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Native microhabitats better predict tolerance to warming than latitudinal macro-climatic variables in arid-zone plants

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ABSTRACT

Aim Understanding species ability to withstand heat stress is paramount for predicting their response to increasing temperatures and decreasing rainfall. Arid systems are subject to climatic extremes, where plants, being immobile, live on the frontline of climate change. Our aim was to investigate whether: (1) warming tolerance [WT = the difference between a species physiological thermal damage threshold (T_{50}) and the maximum temperature within its distribution (T_{hab})] for desert plants is higher at high latitudes, as has been shown for terrestrial ectotherms, and (2) if T_{50} of desert plants better corresponds with broad climatic indicators or species native microhabitats.

Location The Australian Arid Lands Botanic Garden, Port Augusta, South Australia.

Methods Using chlorophyll fluorescence techniques, we measured T_{50} for 42 Australian arid plant species native to different microhabitats based on water availability. WT was calculated ($T_{50} - T_{hab}$) and each metric was compared against microhabitat and broad-scale climatic variables for each species.

Results T_{50} was unrelated to macro-scale climate or latitude, whereas WT increased for species whose distributions extend into higher latitudes, a pattern hitherto not shown for terrestrial plants. We also found that species adapted to higher water availability in their native microhabitat had significantly lower T_{50} and WT than species from drier microhabitats.

Main conclusions (1) Warming tolerance increased with latitude, but the strength of this relationship was related to the way WT was quantified, with T_{hab} and latitude being linked. (2) T_{50} did not correlate with latitude, but both T_{50} and WT were strongly related to their microhabitats. Specifically, water availability is important, such that even within a desert biome, species associated with 'wetter' microhabitats, may be particularly vulnerable to heat stress. Thus, we show that local-scale patterns better capture plant physiological responses to temperature than broad-scale distributions.

Keywords

arid-zone, climate change, desert plants, high temperature stress, species distributions, thermal damage threshold, warming tolerance

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INTRODUCTION

Extreme high temperature events are increasing in both frequency and magnitude world-wide (IPCC, 2014). Identifying the vulnerability of plant species to increasing temperatures is important, as local extinctions can have consequences for

other organisms relying on plants for food or habitat and for carbon cycling and productivity (Walther, 2003). Plant vulnerability to climate change is uncertain and some ecosystems are particularly under-researched; for example, desert and semi-desert (arid) systems. Collectively, these environments comprise approximately one-third of the land surface

area globally (Prentice *et al.*, 2001). Recent studies have shown that some semi-arid regions contribute far more to carbon cycling than previously thought (Cleverly *et al.*, 2013), highlighting the importance of understanding these systems. Plants in these regions must withstand biologically stressful and highly variable conditions, especially with regard to temperature extremes and drought (Noy-Meir, 1973; Wahid *et al.*, 2007). Examining the effects of heat stress on desert vegetation will provide a means of understanding the effects of a changing climate on plants living at the upper edge of what many species can tolerate.

Predictive models characterizing the bioclimatic envelope (or climate niche) of a species are often used to forecast vulnerability and potential distributions under future climate change scenarios (Pearson & Dawson, 2003; Beaumont *et al.*, 2005). While providing useful ecological insight and strong predictive potential, these models largely focus on linking coarse-scale spatial and climatic data to species distribution records (Hampe, 2004; Thuiller *et al.*, 2005; Elith & Leathwick, 2009). Importantly, such models generally do not take into account species unique physiological limits under particular environmental conditions or the scale at which species interact with their surroundings (Ashcroft *et al.*, 2014). Consequently, predictions are constrained by the exclusion of important mechanistic links between species functional traits and their native microhabitat (Kearney & Porter, 2009). Incorporating such links into future models is key to more accurate forecasting of species survival and persistence within a given location.

A popular measure for ranking species vulnerability to a warmer world is warming tolerance (WT): the difference between a measure of a species physiological thermal limit (heat stress damage threshold) and a thermal index of its habitat (Deutsch *et al.*, 2008). An increasing positive value indicates species less vulnerable to effects of climate warming, whereas values close to zero suggest species are more vulnerable (Hoffmann *et al.*, 2013). The use of WT allows multiple species to be ranked by their relative vulnerability to a warming climate. Studies on terrestrial ectotherms (including reptiles, amphibians and insects) have consistently found WT to be greater for species at higher latitudes relative to those nearer the equator (Fig. 1) (Deutsch *et al.*, 2008; Huey *et al.*, 2009; Diamond *et al.*, 2012; Hoffmann *et al.*, 2013). Species at lower latitudes are considered to be most vulnerable because they already are living at the thermal limits of what many organisms can withstand. Importantly, however, higher latitudes are expected to experience larger increases in average temperature and species at these latitudes therefore may be at an increased risk of thermal stress (IPCC, 2007; Deutsch *et al.*, 2008). Being immobile, plants, especially long-lived perennials with limited dispersal, are potentially more vulnerable than organisms that are able to migrate. Warming tolerance, however, has not yet been recorded for plants. Thus, an aim of the current study was to determine whether the well-documented latitudinal trend in WT exhibited for animals (Fig. 1) can be generalized to plants.

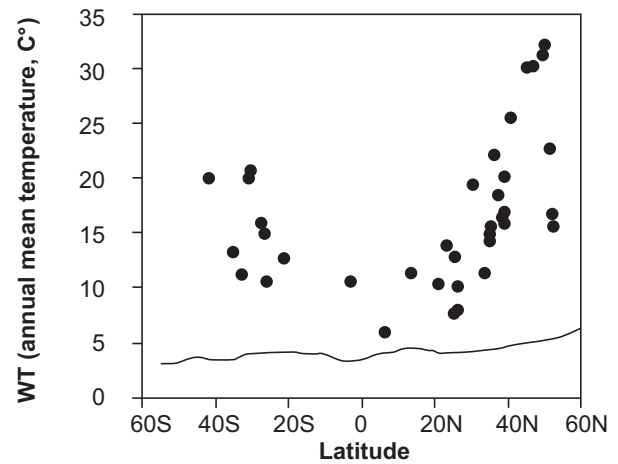


Figure 1 Estimates of the impact of warming on insects by comparing the relationship between warming tolerance (WT, based on the annual mean temperature) and latitude with the projected magnitude of warming expected by 2100 (black line) (adapted from Deutsch *et al.*, 2008, Copyright (2008) National Academy of Sciences, U.S.A.).

In contrast to these broad-scale relationships, recent studies have highlighted the importance of understanding local landscape heterogeneity in the provision of refugia for making more informed predictions of species vulnerability under climate change (Suggitt *et al.*, 2011; Ashcroft *et al.*, 2014). A multidisciplinary approach, integrating species physiology and ecology, together with their known distributions and environmental data at multiple scales, is necessary to improve predictions of community and species-level responses (Cooke *et al.*, 2013). This approach can provide valuable insight for identifying species or functional types that will be most at risk from future temperature regimes (Tsonev *et al.*, 1999; Curtis *et al.*, 2014).

With respect to assessing physiological vulnerability, the availability of water in plant microhabitats is likely to be an important factor influencing the way they experience high temperatures. Although arid biomes are dry on average, water availability can vary at local scales; for example, an ephemeral river bed adjacent to a well-drained hill slope or dune (Morton *et al.*, 2011; Free *et al.*, 2013). Under conditions of water limitation, plants often restrict transpiration to reduce water loss (Barradas *et al.*, 1994; Hamerlynck *et al.*, 2000), but the resulting drop in latent heat loss can cause leaf temperatures to rise considerably above ambient (Ball, 1988; Nobel, 2005), particularly under hot, still conditions (Leigh *et al.*, 2012). Also, multiple stresses can have confounding effects (Suzuki *et al.*, 2014), such that a drought-stressed plant may be more severely damaged by heat stress than a well-watered plant. Accordingly, even within a given bioclimatic envelope, differences in microhabitat type, based on access to water, may influence species ability to cope with heat stress. Such differences at the microhabitat scale could well be independent of any macro-scale latitudinal trends relating to species distribution.

In this study, we investigated whether – and at what scale – species-specific thermal threshold parameters might best be used to predict plant vulnerability under future climate change. Specifically we asked: (1) is WT for desert plants higher at high latitudes, as has been shown for terrestrial ectotherms? and (2) do the physiological thermal damage thresholds of desert plants correspond most strongly with macro-scale, broad climatic indicators or species native microhabitat? We investigated these questions for Australian southern arid species grown under natural conditions in a common environment, but differing with respect to the water availability characterizing the microhabitat in which they normally grow.

MATERIALS AND METHODS

Site description

All species in this study grew in a common arid environment at the Australian Arid Lands Botanic Garden (AALBG) in Port Augusta, South Australia (32°27'56.3" S, 137°44'40.7" E). Mean annual rainfall is *c.* 250 mm and mean maximum summer temperature is *c.* 31.3 °C, with maximum temperatures exceeding 45 °C in summer (AGBoM, 2013a). All measurements took place during summer 2013 (late January to early March).

Measuring species-mean values of T_{50}

We measured T_{50} for 42 Australian arid shrub, tree and one herb species, following the Flora of New South Wales (accessed online via PlantNET, The Royal Botanic Gardens and Domain Trust, 2013) and the Flora of South Australia (accessed online via eFLORA SA, DEWNR South Australia, 2013). Species were selected from 21 plant families to encompass a range of growth forms (Table 1). One of the study species, the herbaceous fern *Marsilea drummondii*, was selected in both its aquatic and terrestrial form as the two different microhabitats of this plant were of interest. Unless otherwise stated, analyses presented only include measurements from the terrestrial form of *M. drummondii*.

To determine species-mean values of T_{50} for each species, maximum quantum yield of PSII of dark-adapted tissue (F_V/F_M) was measured with chlorophyll *a* fluorometry using a pulse modulated fluorometer (Heinz Walz, EffeTrich, Germany), following the protocol of Curtis *et al.* (2014). Values of F_V/F_M are useful for assessing the health of plant photosynthetic reaction centres under numerous stressors, including temperature, by providing an indication of the capacity of PSII to accept light (Baker & Rosenqvist, 2004). Our method of quantifying T_{50} utilizes the temperature-dependent decline in F_V/F_M to determine the temperature at which it drops to 50% of pre-stress levels, a point corresponding to the onset of irreparable thermal damage. Briefly, leaves of each species were subjected to five temperature treatments (46, 48, 50, 52 and 54 °C) and one control (28 °C) using temperature

controlled water baths, accurate to ± 0.2 °C. Leaves were exposed to a subsaturating light level of *c.* 280 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ throughout the process. Applying heat stress at different treatment temperatures was performed on replicate batches of 10 leaves for each species in the following sequence. Pre-stress F_V/F_M was measured after a 30-min period of dark-adaptation. Leaves then were placed for 15 min under control conditions (28 °C, 280 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), allowing them to reach steady state under the treatment light conditions prior to stress. Each batch of replicate leaves then was transferred to one of the five temperature baths for 15 min. Following 90 min of recovery under control conditions (28 °C, 280 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), samples were dark-adapted for 30 min before F_V/F_M was again measured. Final measurements of F_V/F_M were recorded after leaves had recovered overnight in the dark. Previous studies have shown a strong positive correlation across species between T_{50} measured this way and other fluorescence methods (e.g. T_{S20}), which measure whole leaves that remain attached to the plant (Knight & Ackerly, 2003). Our method of assessing T_{50} allows for relatively rapid measurement of a large number of species and appropriate data replication, while controlling for external conditions including light levels.

Bivariate relationships among WT, T_{50} and latitude across species

In this study, WT for a given species was defined as: $WT = T_{50} - T_{\text{hab}}$, where T_{hab} is a thermal index of a species habitat (Deutsch *et al.*, 2008; Diamond *et al.*, 2012). To estimate T_{hab} , studies have used various temperature indices, including the long-term mean annual temperature and mean temperature during the warmest quarter of the year (e.g. Deutsch *et al.*, 2008; Huey *et al.*, 2009; Diamond *et al.*, 2012). We calculated several values of WT for each plant species by selecting four T_{hab} temperature variables. These included maximum values for annual mean, annual maximum mean, warmest maximum period and warmest quarter using occurrence records from across the entire Australia-wide distribution of each species. Climate data were sourced from the online data portal Atlas of Living Australia (ALA) database (<http://www.ala.org.au/>, sourced May, 2013). Species occurrence records suspected of being erroneous are recognized by the ALA and were excluded from our analyses. Retrieved data were plotted and the maximum value for each climatic variable was extracted. Temperature variables defining T_{hab} were generated using distributional data and the software package ANUCLIM 6, which is used for obtaining climate data from climate surfaces built using the ANUSPLIN package (Xu & Hutchinson, 2011).

Data for Australia-wide latitudinal distributions were also obtained from ALA records (sourced May, 2013). Minimum, maximum, mean and range in latitude were quantified for the distributional envelope of each species. Preliminary analysis indicated that WT varied predictably with latitude across species when either the most northerly latitudinal

Table 1 List of the 42 Australian desert plant species used in this study, arranged from lowest to highest thermal damage thresholds (T_{50} , °C). Growth form is given in parentheses: g, grass; h, herb; p, hemi-parasite; s, shrub; t, tree. T_{50} was calculated as the temperature at which maximum quantum yield (F_V/F_M) declines to 50% of the maximum pre-stress F_V/F_M measurement. Native microhabitats were defined as the environments that species naturally tend to occupy and that differ broadly on the availability of water: W_{low} , relatively low water availability; W_{var} , availability of water is variable; W_{high} , relatively high water availability. Warming tolerance (WT) was defined as the difference between a species physiological limit to temperature (T_{50}) and a thermal index of its habitat (T_{hab}). The measure of T_{hab} was based on maximum values across each species Australia-wide distribution using four different thermal indices: annual maximum mean temperature (amm); annual mean temperature (am); warmest maximum period (wmp); warmest quarter (wq).

Species	Family	* T_{50} (°C)	Native microhabitat	WT _{amm}	WT _{am}	WT _{wp}	WT _{wq}
<i>Marsilea drummondii</i> (aquatic form) (h)	Marsileaceae	45.1	W_{high}	10	15.6	3.9	12.4
<i>Eremophila bignoniiflora</i> (t)	Scrophulariaceae	47.9	W_{high}	12.5	19.5	6.7	15.2
<i>Pimelea microcephala</i> (s)	Thymeleaceae	48.2	W_{var}	13.4	20.2	7.2	15.5
<i>Amyema quandang</i> subsp. <i>quandang</i> (p)	Loranthaceae	48.3	W_{high}	15.5	21.6	8.2	16.9
<i>Marsilea drummondii</i> (terrestrial form) (h)	Marsileaceae	48.7	W_{high}	13.6	19.2	7.5	16
<i>Amyema miraculosa</i> subsp. <i>miraculosa</i> (p)	Loranthaceae	48.9	W_{high}	22.1	28.2	11.8	21.5
<i>Hakea francisiana</i> (s)	Proteaceae	48.9	W_{var}	19	25.8	9.4	18.3
<i>Bauhinia gilva</i> (s-t)	Fabaceae – Caesalpinioideae	49.4	W_{high}	15	26.8	8.4	16.6
<i>Acacia papyrocarpa</i> (t)	Fabaceae – Mimosoideae	49.6	W_{var}	19.9	26.5	9.7	18.6
<i>Gyrostemon ramulosus</i> (t)	Gyrostemonaceae	49.6	W_{low}	16.2	23.1	8.4	17.1
<i>Dodonaea viscosa</i> subsp. <i>angustissima</i> (s)	Sapindaceae	49.8	W_{low}	15	21.8	8.9	17.1
<i>Eucalyptus camaldulensis</i> subsp. <i>camaldulensis</i> (t)	Myrtaceae	50.0	W_{high}	20.5	26.6	10.9	18.9
<i>Atriplex nummularia</i> (s)	Chenopodiaceae	50.2	W_{high}	18.6	25.5	10.2	18.7
<i>Senna pleurocarpa</i> var. <i>pleurocarpa</i> (s)	Fabaceae – Caesalpinioideae	50.5	W_{low}	17.3	24.3	9.6	18.2
<i>Exocarpos aphyllus</i> (s)	Santalaceae	50.6	W_{low}	19.5	24.2	10.6	19.2
<i>Eremophila longifolia</i> (t)	Scrophulariaceae	50.6	W_{var}	15.3	22.6	9	17.8
<i>Cassinia laevis</i> (s)	Asteraceae	50.7	W_{low}	21.6	27.8	12.8	21.1
<i>Eucalyptus pimpiniana</i> (t)	Myrtaceae	50.7	W_{low}	23.6	30.7	14.4	24.1
<i>Geijera parviflora</i> (t)	Rutaceae	50.7	W_{low}	19.8	26.7	12	20
<i>Nitraria billardiieri</i> (s)	Nitrariaceae	50.8	W_{var}	20.6	27.3	10.3	19.5
<i>Cymbopogon obtectus</i> (g)	Poaceae	50.9	W_{high}	16.7	23	9.5	18.3
<i>Acacia ligulata</i> (t)	Fabaceae – Mimosoideae	51.1	W_{low}	16.3	23.1	9.9	18.5
<i>Melaleuca uncinata</i> (s)	Myrtaceae	51.3	W_{high}	18.9	25.2	11.4	19.1
<i>Casuarina pauper</i> (t)	Casuarinaceae	51.6	W_{low}	22.1	28.7	11.8	20.7
<i>Solanum orbiculatum</i> subsp. <i>orbiculatum</i> (s)	Solanaceae	51.6	W_{low}	21.1	27.6	11.1	20.3
<i>Santalum acuminatum</i> (t)	Santalaceae	51.7	W_{var}	18.5	25.4	10.9	19.2
<i>Commersonia magniflora</i> (s)	Malvaceae	51.8	W_{var}	22	29	13.7	22
<i>Callistemon teretifolius</i> (s)	Myrtaceae	51.8	W_{low}	25.3	31.2	15.4	24.4
<i>Grevillea stenobotrya</i> (s)	Proteaceae	51.8	W_{var}	17.3	24	10.3	19.2
<i>Brachychiton gregorii</i> (t)	Malvaceae	52.0	W_{var}	21.1	27.9	11.9	20.5
<i>Bossiaea walkeri</i> (s)	Fabaceae – Faboideae	52.1	W_{low}	24.5	31.1	14	23.2
<i>Sida ammophila</i> (h-s)	Malvaceae	52.2	W_{high}	17.97	24.79	11.59	19.69
<i>Lasiopetalum behrii</i> (s)	Malvaceae	52.2	W_{low}	27.9	34.3	18.4	26.7
<i>Acacia aneura</i> (t)	Fabaceae – Mimosoideae	52.3	W_{low}	25.5	31.6	15.2	24.9
<i>Callitris glaucophylla</i> (t)	Cupressaceae	52.4	W_{low}	20.8	25.5	12.7	20.9
<i>Atriplex vesicaria</i> (s)	Chenopodiaceae	52.5	W_{var}	19.5	25.5	11.7	20.1
<i>Maireana pyramidata</i> (s)	Chenopodiaceae	52.6	W_{var}	20.1	27.3	11.1	20.1
<i>Cratystylis conocephala</i> (s)	Asteraceae	52.7	W_{low}	25.5	32.5	16.4	25.9
<i>Jasminum didymum</i> (s)	Oleaceae	52.8	W_{var}	17.5	23.2	11.3	20.1
<i>Santalum lanceolatum</i> (t)	Santalaceae	53.1	W_{var}	17.7	24.3	11.4	20.3
<i>Maireana sedifolia</i> (s)	Chenopodiaceae	53.2	W_{var}	24.5	30.9	14	23.4
<i>Xanthorrhoea thorntonii</i> (g)	Xanthorrhoeaceae	53.5	W_{low}	24.1	30.7	15	23.7
<i>Triodia irritans</i> (g)	Poaceae	54.3	W_{low}	23.4	30.5	15.1	23.3

*Thermal threshold measurements for all species (with the exception of *Marsilea drummondii*, aquatic form) are taken from Curtis *et al.* (2014).

distributions or latitudinal range (the most northerly minus the most southerly distribution) was used. We chose to examine the WT–latitude relationship based on most northerly latitudinal distributions as this depicted the relationship most strongly.

We note here that T_{50} values are not fixed for a given species and can depend upon factors such as the length of the applied heat treatment and plant health. Values of WT, incorporating metrics such as T_{50} , are therefore a coarse measurement, which does not represent a species absolute

critical thermal limit with regard to climate warming. That is, WT is not an indication of the absolute amount of climate warming that we could expect species to tolerate before substantial declines are observed. Rather, WT should be thought of as a relative way to rank species potential vulnerability across a large spatial scale.

Relating T_{50} and WT to macro-scale climate and native microhabitat

We wanted to determine if macro-scale climatic indicators or species native microhabitats most strongly corresponded to their resistance to heat stress (T_{50} and WT). To address this question, information on the climatic variables of species Australia-wide distribution and their affinity for a local microhabitat were collated. For broad climate variables, the temperature indices outlined above were used, as well as water availability (mean annual rainfall, mm), solar radiation (annual mean; MJ m⁻² day) and site aridity (mean annual aridity index) (<http://www.ala.org.au/>, sourced May, 2013). Microhabitat determination was based on the availability of water to each species within its native environment. Because transpirational cooling is often reduced under conditions of drought stress, from a plants perspective, apparent temperature is thus likely to be greater in areas with less water. Using this criterion, water availability served as a proxy indicator of potential temperature stress plants may experience in their native microhabitats. To identify each species affinity for a microhabitat, a range of literature was reviewed (Jessop *et al.*, 1986; Cunningham *et al.*, 1992; Department of Parks and Wildlife: Western Australian Herbarium, 2013; DEWNR South Australia, 2013; The Royal Botanic Gardens and Domain Trust, 2013). Species were found to fall into three microhabitats with respect to water access: W_{high} , W_{low} and W_{var} (Table 1). W_{high} ($n = 10$) incorporated microhabitats where water is relatively available and included hemi-parasitic species that have ready access to their hosts' xylem (Ehleringer *et al.*, 1985; Goldstein *et al.*, 1989), species restricted to the banks of seasonally flooded rivers, road-side depressions or wadis, and ephemeral species that tend to respond rapidly to wet weather events. Species naturally found where water is less often available and/or where water drains away readily were classified as W_{low} ($n = 18$). Microhabitats fitting this description included sand dunes and exposed rocky hill slopes. The remaining species were categorized as W_{var} ($n = 14$) and can be found in areas fitting the water availability of both W_{high} and W_{low} species. It is important to note that these native microhabitat details were irrespective of the conditions in which the sample plants used for measurements were grown and therefore represent the innate preference for a given set of microhabitat conditions.

Statistical analyses

Pearson's product-moment correlation coefficient analyses were used to quantify bivariate relationships among the

variables WT, T_{50} and latitude across species. Pearson's correlations were also used to quantify relationships between broad-scale climate variables and T_{50} . One-way ANOVA was used to determine differences in T_{50} and WT among the three native microhabitats (as a fixed factor). Data were tested for assumptions of normality and heterogeneity of variances using Kolmogorov–Smirnov and Levene's tests respectively. Data were analysed using the statistical software IBM SPSS[®] (v19).

RESULTS

Native microhabitats and T_{50}

Species-mean values of T_{50} ranged from 45.1 °C (*M. drummondii*, aquatic form) to 54.3 °C (*Triodia irritans*) and varied significantly with respect to native microhabitat (Table 1, Fig. 2a). We found that W_{high} species had significantly lower T_{50} values on average than either W_{low} or W_{var} species [$F_{(2,38)} = 7.643$, $P = 0.002$]. Thus, we show that our study species can be ranked in terms of their vulnerability to future climate change with respect to their native microhabitats. Interestingly, T_{50} did not covary significantly with any of the climate variables both across and within microhabitats, although for W_{high} species, there was a strong, if non-significant, increase in T_{50} with precipitation (see Appendix S1 in Supporting Information, Table S1).

Warming tolerance, T_{50} and latitude

We found that T_{50} increased significantly with increasing WT (Table 2). This is not surprising, given that T_{50} is used to calculate WT and a relationship should be expected. Nevertheless, it is interesting that the strength of this relationship was dependent on the specific climate variable used to calculate T_{hab} for each species, with the strongest relationship occurring when the warmest maximum period was used (Table 2). We found that T_{50} and latitude were not strongly correlated with one another (Table 2, Fig. 3a), with species-mean values of T_{50} stable across latitude and little variation apparent. In contrast, T_{hab} increased from higher to lower latitudes (Fig. 3a). When WT was calculated by subtracting T_{hab} from T_{50} (the largest and smallest differences indicated by arrows, Fig. 3a), it emerged that the WT–latitude relationship was primarily generated by the inherent relationship between T_{hab} and latitude.

As has been found in previous studies for animals (Fig. 1), we found that WT increased with distance from the equator, regardless of the measurement of T_{hab} used. That is, WT was greatest among species with distributions that extend to higher latitudes (more negative values: southward bearing – Fig. 3b; Table 2). Of the four measurements of WT used in this study, its relationship with latitude was the strongest when T_{hab} was based on the highest annual mean temperature for a given species distribution (WT_{am} , Table 2, Fig. 3b). There was considerable overlap among microhabitat

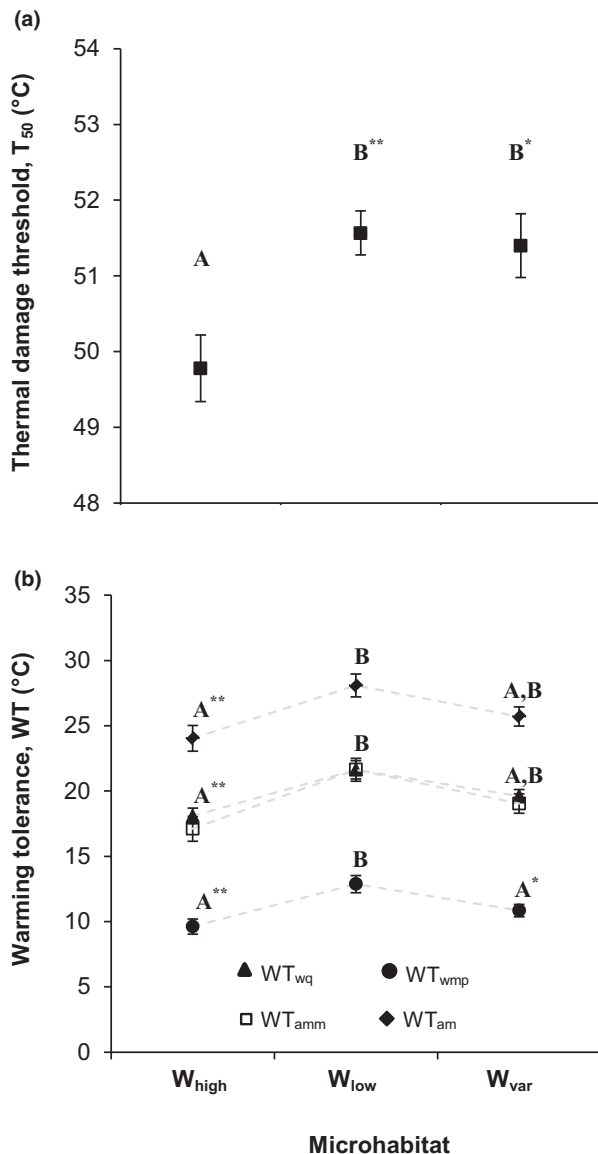


Figure 2 Species variation as a function of microhabitat type: W_{high}, high water availability; W_{low}, low water availability; W_{var}, variable water availability. (a) T_{50} , mean summer thermal damage threshold, (b) WT, mean warming tolerance. Filled diamonds, WT highest annual mean temperature; filled triangles, WT highest warmest quarter; open squares, WT highest mean annual maximum temperature; filled circles, WT highest warmest maximum period. Dashed lines are for ease of reading patterns. Points with letters different from one another are significantly different pairwise comparisons (* $P < 0.05$, ** $P < 0.01$). (Note that the letters above the middle points apply to both sets of data points that overlap: solid triangles and open squares.)

groupings along the WT-latitude spectrum (Fig. 3b). In spite of this overlap, average WT was significantly different among native microhabitat groups: W_{low} species had a significantly higher mean WT than W_{high} species, with W_{var} species WT being intermediate between these two (Fig. 2b).

Table 2 Pearson's correlations ($n = 42$) between warming tolerance (WT) and (1) species thermal damage thresholds (T_{50}) and (2) their mean maximum latitudinal Australia-wide distributions. WT is calculated as the difference between the maximum recorded values of any relevant long-term mean climatic temperature variable across a given species distribution minus its thermal damage threshold (see Table 1 legend). Here, WT was calculated using four different thermal indices: annual maximum mean temperature (amm); annual mean temperature (am); warmest maximum period (wmp); warmest quarter (wq). The strongest relationship for each bivariate combination is shown in bold (** $P < 0.001$).

	WT _{amm}	WT _{am}	WT _{wmp}	WT _{wq}	T_{50}
T_{50}	0.622***	0.595***	0.735***	0.710***	–
Max. latitude	–0.745***	–0.794***	–0.621***	–0.634***	–0.247

Depending on the measure of T_{hab} used to calculate WT, the range of WT within each microhabitat grouping was as much as 15.2 °C (Fig. 2b). Regardless of this variation, the general pattern among microhabitats of lower WT in W_{high} species compared with W_{low} and W_{var} species remained unchanged [Fig. 2b, WT highest annual mean temperature: $F_{(2,38)} = 4.999$, $P = 0.012$; WT highest warmest quarter: $F_{(2,38)} = 7.379$, $P = 0.002$; WT highest mean annual maximum temperature $F_{(2,38)} = 6.261$, $P = 0.004$; WT highest warmest maximum period $F_{(2,38)} = 7.501$, $P = 0.002$]. The order that species were positioned in the relationship did vary among measures of WT; however, only marginally so (see Appendix S1, Tables S2–S5).

DISCUSSION

Species distributions and T_{50}

The average summer T_{50} values of these Australian desert plant species can vary by > 5 °C, with maximum thresholds exceeding 54 °C for some species (Curtis *et al.*, 2014). In the current study, we set out to understand if this variation corresponded more closely with macro-scale, broad climatic indicators or to differences in the microhabitats where species are known to occur naturally. Broad-scale climatic variables change predictably with latitude and often this pattern is used to help explain species distributions (Walther, 2003; Thomas, 2010). One might therefore infer that species physiological thresholds for heat stress would vary with climatic variables or latitude. We found no clear relationship between species upper physiological thermal damage thresholds (T_{50}) and macro-scale climate variables or latitude, both across and within microhabitat groups. These results indicate that the climatic conditions defining a species geographical range are not the best predictors of its physiological heat stress threshold. Instead, we found that species native microhabitat clearly influenced T_{50} . In particular, species adapted to conditions of higher water availability had significantly lower thermal damage thresholds than the other species examined.

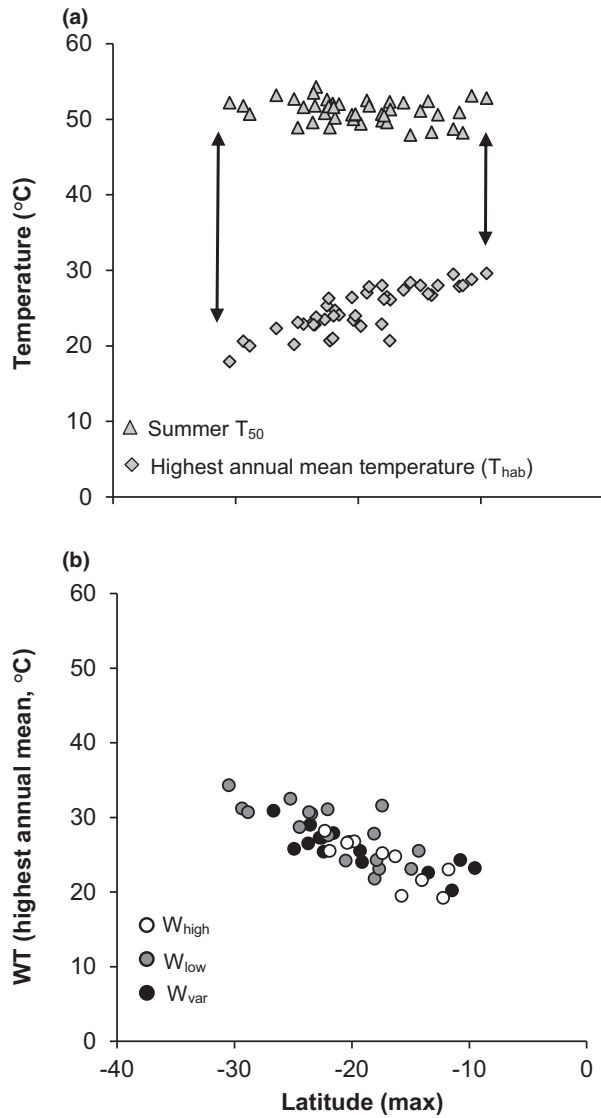


Figure 3 Pearson’s correlation (r) relationship between latitude and: (a) species thermal damage threshold (T_{50}) and the highest annual mean temperature across their Australia-wide distributions (T_{hab}); (b) warming tolerance (WT, based on the highest annual mean). Latitude was defined as the most northerly distribution in Australia for each of the 42 species investigated (see Table 2). More negative latitudinal values indicate that species distributions extend further south. Arrows on panel a are referred to in text in the Results. For panel b, microhabitat preference (see Fig. 1) is indicated for each species: W_{high} (open circles), W_{low} (black circles), W_{var} (grey circles).

As all plants were grown under common climatic conditions, these findings suggest a genotypic effect on plant tolerance to high temperatures.

Maintaining a higher level of protection against thermal damage implies a metabolic cost for plants, as they need firstly to protect against structural and functional disruption of cell membranes and proteins, and secondly to repair damage (Pierce *et al.*, 2005). Such processes may be particularly costly for desert plants, which typically live in resource-poor

environments. If a situation arose in which the ongoing likelihood of heat stress was reduced, unnecessarily sustaining a high thermal damage threshold would not be economical. The lower thermal damage thresholds we observed for W_{high} species therefore might be explained in terms of resource conservation. For example, if the cooling benefits of transpiration afforded by relatively better access to water enabled a lower leaf temperature, then costly high thermal thresholds would be less necessary. The three lowest T_{50} values of all species measured were for *M. drummondii* (W_{high}), *Eremophila bignoniiflora* (W_{high}) and *Pimelea microcephala* ssp. *microcephala* (W_{var}). Although *P. microcephala* is a W_{var} species, resource trade-offs may yet explain its low T_{50} . Under stressed conditions *P. microcephala* plants are known to drop their leaves (B. Haase, pers. comm., 2013; E.M. Curtis, pers. obs., 2013). The leaves of this species are especially thin, with low leaf mass per area (A. Leigh & E.M. Curtis, unpublished data), and therefore may represent a lower production cost than would be required to maintain a long-lived leaf with a higher thermal damage threshold. In contrast, the highest T_{50} values of all species measured were found in *Santalum lanceolatum* and *Maireana sedifolia*, both W_{var} species capable of occupying very dry sites, and *Xanthorrhoea thorntonii* and *T. irritans*, both extremely xerophytic, W_{low} species. With relatively lower access to water, these species may invest resources in higher physiological tolerance and trade-off this cost by having slower growth rates or longer lived leaves (e.g. *Xanthorrhoea* spp. are known to have slow leaf growth rate in warmer temperatures, Lamont *et al.*, 2004).

In addition to some unique adaptive abilities suggested above, species also may have potential to acclimatize via short-term physiological adjustments to changing environmental conditions (Downton *et al.*, 1984). In the present study, evidence was seen for spatial acclimatization as a response to changed water availability in the two *M. drummondii* forms (i.e. aquatic and terrestrial forms). Although the two forms were the same species, and growing within close proximity to one another, T_{50} was > 3 °C lower for the individuals growing in a permanent pool of water compared with those situated on drier sites (Table 1). As both climate and water availability are dynamic through time, the effect of temporal variation with respect to acclimatization must be accounted for to obtain a more holistic understanding of species physiological responses to heat stress, something we investigate in an upcoming paper.

WT: species distribution and T_{50}

Unlike T_{50} , which is a plant-specific, raw measure of a species physiological heat stress threshold, WT integrates this measure with the realized temperature conditions to estimate vulnerability of that species to increased climate warming. We were interested in whether plants mirrored the clear latitudinal trend demonstrated for numerous animal taxa, where species with distributions extending into lower latitudes consistently show lower WT than species at higher latitudes

(Fig. 1). Our results confirmed this pattern in desert plants, a pattern that has not, to our knowledge, previously been shown for terrestrial vegetation. Nevertheless, T_{50} and WT differed in their relationships with broad-scale indicators, causing us to question how the WT–latitude relationship should be interpreted.

To help understand and interpret these WT findings, it is necessary to unpack the method used for obtaining them. Warming tolerance comprises two metrics, the damage threshold, T_{50} , and a measure of a species thermal environment, T_{hab} . Of these two metrics, T_{50} held no relationship with latitude, whereas T_{hab} did (Fig. 3a), something also seen for animal taxa (Addo-Bediako *et al.*, 2000; Sunday *et al.*, 2011; Diamond *et al.*, 2012). Generally speaking, habitats at higher latitudes experience lower temperatures than those closer to the equator (Jones *et al.*, 1999; Sunday *et al.*, 2011). It is this inherent relationship between latitude and temperature that drives the observed pattern between WT and latitude: high latitude species have a larger difference between T_{hab} and T_{50} than low latitude species (see arrows, Fig. 3a).

Because of its dependence on T_{50} , WT also differed with microhabitat, being highest for species adapted to low water access, and this variation was irrespective of latitude (Fig. 3b). That WT varied at this micro-scale has implications for interpreting broad-scale ecological conclusions about the relative vulnerability of species to climate change based on calculations of WT (e.g. Deutsch *et al.*, 2008). From the present study, we might conclude that W_{high} species at lower latitudes are comparatively more vulnerable, particularly if, as suggested, climate change brings localized reductions in rainfall (Hennessy *et al.*, 2007). Such changes may conceivably reduce the availability of specialized habitats, on which W_{high} species rely. On the other hand, higher latitudes are expected to experience larger increases in temperature (Diamond *et al.*, 2012), potentially offsetting the presumed benefits of a higher WT towards the poles.

CONCLUDING SUMMARY

The search for widespread, repeatable patterns to explain species distribution and performance with predicted increases in temperature has become a frontrunner of ecological research. Our findings for desert plants agree with those for various animal taxa that WT is greater at higher latitudes. Far from providing a clear-cut picture of species future vulnerability, however, we suggest interpreting such broad patterns with caution. Rather than macro-climatic measures of temperature, it is with the thermal characteristics of microhabitats that most plants interact at a physiological level. In support of other authors (Biederman & Whisenant, 2011; Ashcroft & Gollan, 2013), our results demonstrate the importance of small-scale differences in the landscape for explaining species ability to cope with high temperature. Therefore, we place much greater importance on our other key finding: that physiological thermal thresholds, indeed

WT, were highest for plant species adapted to microhabitats with lower access to water.

Desert plants, already living in extreme environments, may be especially vulnerable to changes in climate, particularly where increases in temperature are coupled with more variable rainfall (IPCC, 2014). Under such a scenario, species more reliant on the availability of ‘wetter’ microhabitats may therefore be particularly vulnerable to heat stress under conditions of reduced water availability (Suzuki *et al.*, 2014). Ignoring fine-scale thermal attributes (or features that influence heat stress) of a species microhabitat may therefore lead to their persistence with global warming either being under or overestimated (Pincebourde & Casas, 2014). Predictive distribution models would be improved by incorporating local-scale variation in water availability, including soil types and local topography, which influence water holding capacity and run-off respectively. Such an approach would refine predictive outcomes for individual species with respect to not only survival from heat stress, but also to growth, productivity, reproduction and recruitment.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Accessory results including Pearson's correlations among climatic variables, and tabulation of species WT rank.

BIOSKETCHES

This research involved collaboration across different areas of expertise in plant, animal and conservation ecology. The current focus of **E. Curtis** and **A. Leigh** is on understanding how plants withstand stressful conditions, particularly high temperature stress, with an emphasis on arid regions (link: <http://www.uts.edu.au/about/faculty-science/what-we-do/our-research-areas/arid-zone-plant-ecology-and-ecophysiology-group>). The expertise of all authors spans: arid-zone ecology, biological invasions, ecoclimatology, leaf-level to whole plants and cross-species comparisons, plant-fire interactions and vertebrate macroecology.

Author contributions: A.L. and E.C. designed the thermal tolerance work; B.M. contributed to analyses and interpretations; J.G. contributed the ideas on warming tolerance; E.C. collected and analysed the data and led the writing, with all authors contributing to the text.

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