator of physiological stress. Despite the continuing interest in plant sHsps, only a few studies have examined plant sHsp expression in the field. Of the few studies that have, two made no report of Hsp expression in leaves (Hernandez & Vierling, 1993; Stout *et al.*, 1997), and the others examined Hsp expression in agricultural fields (Burke *et al.*, 1985; Kimpel & Key, 1985).

Species with smaller, thicker leaves generally occur in more stressful environments and exhibit lower specific leaf area (SLA). Previous studies observed reduced SLA in experimental water stress treatments (Li *et al.*, 2000) and others found correlations across species between SLA and water availability (Fonseca *et al.*, 2000; Li *et al.*, 2000; Wright *et al.*, 2001).

Higher SLA in environments with greater water availability may be due to enhanced water use efficiency associated with the increase in photosynthetic tissue relative to transpiring area (Givnish, 1987; Cunningham *et al.*, 1999; Fonseca *et al.*, 2000; Wright *et al.*, 2001) suggesting a link between SLA and photosynthetic performance. Others have shown that variation in SLA is correlated with a suite of physiological and plant growth parameters including: slower growth rates, lower leaf nitrogen content, lower light-saturated photosynthetic capacity and dark respiration rates, and longer leaf life spans (Dijkstra & Lambers, 1989; Chapin *et al.*, 1993; Reich *et al.* 1997, 1998). The fact that SLA is related to these traits may be coincidental, or perhaps due to functional interrelationships that may represent both evolutionary constraints and correlated responses to the environment.

Materials and Methods

This study involved several phylogenetically independent contrasts (PICs) involving congeneric *Atriplex*, *Encelia*, *Eriogonum* and *Salvia* species pairs that differed in mean July maximum temperature inside their geographic ranges by close to 10°C. See Knight & Ackerly (2002b) for a detailed description of how we calculated these species level estimates. Figure 1a highlights the contrasting realized niche spaces of the PICs (*sensu* Austin *et al.*, 1990). We chose PICs with minimal differences in annual precipitation while maximizing differences in July maximum temperature.

Desert populations were collected in the Mojave Desert near the Desert Studies Center (operated by California State University, 35°11′ N, 116°4′ W). Coastal populations were collected in the Santa Monica and Santa Ynez Mountains north of Santa Barbara. Physiological work for these populations was conducted at the Sedgwick Reserve (operated by the University of California Natural Reserve System, 34°37′ N, 120°5′ W). Temperatures at the desert field site are on average 10°C warmer than the coastal field site in July, but 4°C cooler in December and January. The coastal field site gets twice the precipitation in the winter, but both field sites receive little precipitation in the summer (Fig. 1b).

Seeds were collected in the spring of 1998, germinated in vermiculite and later transplanted to variable grain size sand in 20-cm diameter and 50-cm deep pots in a glasshouse at the Plant Growth Facility on the campus of Stanford University. Separate pots for approximately 50 individuals of each species within a congeneric pair (PIC) were established together in a rectangular block. Each genus had its own block. Within a block, pots for the two species were arranged in an alternating matrix.

The mean daytime temperature in the glasshouse was 25°C during the day and 15°C during the night. Plants were watered approximately once every week. Therefore they experienced fluctuating water availability but were never as water stressed as they sometimes are in the field. The plants were fertilized monthly. The amount of nutrient addition was determined so that adequate growth and healthy foliage was maintained with minimal fertilizer (based on information from test plantings and by visual inspection of the plants in our experiment). Nutrient addition was identical within congeneric pairs. The plants were grown in the CE for over a year before the first measurements were made. In May 2000, the parent field populations of the common garden species pairs were revisited and the physiological parameters listed below were measured with identical methodology as measurements made in the CE. Most of the co-occurring dominant species at the two field sites were also measured. The full species names, along with each of the variables described below, are listed in Table 1.

Table 1 The mean specific leaf area (SLA) and F_v/F_m T_{50} for desert and coastal species in the common environment (CE), and at the desert (D) and coastal (C) field sites

Congeneric species in the common environment	SLA (mm^2 mg^{-1})		F_v/F_m T_{50}	
	CE	Field	CE	Field
Atriplex hymenelytra (D)	11.5	8.1*	41.5	46.2*
Atriplex leucophylla (C)	16.9	16.5	41.7	42.5
Encelia farinosa (D)	12.9	10.4*	42.6	45.2*
Encelia californica (C)	21.6	18.8*	42.3	40.9*
Eriogonum fasiculatum (D)	8.3	8.2	44.9	45.9
Eriogonum latifolium (C)	9.4	8.5*	44.4	43.9
Salvia mohavensis (D)	12.0	9.8*	41.3	42.9*
Salvia leucophylla (C)	15.6	12.6*	41.9	41.2
Additional species at the field sites				
Artemesia californica (C)		–		41.6
Ambrosia dumosa (D)		12.3		43.5
Baccharis pilularis (C)		14.0		42.6
Brickellia arguta (D)		14.0		42.8
Encelia frutescens (D)		9.7		42.6
Hazardia sqr. var. *sqr.* (C)		14.4		41.5
Isocoma acradenia (D)		9.6		43.3
Isocoma menziesii (C)		10.8		39.3
Larrea tridentata (D)		8.0		46.9
Salvia dorrii var. *dorrii* (D)		10.7		42.7
Salvia mellifera (C)		15.1		40.9

Measurements for SLA and F_v/F_m T_{50} that are significantly different between the CE and field sites are indicated by an asterisk (*).

Specific leaf area (SLA) and leaf size

We collected 15–20 randomly selected, mature, healthy, fully exposed leaves from each species – each leaf was collected from a different plant to avoid pseudo-replication. Our method was to blindly reach into the exposed canopy and then to ensure that the leaf we picked conformed to the rest of our criteria. We selected fully exposed leaves because these species have relatively open canopies (i.e. few leaves could be classified as 'shade leaves') and to standardize measurements between individuals and between species. We did not sample

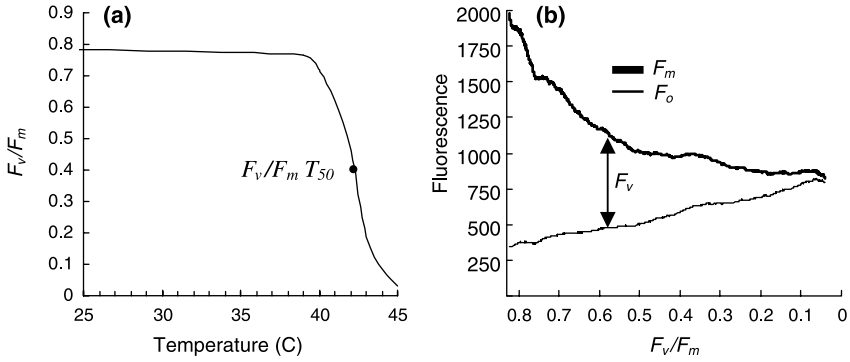

Fig. 2 (a) A typical curve for the temperature dependent decline in F_v/F_m. The temperature at which F_v/F_m declined to 50% of its maximum (F_v/F_m T_{50}) was used for comparison between species and treatments. (b) The relative contribution of F_o and F_m to the decline in F_v/F_m. Notice that the F_v/F_m axis is plotted in reverse (high to low), representing increasing stress (higher temperatures) to the right. The data plotted here are averages of > 1080 individual F_v/F_m measurements taken for this study.

for developmental variation in SLA. However, results from a previous study involving 20 chaparral species suggested that species to species differences for SLA and leaf size can be detected with small sample sizes for mature leaves because between species variance is much greater than within species variance (Ackerly *et al.*, 2002). We measured SLA and leaf size for CE plants when they were approximately two years old. In the CE congeneric species were sampled on the same day. For plants in the CE, leaf area was determined using a Li-3100 leaf area meter (LiCor, Lincoln, Nebraska, USA). Leaf area for leaves collected in the field was determined using an AM100 portable leaf area meter (ADC Bioscientific, Hoddeson, UK). Leaves were weighed using an analytical balance (Mettler-Toledo, Columbus, OH, USA) after drying for 5 days in an oven at 80°C. Specific leaf area (SLA) is expressed in mm^2 leaf area mg^{-1} d. wt.

Photosynthetic thermal tolerance – F_v/F_m

We used the temperature dependent decline in the photochemical efficiency of photosystem II (PSII) as a metric of comparison between species for photosynthetic thermal tolerance. We quantified the photochemical efficiency of PSII using the ratio of variable to maximal fluorescence (F_v/F_m) following actinic light pulses (12 000 µmol m^{-2} s^{-1}, 0.7 s, Fig. 2) using a Hansatech FMS2 fluorometer (King's Lynn, Norfolk, UK). Excitation is the first step in photosynthesis while carbon fixation can be considered the last. Most literature suggests that the D1 protein and the oxygen evolving proteins of PSII are the most thermally labile components of photosynthesis (Berry & Bjorkman, 1980; Weis & Berry, 1988; Havaux, 1993; Heckathorn *et al.*, 1998), therefore we chose the initial photochemistry of PSII as our metric of comparison for PT. However, photosynthetic thermal tolerance can be defined at several levels, from a change in excitation capacity, as we did, to a change in carbon assimilation or biomass accumulation. We chose F_v/F_m because its measurement is rapid (enabling comparisons of multiple species and larger sample sizes) and adaptable to field conditions.

Stems with several healthy leaves were collected early in the morning and kept in the dark. Five leaves of each species were placed on moist filter paper in a small plastic chamber submerged in a temperature controlled water bath. Outside air was circulated through the chamber during the temperature treatment. Leaf temperatures inside the chambers did not vary by more that 0.1°C and equilibrated to water bath temperatures in less than 5 min. A proportional, integrated and differential temperature controller was used to maintain water bath temperatures (Oven Industries, Mechanicsburg, PA, USA). Four-hour heat treatments were carried out between 39 and 46°C at 1°C intervals, as well as at room temperature (approximately 28°C). For a given set temperature, the actual treatment temperature did not vary by more than 0.1°C. Five replicates of each temperature treatment were conducted both in the CE and in the field. F_v/F_m was quantified 4 h after the heat treatments ranging from 28 to 46°C. The last hour of recovery was in the dark. The treatment temperature at which F_v/F_m declined 50% from the species maximum, here referred to as F_v/F_m T_{50}, was estimated for each species by linear interpolation between the temperature treatments that bracketed the 50% decline (Fig. 2a). Knight & Ackerly (2002a) measured the temperature at which the steady state fluorescence F_o reached 20% of it's maximum (T_{S20}). Here we present correlated relationships of T_{S20} with SLA and F_v/F_m T_{50}.

sHsp expression

We quantified standing levels of sHsp expression for leaves collected in the CE and in the field at the same time as our F_v/F_m T_{50} measurements. Small Hsp expression was quantified for seven samples of each species both in the CE and in the field. Each sample consisted of 5–10 randomly chosen leaves. Protein extraction followed the methods of Knight & Ackerly (2001). We used a polyclonal antibody that detects multiple sHsps in heat-stressed plant tissue (provided by S. A. Heckathorn). It was produced using an oligopeptide of the conserved heat-shock domain found in all plant sHsps (as in Downs *et al.*, 1998, except that the antiserum was raised in rabbits and the peptide was conjugated to keyhole limpet hemocyanin). The antibody cross-reacts with several sHsps. Because we used one-dimensional

electrophoresis we could not precisely quantify variation in the number of sHsps recognized. The comparison data consisted of the optical density of sHsp 'bands' developed using the alkaline phosphatase reaction following incubation with a secondary antibody conjugated to the alkaline phosphatase enzyme. For each species, the samples from the field and common garden were run on the same gel to highlight relative differences for sHsp accumulation. A positive control run on each gel to normalize gel-to-gel variation in band intensity.

Statistical analysis

The questions raised by this study primarily relate to whether there are significant differences for four traits (SLA, leaf size, F_v/F_m T_{50}, and sHsp expression) between congeneric species native to desert and coastal environments when grown in a common environment (CE) and when measured in the field. We performed two-way ANOVAs for each trait measured in the CE, and additional two-way ANOVAs for field measurements (eight total for native environment comparisons). Genus, native environment were modelled as fixed factors. The interaction term was included. We were primarily interested in the native environment factor (differences between species within genera). To further examine significant differences for the native environment factor we performed planned comparisons in each of the two-way ANOVAs following Underwood (1997). There were four planned comparisons in the CE, comparing desert to coastal species within each genus. In the field there was an additional comparison between desert and coastal species of *Isocoma*. The numerator when calculating the F statistic for each of these planned comparisons is the mean square difference between species values, and the denominator is the error mean square from the full model. All of the planned comparisons are orthogonal so we could use $\alpha = 0.05$ level for each of the planned comparisons (Underwood, 1997). However, all significant planned comparisons were also significant using the Bonferroni adjustment of α. Data for SLA, leaf size, and F_v/F_m T_{50} were normally distributed and conformed to the assumptions of the ANOVA. We used Data desk for the two-way ANOVAs and computed the planned comparisons by hand.

There were several additional unpaired species at the desert and coastal field sites. Therefore, to test for over-all differences for these traits at our field sites, we also performed nested ANOVAs where species were nested in native environment. We also calculated Pearson's correlation coefficient for relationships between SLA, F_v/F_m T_{50}, and T_{S20} – a trait measured for these same species pairs and presented in Knight & Ackerly (2002a).

Plasticity for each trait between the CE and the field was also analysed with two-way ANOVAs. Plasticity for desert and coastal species was modelled separately because the field environments were different. Genus and growth environment (CE or field) were modelled as fixed factors. Our model included the interaction term. We also performed planned comparisons as described above with the exception that that growth environment (CE vs field) was substituted for native environment (desert vs coast).

Results

Field

Comparisons of congeners in the field indicated that native environment was a significant factor for two-way ANOVAs involving SLA, leaf area, and F_v/F_m T_{50} (Appendix 1a–c). Desert species had lower SLA, smaller leaf areas, and greater F_v/F_m T_{50} (Fig. 3a,b). There was also a significant difference between genera and significant interactions between genera and environment for all three factors. Planned comparisons for SLA indicated that there was a highly significant difference between the desert and coastal *Atriplex*, *Encelia* and *Salvia*, but not for *Eriogonum* and *Isocoma*. Leaf area was also greater for the coastal *Atriplex*, *Eriogonum* and *Salvia*, not different for the *Isocoma* PIC, and smaller for the coastal *Encelia*. Planned comparisons for F_v/F_m T_{50} indicated that all of the desert species had greater F_v/F_m T_{50} when compared to their coastal congeners when measured in the field.

Across all species at the desert and coastal field sites (PICs and unpaired species), ANOVAs with species nested in environment (desert or coast) indicated that SLA and leaf area were significantly lower, and F_v/F_m T_{50} was significantly greater for species at the desert field site (Fig. 3a,b, Appendix 1d–f). Interspecific variation for SLA was nearly twice as great at the coastal field site than in the desert (10.3 mm^2 mg^{-1} and 5.8 mm^2 mg^{-1}, respectively) but variation within both communities was greater than the mean difference between communities (3.8 mm^2 mg^{-1}). Within field site variation for F_v/F_m T_{50} (4.3°C and 4.6°C, respectively, for desert and coastal field sites, respectively) was greater than mean difference between communities (2.6°C). The mean F_v/F_m T_{50} and SLA for each species is listed in Table 1.

Common environment (CE)

Native environment was a significant factor in the CE for two-way ANOVAs involving SLA and leaf area (Fig. 3c, Table 1, Appendix 1g,h). There was also a significant difference between genera and a significant interaction between genus and environment for SLA and leaf area. Planned comparisons indicated that the desert *Atriplex*, *Encelia* and *Salvia* species all had lower SLA compared to their coastal congener, but there was not a significant difference between the desert and coastal *Eriogonum* species. Leaf areas for the coastal *Atriplex*, *Eriogonum* and *Salvia* species were greater than their desert counterparts but smaller for the coastal *Encelia*.

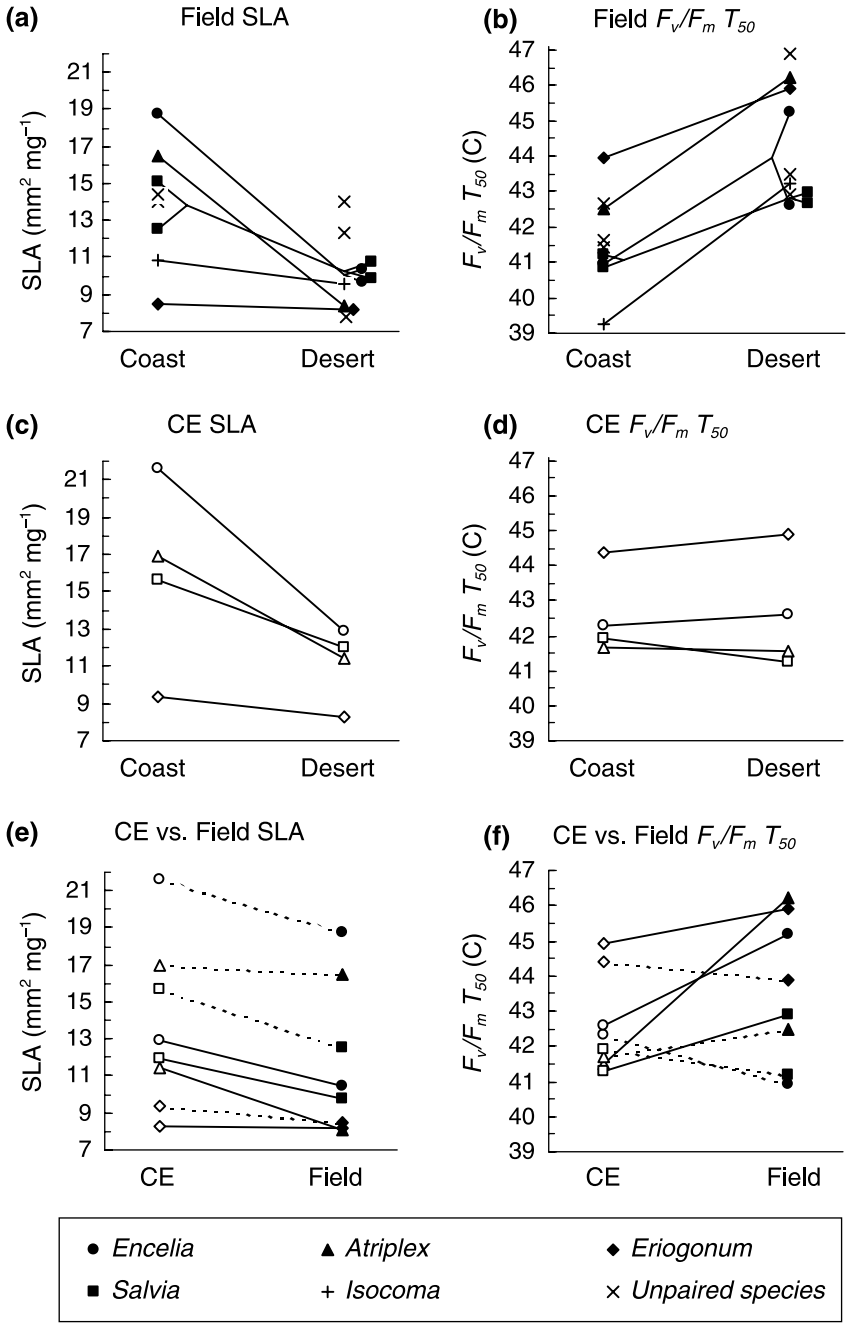

Fig. 3 Differences between congeneric species at the desert and coastal field sites for SLA (a) and F_v/F_m T_{50} (b). Congeneric species are connected by solid lines. There were two *Salvia* and *Encelia* species at the desert field site and two *Salvia* species at the coastal field site; the mean of these pairs are connected to the congener(s) in the opposite environment by a solid line. Unpaired species are represented by an x. Genetic differences for SLA (c) and F_v/F_m T_{50} (d) for the congeneric pairs in the common environment (CE). Plasticity for SLA (e) and F_v/F_m T_{50} (f) between the CE and the field. Measurements in the CE are represented by open symbols and measurements in the field are closed symbols. Dashed lines connect measurements for the coastal species between the CE and field in (e) and (f). For full species names of the desert and coastal congeners refer to Table 1.

When measured in the CE, native environment was not a significant factor for F_v/F_m T_{50} (Appendix 1i, Fig. 3d). However, there were significant differences between genera. Knight & Ackerly (2002a) found that there was a significant difference between the desert and coastal species for T_{S20}. However, the effect was largely driven by a highly significant difference between the desert and coastal *Atriplex*. We did not perform planned comparisons for F_v/F_m T_{50} because the native environment term was not significant. There was a significant positive correlation between T_{S20} and F_v/F_m T_{50} in the CE. However, within PICs there were both positive and negative relationships (Fig. 4d).

Plasticity between the CE and field

SLA was lower in the field than in the CE for both coastal and desert species (two-way ANOVA, Appendix 1j,m, Fig. 3e). Planned comparisons indicated that the differences for the coastal *Salvia* and *Encelia* species and the desert *Atriplex* and *Encelia* species were significant. Leaf areas were often slightly

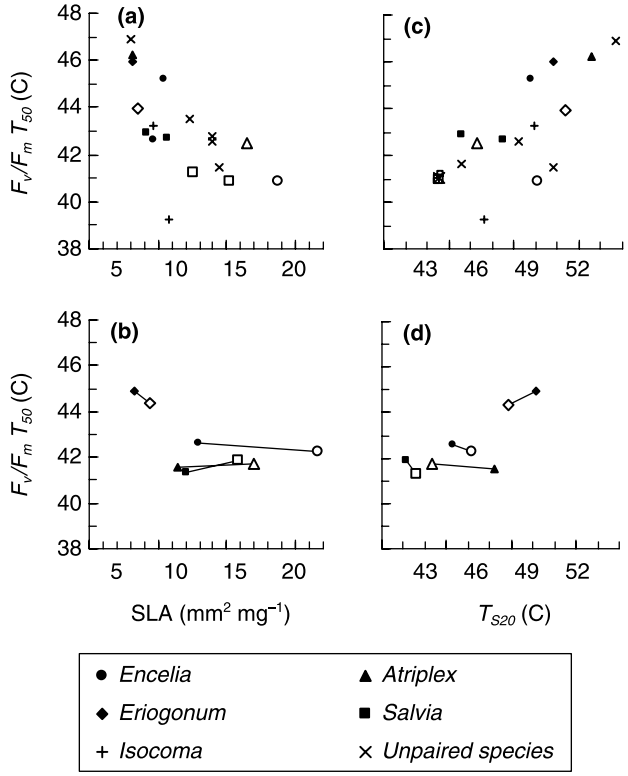

Fig. 4 Relationships between SLA and F_v/F_m T_{50} in the field (a) and in the common environment (CE) (b) and between T_{S20} and F_v/F_m T_{50} in the field (c) and in the CE (d). In b and d congeneric species are connected with a line. Open symbols are coastal species, closed symbols are desert species. Refer to the legend and Table 1 for species names.

larger in the CE compared to field measurements for both desert and coastal species. There was a significant difference between CE and field measurements for F_v/F_m T_{50}. However, the coastal *Encelia*, *Eriogonum* and *Salvia* species had greater F_v/F_m T_{50} in the CE compared to field measurements while desert species all had lower F_v/F_m T_{50} in the CE (Fig. 3f, Appendix 1f,h). Post-hoc multiple comparisons for the coastal species indicated that only the *Encelia* species had a significantly greater F_v/F_m T_{50} in the CE compared to the field, while the desert *Atriplex*, *Encelia* and *Salvia* species all had significantly lower F_v/F_m T_{50} in the CE.

Correlations among SLA, T_{S20}, and F_v/F_m T_{50}

For field measurements, there was a significant negative correlation between SLA and F_v/F_m T_{50} (Fig. 4a). This relationship was not significant for CE measurements (Fig. 4b). The correlation between SLA and T_{S20} both for CE and field measurements was not significant, but in both cases there was a negative trend. There was a positive correlation between T_{S20} and F_v/F_m T_{50} both for field measurements and in the CE (Fig. 4c,d).

sHsp expression in the common environment and in the field

Small Hsp expression levels were significantly different between CE and the field (two-way ANOVA with interaction term, environment (CE or field) and genus as fixed factors, $F_{5,72} = 112.3$, $P \leq 0.001$). We were unable to obtain sufficient soluble protein extractions to quantify sHsp expression for the *Saliva* species, perhaps because of high concentrations of phenolics, which may have contributed to sample degradation. For the desert *Encelia*, *Atriplex* and *Eriogonum* species, *sHsp* expression in the field was significantly greater than in the CE (Fig. 5, planned comparisons, $P < 0.001$ in all cases). In the CE, only the desert and coastal *Eriogonum* species had low levels of sHsp expression. Of the coastal species, only *Encelia californica* had significantly greater sHsp expression in the field (planned comparison, $P = 0.035$). Expression levels were largely unchanged for *Eriogonum latifolium* between the CE and field. We did not detected sHsp expression for *Atriplex leucophylla* in the CE or in the field, despite the fact that we were able to extract and separate proteins for Coomassie stained gels.

Discussion

The most interesting result from this study was the lack of genetic variation for photosynthetic thermal tolerance between desert and coastal congeneric species (i.e. from common environment measurements). There are several possible explanations for this. Thermal environments are highly variable across the entire range of spatial and temporal scales, which may allow species with various tolerances to persist in both desert and cooler coastal environments. In addition, whole plant thermal tolerance (i.e. survival) and PT may not be correlated because photosynthesis occurs only when environmental conditions are favourable. Favourable conditions may be frequent enough even in environments with frequent and extreme high temperature stress – therefore, there may not be selection pressures for increased PT. It is also possible that the plasticity we observed for PT was adaptive and that plasticity for PT precluded genetic divergence (Sultan, 1987). Another possibility is that there has been genetic divergence for PT, but we did not detect it because the norms of reaction for PT converged to similar phenotypic states in the CE we used. If the congeneric species had been grown in a different CE with a different combination of abiotic factors perhaps we would have found significant differences (i.e. if we had tested the entire norm of reaction).

Evolutionary studies concerning photosynthetic thermal tolerance are also complicated by the fact that a variety of environmental factors can affect photosynthesis, including plant water status (Seemann *et al.*, 1979, 1986; Havaux,

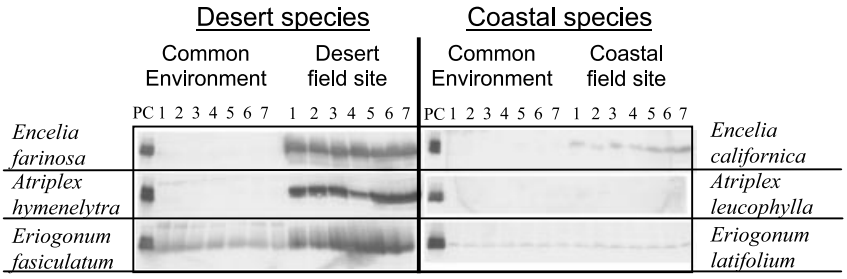

Fig. 5 Small hsp expression for desert and coastal species in the common environment (CE) and in the field. There are seven replicates for each species in each environment. PC is a positive control run on each gel to normalize gel-to-gel variation in band intensity. The CE and field samples for each species were run on the same gel.

1992; Valladares & Pearcy, 1997), soil salinity (Larcher *et al.*, 1990), light levels (Schreiber & Berry, 1977; Weis, 1982; Havaux & Strasser, 1992), nutrient availability (Field & Mooney, 1986), and growth temperature (Schreiber & Berry, 1977; Seemann *et al.*, 1979, 1986; Downton *et al.*, 1984). In addition, photosynthetic acclimation can occur on the scale of minutes to hours in response to moderately elevated temperatures (Havaux, 1993), and comparable leaves from different individuals of a single species in the same environment can also exhibit considerable variation (Knight & Ackerly, 2002a).

The 25°C daytime temperatures in our CE were higher than the average growing season temperatures at the coastal field sites, but lower than those in the desert. Water availability in our CE was probably higher than usual levels at the desert and coastal field sites. Both of these factors (temperature and water availability) probably contributed to PT differences between the CE and the field, as well as from decreased light availability due to greenhouse shading (the CE was under glass, which blocked approximately 20% of full sunlight).

To interpret trait variation between species it may be important to consider the evolutionary trajectory of these species with respect to their current environment (e.g. did they move into the desert from a cooler environment or into a cooler environment from the desert?). If an ancestral species inhabited a hot desert, and the capacity for high temperature photosynthetic acclimation was selectively neutral, when a daughter species later encountered a cooler environment it may have retained the same PT. It is also possible that the congeneric species we studied were too recently related for substantial phenotypic divergence for PT. However, it is interesting to note that SLA and leaf size did exhibit genetic divergence. We did observe genetic variation for PT among genera in the CE, suggesting that at deeper levels of evolutionary relatedness PT does evolve.

It is thought that the Mojave Desert was formed by the rapid geologic uplift of the Sierras approximately 1–2 mya, forming a large rain shadow (Oakeshott, 1971; Thorne, 1986). Packrat middens suggest great climatic changes in the last 40 000 yr, with a significant warming trend in the last 10 000 yr (Spaulding, 1990). However, due to shifts in plant distributions, climatic conditions in the Mojave region do not represent the historical conditions experienced by these populations. Paleoecological analyses suggest that in cooler and wetter times (i.e. > 10 000 ybp) elements of the Mojave flora were found at lower elevations and to the south in Mexico and parts of Central America (Axelrod, 1950, 1979; Thorne, 1986). Unfortunately there is a paucity of phytogeographic or historical biogeographic information for the groups that we studied. Of these, *Encelia* is the one most likely to have originated in the south-western deserts of North America from desert dwelling species (Bruce Baldwin, personal communication). Thus, despite the geologic youth of the deserts, it is not possible to state with confidence the direction of divergence for our species pairs.

Several morphological and biochemical processes may contribute to plastic acclimation of photosynthesis. We found differences in standing levels of sHsp expression between the CE and the field populations. These differences were associated with increased PT in the field, which is consistent with the hypothesis that sHsps play a role in the acclimation of photosynthesis to high temperature. We also demonstrate that plants in their native environment express sHsps that are often found to be strictly inducible in controlled environment studies, which highlights the importance of expression profiling under native environmental conditions to fully understand the cellular function of candidate genes.

F_v/F_m T_{50} was positively correlated with the fluorescence rise parameter T_{S20} (data from Knight & Ackerly, 2002a, Fig. 4c,d), though this relationship was only significant in the CE. Under the protocol of Knight & Ackerly (2002a) T_{S20} differs from F_v/F_m T_{50} in that it involved a rapid (1°C min^{-1}) increase in temperature, T_{S20} measurements did not involve a recovery period, and F_m was not measured. The temperature dependent decline in F_v/F_m is both a function of increasing basal fluorescence (F_o), indicating a decline in photochemical quenching with increasing temperature, and a decline in excitation capacity (F_m, Fig. 2b), which may represent a dissociation of light harvesting complexes from the PSII reaction centre core (Yamane *et al.*, 2000), increased membrane fluidity (Raison *et al.*, 1982), the temperature dependent denaturing of the D1 or oxygen evolving proteins, or the dissociation of primary electron acceptors Q_A and Q_B (Bilger *et al.*, 1984; Bukov *et al.*, 1990).

Our study supports the hypothesis that reduced SLA is a convergent trait in plant lineages evolving into thermally stressful environments with lower annual precipitation. Our

results concur with other studies indicating that, within the same habitat, variation among species for SLA is considerable, reflecting the diversity of growth strategies and life histories within the same community (Reich *et al.*, 1997; Ackerly *et al.*, 2002; Ackerly, 2003). The reduction in SLA in the desert primarily represents an absence of species with high SLA; there were species with low SLA at the coastal field site (e.g. *Eriogonum latifolium*) but species at the coastal field site also had the greatest SLA (e.g. *Encelia californica*).

Leaves with lower SLA were better able to withstand and recover photosynthetic electron transport after high temperature stresses than species with greater SLA (Fig. 4a). In the field this correlation was apparent for all species pairs as well as across all taxa (paired and unpaired). In the CE the correlation was not robust within congeneric pairs because of the lack of genetic variation for photosynthetic thermal tolerance, but there was a negative trend (Fig. 4b). Knight & Ackerly (2001) found that after identical heat stresses, species with lower SLA accumulated greater levels of a chloroplast sHsp compared to species with higher SLA. Other studies suggest that greater leaf longevity, which is associated with low SLA (Reich *et al.*, 1997), promotes nutrient retention, enhancing long-term photosynthetic nitrogen-use efficiency (Field & Mooney, 1986; Chapin *et al.*, 1993). Perhaps it is not surprising that leaves with stress tolerant life histories (indicated by low SLA) are resilient to thermal damage of photosynthesis.

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Appendix 1

Two-way ANOVA tables for variation in specific leaf area (SLA), leaf area, and F_v/F_m T_{50} with genus (G) and native environment (NE) as fixed factors and their interaction (G × NE) in the field for just the congeneric pairs (A, B, C) and for measurements in the CE (G, H, I). Two-way ANOVAs for plasticity between the CE and the field are also presented where growth environment (GE) and genus (G) are modelled as fixed factors with their interaction (G × GE). Because field environments were different separate two-way ANOVAs for plasticity between GE are presented for desert species (J, K, L) and coastal species (M, N, O). Nested anovas for all species at the field sites (including the unpaired species) are presented for SLA (D), leaf area (E) and F_v/F_m T_{50} (F). The last row of each Table 1 lists the error degrees of freedom (d.f) and the error mean square (MS). $^{**}P < 0.001$, $^{*}0.05 > P > 0.001$. Field measurements, PICs, two-way ANOVAS – A,B,C

A. SLA				B. Leaf area				C. F_v/F_m T_{50}			
	d.f.	*F*	*P*		d.f.	*F*	*P*		d.f.	*F*	*P*
G	4	45.65	**	G	4	35.5	**	G	4	43.31	**
NE	1	129.1	**	NE	1	100.1	**	NE	1	197.1	**
G × NE	4	29.09	**	G × NE	4	30.06	**	G × NE	4	4.77	*
Error	119	MS = 333.2		Error	119	MS = 270.9		Error	55	MS = 0.59	
Nested ANOVAS, all species, field measurements – D,E,F											
D. SLA				E. Leaf area				F. F_v/F_m T_{50}			
	d.f.	*F*	*P*		d.f.	*F*	*P*		d.f.	*F*	*P*
NE	1	9.47	*	NE	1	18.74	*	NE	1	14.4	**
Sp(NE)	16	21.38	**	Sp(NE)	16	7.65	*	Sp(NE)	17	29.1	**
Error	161	MS = 310.6		Error	161	MS = 120.7		Error	76	MS = 0.37	
Common Environment measurements, PICs, two-way ANOVAS – G,H,I											
G. SLA				H. Leaf area				I. F_v/F_m T_{50}			
	d.f.	*F*	*P*		d.f.	*F*	*P*		d.f.	*F*	*P*
G	3	49.42	**	G	3	43.12	**	G	3	41.02	**
NE	1	98.64	**	NE	1	78.34	**	NE	1	0.003	NS
G × NE	3	8.67	*	G × NE	3	6.56	*	G × NE	3	1.20	NS
Error	112	MS = 448.7		Error	113	MS = 67.06		Error	32	MS = 0.50	
Plasticity between the CE and field, desert species – J,K,L											
J. SLA				K. Leaf area				L. F_v/F_m T_{50}			
	d.f.	*F*	*P*		d.f.	*F*	*P*		d.f.	*F*	*P*
G	3	16.94	**	G	3	12.36	*	G	3	34.31	**
GE	1	20.97	**	GE	1	9.05	*	GE	1	115.9	**
G × GE	3	4.60	*	G × GE	3	7.06	*	G × GE	3	12.74	**
Error	112	MS = 239.8		Error	113	MS = 56.04		Error	32	MS = 0.52	
Plasticity between the CE and field, coastal species – M,N,O											
M. SLA				N. Leaf area				O. F_v/F_m T_{50}			
	d.f.	*F*	*P*		d.f.	*F*	*P*		d.f.	*F*	*P*
G	3	86.97	**	G	3	10.05	*	G	3	43.20	**
GE	1	16.57	**	GE	1	7.68	*	GE	1	5.14	*
G × GE	3	1.54	NS	G × GE	3	5.48	*	G × GE	3	5.66	*
Error	112	MS = 438.6		Error	113	MS = 23.67		Error	32	MS = 0.33	