

# Weak coordination between leaf structure and function among closely related tomato species

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## Summary

- Theory predicts that natural selection should favor coordination between leaf physiology, biochemistry and anatomical structure along a functional trait spectrum from fast, resource-acquisitive syndromes to slow, resource-conservative syndromes. However, the coordination hypothesis has rarely been tested at a phylogenetic scale most relevant for understanding rapid adaptation in the recent past or for the prediction of evolutionary trajectories in response to climate change.
- We used a common garden to examine genetically based coordination between leaf traits across 19 wild and cultivated tomato taxa.
- We found weak integration between leaf structure (e.g. leaf mass per area) and physiological function (photosynthetic rate, biochemical capacity and CO<sub>2</sub> diffusion), even though all were arrayed in the predicted direction along a 'fast-slow' spectrum.
- This suggests considerable scope for unique trait combinations to evolve in response to new environments or in crop breeding. In particular, we found that partially independent variation in stomatal and mesophyll conductance may allow a plant to improve water-use efficiency without necessarily sacrificing maximum photosynthetic rates. Our study does not imply that functional trait spectra, such as the leaf economics spectrum, are unimportant, but that many important axes of variation within a taxonomic group may be unique and not generalizable to other taxa.

## Introduction

The ecology of organisms critically depends on their ability to obtain energy for growth and reproduction. In C<sub>3</sub> plants, both passive diffusion of CO<sub>2</sub> from the atmosphere to chloroplasts, via stomata and the leaf mesophyll, and biochemical capacity limit photosynthetic rates (Farquhar & Sharkey, 1982; Parkhurst, 1994; Evans *et al.*, 2009; Flexas *et al.*, 2015). Optimization theory predicts that these functions should be tightly coordinated: leaf anatomies that allow rapid diffusion of CO<sub>2</sub> should also have the biochemical capacity to take advantage of it, and vice versa (Givnish, 1986; Wright *et al.*, 2004; Reich, 2014). Natural selection on trait coordination, also known as phenotypic integration (Pigliucci, 2003), should thus generate major functional trait spectra associated with different ecological strategies (Grime, 1977; Westoby *et al.*, 2002; Chave *et al.*, 2009; Reich, 2014). Indeed, intrinsic photosynthetic capacity varies widely between species, indicating that leaf-level CO<sub>2</sub> diffusion and biochemistry are major levers through which natural selection and crop breeders can alter plant performance and fitness in different environments. For example, selection might favor leaf traits that inhibit rapid CO<sub>2</sub> diffusion (e.g. lower stomatal density or thicker

leaves) if doing so has an adaptive benefit, bringing about a trade-off (e.g. sclerophyll leaves of species from water-limited environments; Medrano *et al.*, 2009).

Trait coordination is evident when several tightly correlated traits are well described by a single axis, such as a principal component, that uses multiple traits to position species along a trait spectrum. The most widely studied coordinated trait spectrum is the 'leaf economics spectrum' (LES) (Wright *et al.*, 2004), which runs from resource acquisitive (tender, short-lived leaves with high mass-based photosynthetic rates) to resource conservative (robust, long-lived leaves with low photosynthetic rates). Although several leaf structural and chemical traits comprise the LES, most species fall along a single, dominant axis of trait variation when viewed at broad phylogenetic scales. A central component of the LES is that structurally robust leaves with greater leaf mass per area (*LMA*) do not have concomitantly greater photosynthetic rates. This is not simply because greater allocation to leaf structure 'dilutes' photosynthetically active tissue (*sensu* Edwards *et al.*, 2014), but rather that more robust structure makes achieving higher photosynthetic rates physically challenging because 'leaves with high mass per area hav[e] longer diffusion paths from stomata to chloroplasts or greater internal

shading of lower chloroplasts' (Wright *et al.*, 2004). Even if robust leaf structure were accompanied by greater allocation of nitrogen to photochemistry, the physical properties of these leaves would hinder carbon assimilation (Evans, 1989). Hence, a major, but poorly tested, premise of the LES is that *LMA* sets a functional constraint on the evolution of photosynthetic capacity that shapes the dominant axis of trait variation.

Leaf mass per unit area may limit mesophyll  $\text{CO}_2$  diffusion under saturating irradiance, when internal shading is negligible. Hence, *LMA* may functionally constrain (*sensu* Arnold, 1992) phenotypic evolution between leaf structure and function as a result of fundamental physics and chemistry. Although it is often assumed that greater *LMA* decreases mesophyll  $\text{CO}_2$  diffusion, this is not necessarily the case because *LMA* is a highly integrated trait; different anatomical changes lead to different relationships (Niinemets *et al.*, 2009a and references therein). For example, increased mesophyll cell surface area would increase both *LMA* and mesophyll  $\text{CO}_2$  diffusion, whereas increased mesophyll cell wall thickness would increase *LMA* but decrease mesophyll  $\text{CO}_2$  diffusion. Hence, it is an empirical question whether the evolution of leaf structure imposes strong constraints on leaf function. Although LES traits are often analyzed on a mass basis (e.g.  $\text{CO}_2$  assimilation per gram of leaf), for biological and statistical reasons, we analyzed the effect of leaf structure on area-based photosynthetic traits following the recommendations of Lloyd *et al.* (2013) and Osnas *et al.* (2013). Although mass-based traits are important for whole-plant resource economy (Poorter *et al.*, 2014), the analysis of area-based photosynthetic traits is more appropriate for addressing whether the evolution of a thicker or denser leaf necessarily impedes  $\text{CO}_2$  diffusion and carbon assimilation.

Although the LES was originally formulated at broad phylogenetic scales, coordination between leaf structure and photosynthetic function is also predicted to be a fundamental constraint within species (Martin *et al.*, 2007; Vasseur *et al.*, 2012; Aspinwall *et al.*, 2013; Blonder *et al.*, 2013, 2015; Grady *et al.*, 2013; Brouillette *et al.*, 2014; Hu *et al.*, 2015; Niinemets, 2015) and genera (Givnish *et al.*, 2004; Dunbar-Co *et al.*, 2009; Santiago & Kim, 2009; Edwards *et al.*, 2014; Mason & Donovan, 2015). If there is a hard trade-off between *LMA* and mesophyll  $\text{CO}_2$  diffusion and strong selection for coordination between  $\text{CO}_2$  diffusion and biochemical capacity, the axes of trait variation within and between species will be largely concordant. Conversely, if leaf structure merely sets an upper bound on  $\text{CO}_2$  diffusion and selection on coordination between diffusion and biochemistry is weak, there is ample scope for different groups of organisms to find unique adaptive solutions, leading to multiple trait spectra (Grubb, 2015). Indeed, the LES is not a one-size-fits-all trait axis, but rather a set of axes that vary between communities (Funk & Cornwell, 2013; Messier *et al.*, 2016) and taxa (Edwards *et al.*, 2014; Mason & Donovan, 2015; Niinemets, 2015). Such variation in the direction and strength of leaf trait variation does not devalue the broad-scale pattern, but suggests that bulk leaf structure (i.e. *LMA*) sets a soft ceiling, with lots of room to maneuver below, rather than a hard constraint on photosynthetic function. A soft ceiling is plausible if species with similar *LMA* achieve it

with different underlying anatomies (e.g. leaf thickness vs density) that have different effects on  $\text{CO}_2$  diffusion. This idea has not been well studied, particularly among closely related species.

Data on genetically based variation among closely related species, rather than broad comparisons across disparate families and plant functional types, are most appropriate for certain ecological questions (Donovan *et al.*, 2014). For example, the evolutionary routes (not) taken in the recent past among closely related species may help to predict how species will respond to natural and anthropogenic climate change (Kellermann *et al.*, 2012; Donovan *et al.*, 2014). Crop breeders can take advantage of existing trait variation in crop-wild relatives to develop new varieties for sustainable agriculture (Moyle & Muir, 2010; Denison, 2012; Giuliani *et al.*, 2013). Hence, if coordination among leaf traits is limited among closely related species, broad-scale or global trait spectra may be of little use in addressing fundamental challenges, such as the ecology of local adaptation (Kawecki & Ebert, 2004), near-term responses to climate change and crop breeding. Conversely, weak coordination may indicate significant opportunities for natural and artificial selection to fine tune leaf traits in response to different ecological circumstances. For example, independent modification of  $\text{CO}_2$  diffusion components and biochemical properties would allow for a wider range of water-use efficiency, nitrogen-use efficiency and assimilation rate combinations (Buckley & Warren, 2014; Flexas *et al.*, 2015).

Common garden experiments of closely related species, such as the wild tomato clade, can measure how strongly diffusive and biochemical limitations on photosynthesis, water-use efficiency, and leaf structure (e.g. *LMA*, thickness, etc.), are coordinated over short evolutionary timescales. Tomato species (*Solanum* sect. *Lycopersicon*) and their relatives (*Solanum* sect. *Juglandifolia* and *Lycopersicoides*) are closely related herbaceous perennials: fossil-calibrated molecular clocks put section *Lycopersicon* at c. 2.48 million yr (Ma) old, and the split between *Lycopersicon* and *Lycopersicoides* at c. 4.7 Ma ago (Särkinen *et al.*, 2013). Despite sharing a recent common ancestor, tomato species are ecologically and phenotypically diverse (Moyle, 2008; Peralta *et al.*, 2008; Nakazato *et al.*, 2008, 2012; Chitwood *et al.*, 2012; Haak *et al.*, 2014; Muir & Thomas-Huebner, 2015; Pease *et al.*, 2016). Adaptation to different climates, especially along temperature and precipitation gradients, may have played an important role in diversification (Nakazato *et al.*, 2010). Most wild tomato species are interfertile with each other (Baek *et al.*, 2015) and with domesticated tomato (*S. lycopersicum* var. *esculentum*), providing a valuable source of germplasm for crop improvement. In particular, alteration of leaf  $\text{CO}_2$  diffusion properties may enhance photosynthetic rate and/or water-use efficiency (Tholen *et al.*, 2012; Flexas *et al.*, 2013, 2015; Buckley & Warren, 2014; Gago *et al.*, 2014). Diffusion limits photosynthesis in some wild tomato species (Muir *et al.*, 2014) and cultivars (Galmés *et al.*, 2011, 2013), but we know little about the ecological and evolutionary significance of this variation.

More generally, we know relatively more about the mechanistic basis of variation in leaf photosynthesis than its ecological or adaptive significance. Stomatal and mesophyll  $\text{CO}_2$  diffusion are

strongly affected by leaf anatomy (Brown & Escombe, 1900; Franks & Farquhar, 2001; Nobel, 2009; Tholen & Zhu, 2011; Tomás *et al.*, 2013); biochemical limitations depend on the amount of Rubisco, its activation and catalytic rates (Farquhar *et al.*, 1980; von Caemmerer, 2000; Galmés *et al.*, 2014). Area-based maximum photosynthetic rates are generally higher among certain plant functional types (herbs,  $C_3$  graminoids and crop plants) than others (trees, sclerophylls and succulents) because of increased  $CO_2$  diffusion rates and biochemical capacity (Körner *et al.*, 1979; Wullschlegel, 1993; Flexas *et al.*, 2008). Similar patterns hold for mass-based photosynthesis (Reich *et al.*, 1997). These broad-scale correlations suggest that slow-growing plants trade off high photosynthetic rate in favor of leaf traits that may have other fitness benefits, such as durability, defense against herbivores or increased resource-use efficiency (Turner, 1994; Aerts, 1995; Onoda *et al.*, 2011).

The prevailing set of hypotheses tested here can be summarized as follows: there is much variation in leaf form and function, but this variation can be largely reduced to a single spectrum ranging from resource acquisitive to resource conservative, centered around a strong trade-off between robust leaf structure and  $CO_2$  diffusion. Selection favors the maximization of  $CO_2$  diffusion to enhance photosynthesis of a given leaf area and robust leaf structure to protect against damage or stress, but functional constraints prevent the evolution of leaves that do both, so there is a trade-off. Climate, in part, determines the position along this leaf trait spectrum. In particular, arid environments favor robust leaves on the resource-conservative end of the spectrum. Finally, trade-offs between leaf structure and function are widespread and similar, even among distantly related species, leading to convergent evolution of similar trait spectra. We do not test every prediction of these hypotheses, but address them by answering the following questions. (1) How much variation is there in diffusional and biochemical limitations on photosynthesis and water-use efficiency among diverse and agronomically important tomato species? (2) Are leaf structure and physiological function strongly coordinated along a resource-conservative to resource-acquisitive axis? (3) Are 'resource-conservative' trait syndromes associated with arid environments? (4) Are trade-offs between leaf structure and  $CO_2$  diffusion in tomatoes consistent with global patterns?

## Materials and Methods

### Plant materials and cultivation

We obtained seeds of 19 taxa: 16 wild species (Supporting Information Table S1) from the Tomato Genetics Resource Center at the University of California, Davis, CA, USA (TGRC; <http://tgrc.ucdavis.edu>) and three cultivated accessions of *S. lycopersicum* var. *esculentum*, cv 'Roma VF' (Batlle SA) and two Mediterranean 'Tomàtiga de Ramellet' accessions from the University of the Balearic Islands seedbank collection (UIB1-30 and UIB1-48). To even out plant size during measurements, and following TGRC indications, slower growing species were germinated 2 wk ahead of faster growing species. Seeds were soaked in

2.5% sodium hypochlorite (household bleach) for 30 or 60 min (following TGRC instructions for each species), rinsed thoroughly and placed on moist paper to germinate. After 1 wk, seedlings were transplanted to cell-pack flats. Two weeks later, five plants of each taxa were transplanted to 19-l pots in which the experiment was performed. Pots contained a 4 : 1 (v/v) mixture of peat-based horticultural substrate (Prohumin-Potting Soil Klasmann-Deilmann, Projar SA, Valencia, Spain) and perlite (granulometry A13, Projar SA). Plants were grown outdoors in an open experimental field under typical Mediterranean conditions at the University of the Balearic Islands (39°38'14.9"N, 2°38'51.5"E) during the spring–summer season. Plants were irrigated to field capacity daily to prevent drought stress and fertilized weekly with an NPK solution (50% Hoagland's solution).

### Diffusional and biochemical constraints on photosynthesis and water-use efficiency

We measured stomatal ( $g_s$ ) and mesophyll ( $g_m$ ) conductance to  $CO_2$ , the net  $CO_2$  assimilation rate ( $A_N$ ) at ambient  $CO_2$  concentrations and the intrinsic water-use efficiency ( $iWUE = A_N/g_{sw}$ ) using an open-path infrared gas exchange analyzer (LI-6400 or LI-6400XT, Li-Cor Inc., Lincoln, NE, USA) with a 2-cm<sup>2</sup> leaf chamber fluorometer. It should be noted that  $g_s$  and  $g_{sw}$  refer to the stomatal conductance to  $CO_2$  and  $H_2O$ , respectively. Each leaf was acclimatized in the chamber until steady state (usually 15–30 min) under standardized conditions: ambient  $CO_2$  ( $C_a = 400$  ppm); constant leaf temperature ( $T_{leaf} = 25^\circ C$ ), saturating irradiance (photosynthetically active radiation,  $PAR = 1500 \mu mol\ quanta\ m^{-2}\ s^{-1}$ ) and moderate humidity (relative humidity = 40–60%, vapor pressure deficit = 1.45 kPa  $\pm$  0.30 SD). We took point measurements of all traits under these steady-state conditions. In addition, we calculated the maximum rate of carboxylation ( $V_{cmax}$ ) using  $A-C_c$  curves ( $C_c$  is the chloroplastic  $CO_2$  concentration) and leaf dark respiration ( $R_{dark}$ ) at predawn. The estimation of  $g_m$  can be particularly sensitive to the assumed parameter values. To accurately measure  $g_m$ , we estimated species-specific parameters of leaf respiration, light absorbance/photosystem partitioning and Rubisco kinetic parameters. Further details on the measurement of  $g_m$  using combined gas exchange and chlorophyll fluorescence are provided in Methods S1. To investigate Rubisco kinetics, we also sequenced the *rbcl* gene encoding the Rubisco large subunit (LSu) from each species to identify amino acid sequence changes that could alter Rubisco kinetics (see Methods S1 for further details). Next, we characterized Rubisco kinetic parameters directly from two species (*S. lycopersicum* var. *esculentum*, cv 'Roma VF' and *S. lycopersicoides*), representing the two Rubisco LSu types identified from sequence analysis (Methods S1). The kinetic properties of the two Rubisco LSu types were compared using ANOVA.

### Leaf anatomical measurements

We used the youngest, unshaded, fully expanded leaf from each individual (same leaves as those used in gas exchange measurements). Leaves were immediately weighed and scanned to obtain



fresh mass (FM) and leaf area (LA), respectively. Afterwards, leaves were dried for at least 48 h in a drying oven at 60°C to obtain dry mass (DM). We report *LMA* (as DM/LA) on whole leaves, which, in tomato, are pinnately compound, but we exclude structural, non-laminar portions (petiole, rachis and petioles) because we are particularly interested in trade-offs between leaf structure and diffusive conductance within the lamina. Because leaf thickness measurements using a micrometer are unreliable in tomato leaves (C. D. Muir, pers. obs.), we estimated leaf thickness (*LT* in  $\mu\text{m}$ ) using the method of Vile *et al.* (2005):

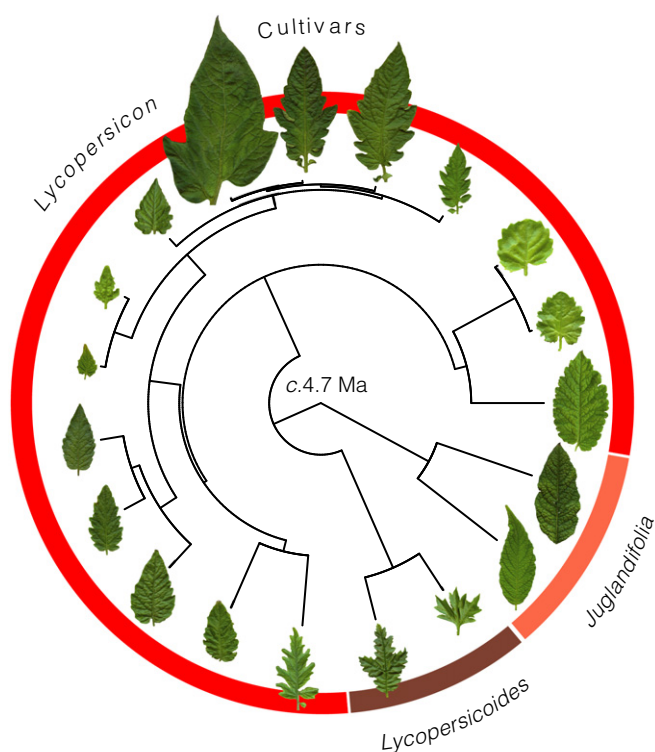
$$LT = \frac{LMA}{LDMC} \quad \text{Eqn 1}$$

*LDMC* is the leaf dry matter content, the ratio of leaf DM to saturated FM. Leaf thickness calculated using Eqn 1 is closely correlated with leaf thickness measured from sections in tomatoes (Muir *et al.*, 2014). We obtained leaf morphological data from 80 of 82 individuals.

### Statistical analyses

We used phylogenetic linear mixed effects models ('phyloLME') to estimate key relationships among traits, whilst accounting for phylogenetic non-independence. Specifically, we fitted statistical models using a Bayesian Markov chain Monte Carlo (MCMC) algorithm implemented in the R package MCMCGLMM v.2.21 (Hadfield, 2010). For all models, we ran the MCMC chain under diffuse priors for  $10^6$  steps after a burn-in of  $10^5$  steps, sampling the posterior distribution  $10^4$  times every  $10^2$  steps. We tested whether stomatal and mesophyll conductance were significantly correlated with  $A_N$  and  $iWUE$ . These variables were log-transformed for linearity and homoscedasticity. We estimated the effect of these diffusion traits on  $A_N$  and  $iWUE$  from the mode of the posterior distribution, and inferred statistical significance if the 95% highest posterior density (HPD) interval did not overlap zero. We simultaneously tested whether phylogeny explained photosynthetic trait variation by including 'species' as a phylogenetically structured random effect and compared that with a model without 'species' using the deviance information criterion (DIC), where a decrease of two or more is interpreted as a significant increase in model fit. We used a maximum likelihood phylogenetic tree inferred from 18 genes (Fig. 1; Haak *et al.*, 2014). Maximum likelihood analyses were conducted using RAxML v.8.1.24 (Stamatakis, 2014). The topology of the best tree agreed with previous Bayesian estimates (Rodríguez *et al.*, 2009).

We used principal component analysis (PCA) to identify major axes of variation among nine leaf traits:  $g_m$ ,  $g_s$ ,  $A_N$ ,  $V_{\text{cmax}}$ ,  $R_{\text{dark}}$ ,  $iWUE$ , *LMA*, *LT*, *LDMC*. All traits, except  $V_{\text{cmax}}$  and  $R_{\text{dark}}$ , were log-transformed to make the distribution approximately multivariate normal. Parallel analysis of the trait correlation matrix using the 'parallel' function from the R package NFACTORS v.2.3.3 (Raiche, 2010) indicated that the first four principal components explained significantly more variance, 90.0% cumulatively, than expected by chance from an uncorrelated matrix with rank 7 (we used nine traits, but  $iWUE$  and *LT*



**Fig. 1** Wild tomatoes and cultivars are closely related, yet phenotypically diverse, taxa. Among the species investigated here, the oldest split is c. 4.7 million yr (Ma) ago based on a fossil-calibrated phylogeny (Särkinen *et al.*, 2013). Functional traits, such as terminal leaflet size, depicted for each species at the tips of the phylogeny, as well as leaf physiological and structural traits used in this study, vary widely among species.

are linear combinations of other traits). The first four principal components also had eigenvalues greater than one (Table S2) and should therefore be retained according to Kaiser's rule (Kaiser, 1960). We focus on the first principal component, denoted PC1, which explained a moderate amount of variation (35.5%). For reference, PC1 loaded positively with *LT* and *LMA*, but negatively with  $A_N$  and components of  $\text{CO}_2$  diffusion ( $g_m$ ,  $g_s$ ). We did not use phylogenetic PCA because currently available methods do not account for intraspecific variation, which can lead to model misspecification (Pennell *et al.*, 2015), and are likely to be misleading because they do not implement appropriate multivariate models of trait evolution (Uyeda *et al.*, 2015).

To test the hypothesis that species from dry and/or hot environments trade off efficient  $\text{CO}_2$  diffusion for a more robust, stress-tolerant leaf anatomy, we looked at the correlation between mean annual precipitation and temperature relative to the average position of the species along PC1. For this analysis, we removed the three cultivars of *S. lycopersicum* var. *esculentum* and one accession *S. lycopersicum* var. *cerasiforme*, an unimproved landrace. To reduce the influence of a single outlier species (*S. juglandifolium*), we shrank the interspecific variance of PC1 using a signed logarithm transformation:

$$\text{PC1}_T = \text{sign}(\text{PC1}) \log |\text{PC1}| \quad \text{Eqn 2}$$

We also performed analyses with and without this species. We obtained mean annual precipitation and temperature at

the habitat of origin for each source population from Worldclim (Hijmans *et al.*, 2005), which has been used previously to study climatic adaptation in wild tomatoes (Nakazato *et al.*, 2008; Chitwood *et al.*, 2012). Phylogenetic regression was carried out with the R package *PHYLOLM* v.2.2 (Ho & Ané, 2014) using the 'OUrandomRoot' model to account for phylogenetic non-independence. The Ornstein–Uhlenbeck (OU) model was a better fit than traditional Brownian motion models of trait evolution because there was little phylogenetic signal in all traits. Other trait–climate relationships were tested in the same way.

### Comparison with global patterns

A central part of the hypotheses being tested is that more robust leaf structure decreases mesophyll CO<sub>2</sub> diffusion ( $g_m$ ). In broad comparative studies, this trade-off is present, but is not especially strong (Flexas *et al.*, 2008). However, apparently weak trade-offs may be an artifact of combining studies, which introduces many sources of methodological variation (e.g. different protocols or statistical analysis) that are not biologically informative. Our experimental design eliminates much of this variation and hence should be better able to identify a true trade-off between leaf structure and CO<sub>2</sub> diffusion. We therefore compared our data with a global dataset (Niinemets *et al.*, 2009a supplemented with data from Niinemets *et al.*, 2009b; Galmés *et al.*, 2011; Muir *et al.*, 2014). These data are available in Table S3. From this global dataset, we fitted a linear model of  $\log(LMA)$  against  $\log(g_m)$ . We used predictions from the fitted model to test whether the trade-off in tomatoes was significantly shifted or more/less variable than the global dataset. In particular, we predicted that, if strong trade-offs shape a coordinated 'fast–slow' axis, the relationship between  $\log(LMA)$  against  $\log(g_m)$  should be stronger in tomatoes compared with the global dataset. We tested this by asking whether the residual variance in tomatoes was less than that predicted from the

model fitting to the global dataset. For comparability with the global dataset, we used species-average  $LMA$  and  $g_m$  rather than individual plant values. Further details of the statistical methods are provided in Methods S1.

### Data availability

All data for these analyses are available on Dryad (Muir *et al.*, 2016).

## Results

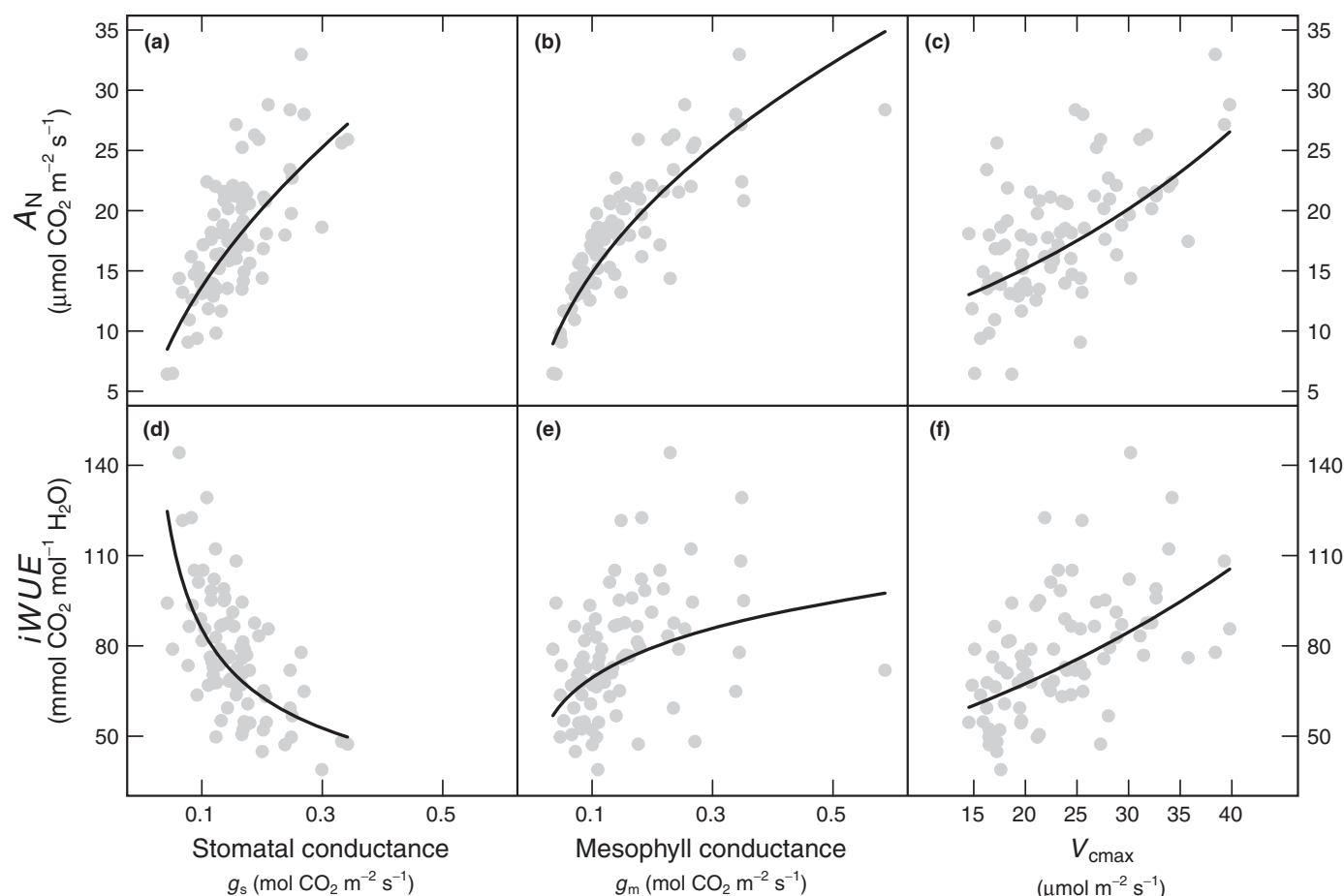
### CO<sub>2</sub> diffusion and biochemistry limit photosynthesis and alter water-use efficiency

Tomato species vary considerably in photosynthetic rate ( $A_N$ ) and intrinsic water-use efficiency ( $iWUE$ ), driven by constraints on leaf CO<sub>2</sub> diffusion and the maximum rate of carboxylation ( $V_{cmax}$ ). Phylogenetic linear mixed effects models ('phyloLME') showed that, between individual plants, increased stomatal ( $g_s$ ) and mesophyll ( $g_m$ ) conductance increased  $A_N$  (Table 1; Fig. 2a, b), but had opposing effects on  $iWUE$  (Table 1). However, the phylogenetic relationship explained little of the trait variation, and thus phylogenetic and non-phylogenetic gave nearly identical results (Table 1). Increased  $g_s$  was associated with lower  $iWUE$  (Fig. 2d), whereas greater  $g_m$  was associated with greater  $iWUE$  (Fig. 2e).  $V_{cmax}$ , an indicator of biochemical capacity, had a similar effect to  $g_m$ , and was associated with significantly greater  $A_N$  and  $iWUE$  (Fig. 2c,f). Figure S1 shows the trait relationships depicted in Fig. 2 grouped by species' averages rather than by individual plants. The data show that tomato species achieved high photosynthetic rates via two routes: high  $g_s$ , but lower  $iWUE$  or high  $g_m$ , but relatively high  $iWUE$ . The drawdown of CO<sub>2</sub> concentration from the leaf interior ( $C_i$ ) to the chloroplast ( $C_c$ ), another indicator of diffusional constraint in the leaf mesophyll, was even more strongly correlated with  $iWUE$  than

**Table 1** Diffusional and biochemical constraints drive variation in photosynthetic rate ( $A_N$ ) and intrinsic water-use efficiency ( $iWUE$ ) among tomato species

Response variable	Fixed effects	Random effects	DIC	Parameter estimate		95% HPD interval
$\log(A_N)$	$\log(g_s)$	(none)	–233.4	$\log(g_s)$	0.39	0.35 to 0.43
	$\log(g_m)$			$\log(g_m)$	0.31	0.28 to 0.34
	$V_{cmax}$			$V_{cmax}$	$9.7 \times 10^{-3}$	7.2 to $12.5 \times 10^{-3}$
	$\log(g_s)$	Species	–250.5	$\log(g_s)$	0.36	0.32 to 0.40
	$\log(g_m)$			$\log(g_m)$	0.32	0.28 to 0.35
	$V_{cmax}$			$V_{cmax}$	$9.2 \times 10^{-3}$	6.4 to $12.0 \times 10^{-3}$
$\log(iWUE)$	$\log(g_s)$	(none)	–233.1	$\log(g_s)$	–0.61	–0.64 to –0.57
	$\log(g_m)$			$\log(g_m)$	0.31	0.28 to 0.34
	$V_{cmax}$			$V_{cmax}$	$9.7 \times 10^{-3}$	7.1 to $12.3 \times 10^{-3}$
	$\log(g_s)$	Species	–250.5	$\log(g_s)$	–0.64	–0.67 to –0.60
	$\log(g_m)$			$\log(g_m)$	0.32	0.28 to 0.35
	$V_{cmax}$			$V_{cmax}$	$9.2 \times 10^{-3}$	6.3 to $12.0 \times 10^{-3}$

For both  $A_N$  and  $iWUE$ , phylogenetic linear mixed effects models including 'species' as a random effect improved the model fit (lower deviance information criterion (DIC)) over non-phylogenetic linear models, indicating some effect of phylogenetic relatedness. Greater stomatal ( $g_s$ ) and mesophyll ( $g_m$ ) conductance significantly increased  $A_N$ , but had opposing effects on  $iWUE$ . Likewise, greater maximum carboxylation rates ( $V_{cmax}$ ) increased both  $A_N$  and  $iWUE$ . Parameters were estimated from the mode of  $10^4$  samples drawn from the posterior distribution using MCMCglmm. The effects of  $g_s$ ,  $g_m$  and  $V_{cmax}$  are highly significant (95% highest posterior density (HPD) intervals do not overlap zero) in all models.



**Fig. 2** Leaf CO<sub>2</sub> diffusion and biochemistry limit net CO<sub>2</sub> assimilation rates ( $A_N$ ) and alter intrinsic water-use efficiency ( $iWUE$ ). Each point shows the data from one of 82 individual plants across 19 wild and cultivated tomato taxa. (a, b) Faster diffusion through stomata ( $g_s$ ) and mesophyll ( $g_m$ ) resulted in higher  $A_N$ . However, these parameters had opposing effects on  $iWUE$ . Greater  $g_s$  lowered  $iWUE$  (d), whereas greater  $g_m$  increased  $iWUE$  (e). Leaf biochemistry, specifically the maximum rate of carboxylation ( $V_{cmax}$ ), also increased  $A_N$  (c) and  $iWUE$  (f). Fitted lines are based on a Bayesian phylogenetic mixed model treating 'species' as a random effect:  $\log(A_N) \sim \log(g_s) + \log(g_m) + V_{cmax} + (1|\text{species})$  and  $\log(iWUE) \sim \log(g_s) + \log(g_m) + V_{cmax} + (1|\text{species})$ . For visual aid, lines are drawn to account for positive co-variation between  $g_s$ ,  $g_m$  and  $V_{cmax}$  (data not shown). For example, (a) shows the predicted  $A_N$  at a given  $g_s$  with  $g_m$  and  $V_{cmax}$  set to the predicted value at a given  $g_s$ . All slopes were significantly different from zero ( $P < 0.0001$ ) based on  $10^4$  Markov chain Monte Carlo (MCMC) samples from the posterior distribution of the model.

was  $g_m$  (Fig. S2), arguing that reduced internal diffusion enhances  $iWUE$ .

### Limited variation in Rubisco biochemistry between tomato species

The two Rubisco LSU types showed significant differences in some kinetic parameters (Table 2; see Methods S1 for a description of how types were identified and Notes S1 for further details on the molecular evolution of *rbcL* in tomatoes). Interestingly, the Rubisco LSU type 2, which occurred in the domesticated clade species (*S. pimpinellifolium*, *S. cheesmaniae*, *S. galapagense*, *S. lycopersicum* var. *cerasiforme* and the cultivars; see Fig. S3) and *S. habrochaites*, had a higher  $S_{c/o}$  value, which was a result of a lower Michaelis–Menten constant for CO<sub>2</sub> under atmospheric conditions ( $K_c^{\text{air}}$ ) and a higher catalytic turnover rate for the oxygenase reaction ( $k_{\text{cat}}^o$ ), compared with the Rubisco LSU type 1. Non-significant differences were observed between the two Rubisco LSU types in the remaining kinetic parameters.

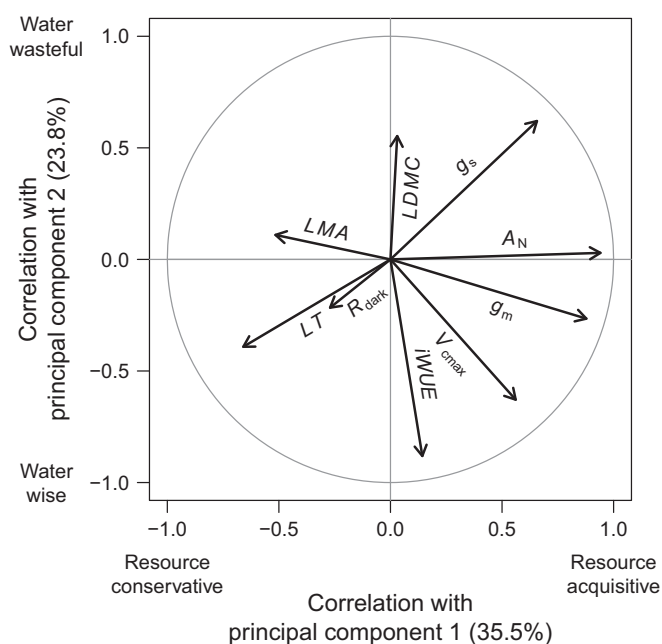
### Modest coordination between leaf physiological and structural traits

The first principal component (PC1) accounted for 35.5% of the variation among individual leaf physiology ( $g_m$ ,  $g_s$ ,  $A_N$ ,  $V_{cmax}$ ,  $R_{\text{dark}}$ ,  $iWUE$ ) and bulk anatomy ( $LMA$ ,  $LT$ ,  $LDMC$ ). On one end of this axis were thin leaves with fast CO<sub>2</sub> diffusion and high  $A_N$ ; on the other, were thick leaves with slower CO<sub>2</sub> diffusion and lower  $A_N$  (Fig. 3). This principal component indicates an axis of leaf trait variation probably mediated by trade-offs between more robust leaf structure (i.e. higher  $LMA$  and  $LT$ ) and CO<sub>2</sub> diffusion. However, the modest amount of trait variance explained indicates that this trade-off does not tightly constrain leaf trait evolution in tomatoes. The second principal component (PC2; 23.8% variance explained) was most strongly associated with  $iWUE$  and showed that greater  $iWUE$  was associated with leaves with higher water content (lower  $LDMC$ ) (Fig. 3). Figure S4 shows the average position of tomato species in PC1–PC2 space.

**Table 2** Comparison of the Rubisco kinetics at 25°C for the two Rubisco large subunit (LSu) types detected among wild and domesticated tomatoes based on the amino acid sequence, namely LSu types 1 and 2

Rubisco LSu type	1	2	P-value
Species measured	<i>Solanum lycopersicoides</i>	<i>Solanum lycopersicum</i>	
$K_c$ (mM)	$9.3 \pm 0.9$	$8.2 \pm 0.7$	0.308
$K_c^{\text{air}}$ (mM)	$16.6 \pm 0.8$	$13.5 \pm 0.8$	0.020*
$k_{\text{cat}}^c$ ( $\text{s}^{-1}$ )	$2.430 \pm 0.138$	$3.142 \pm 0.518$	0.176
$k_{\text{cat}}^o$ ( $\text{s}^{-1}$ )	$0.892 \pm 0.099$	$1.359 \pm 0.108$	0.010**
$k_{\text{cat}}^c/K_c$ ( $\text{s}^{-1} \text{mM}^{-1}$ )	$0.261 \pm 0.026$	$0.391 \pm 0.075$	0.127
$S_{c/o}$ ( $\text{mol mol}^{-1}$ )	$99.4 \pm 3.5$	$115.0 \pm 2.1$	0.007**

Parameters, measured in a representative species of each Rubisco type, describe the Michaelis–Menten constant for  $\text{CO}_2$  under 0%  $\text{O}_2$  ( $K_c$ ) and 21%  $\text{O}_2$  ( $K_c^{\text{air}}$ ), the maximum rates of carboxylation ( $k_{\text{cat}}^c$ ) and oxygenation ( $k_{\text{cat}}^o$ ), carboxylation catalytic efficiency ( $k_{\text{cat}}^c/K_c$ ) and specificity factor ( $S_{c/o}$ ). Data are means  $\pm$  SE of four replicates per Rubisco type. Significant differences between both Rubisco types are indicated by the ANOVA P-value: \*,  $<0.05$ ; \*\*,  $<0.01$ .



**Fig. 3** Major axes of leaf trait variation in tomato. The first axis, principal component 1, explains 35.5% of the total variation. It delineates plants along a continuum from resource acquisitive (higher PC1, greater  $A_N$ , thinner leaves) to resource conservative (lower PC1, lower  $A_N$ , thicker leaves). The second axis, principal component 2, explains 23.8% of the trait variation and delineates an axis from more water wise (lower PC2, greater  $iWUE$ ) to water wasteful (higher PC2, lower  $iWUE$ ). The arrows are vectors showing the correlation across individual plants between a trait and both principal components. For example,  $A_N$  is positively correlated with PC1, but uncorrelated with PC2. The gray circle shows the outer possible set of correlation combinations.  $R_{\text{dark}}$ , dark respiration rate;  $iWUE$ , intrinsic water-use efficiency;  $V_{\text{cmax}}$ , maximum rate of carboxylation;  $g_m$ , mesophyll conductance;  $A_N$ , net  $\text{CO}_2$  assimilation rate;  $g_s$ , stomatal conductance;  $LDMC$ , leaf dry matter content;  $LMA$ , leaf mass per area;  $LT$ , leaf thickness.

### Limited evidence of leaf trait–climate associations

Contrary to the hypothesis that dry, hot environments select for a stress-tolerant, robust leaf structure, thinner leaves and more

rapid  $\text{CO}_2$  diffusion (i.e. higher values of PC1) were associated with drier habitats (PC1–Precip,  $P=0.006$ ), but not temperature (PC1–Temp,  $P=0.092$ ). However, this correlation between PC1 and precipitation was strongly influenced by a single species, *S. juglandifolium* (Fig. 4), and was not significant if this species was removed (PC1–Precip,  $P=0.163$ ). Certain traits that loaded strongly with PC1, especially  $LMA$  and  $LT$ , were strongly associated with precipitation. Specifically, species from the driest habitats had the thinnest leaves, even when *S. juglandifolium* was excluded (Fig. S5). Our data therefore do not support the hypothesis that species trade off slow  $\text{CO}_2$  diffusion and lower metabolic rates for robust leaf structure in stressful environments. All trends in the data are actually in exactly the opposite direction.

### Structure–function relationships in tomato are consistent with global patterns

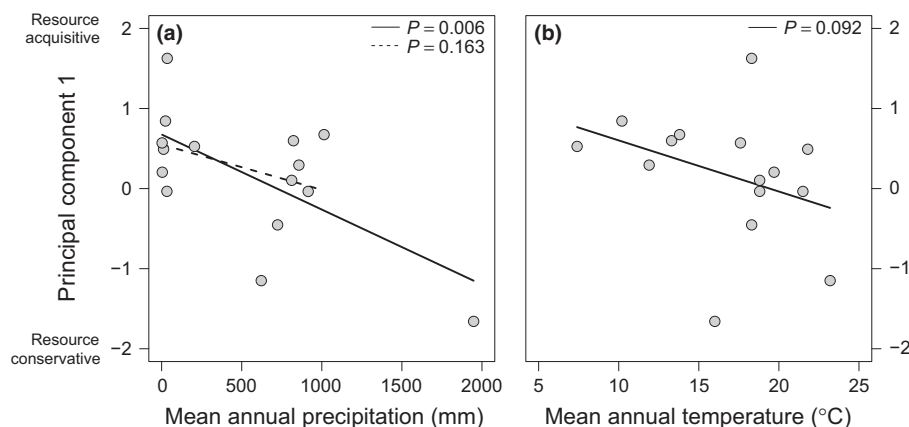
Globally,  $LMA$  is weakly negatively correlated with  $g_m$  (Fig. 5; linear regression  $r^2=0.44$ ,  $P<10^{-13}$ ). Despite the fact that tomato species are closely related and our common garden design eliminated sources of variation that afflict cross-study comparisons, the relationship between  $LMA$  and  $g_m$  in tomatoes is entirely consistent with the global pattern. Qualitatively, trait values for tomato species fall within the 95% prediction intervals of the global dataset (Fig. 5). This is borne out with quantitative measures. Residual differences between actual  $g_m$  and model-predicted  $g_m$ , conditional on the  $LMA$  of species, were not significantly different from zero ( $t$ -test,  $P=0.13$ ). Further, the sum of squared residual variance was exactly what one would expect ( $q=0.38$ ; for reference,  $q=0.5$  is the median quantile) for new observations from the same underlying model. It should be noted that data from this study were not used to estimate the model parameters.

### Discussion

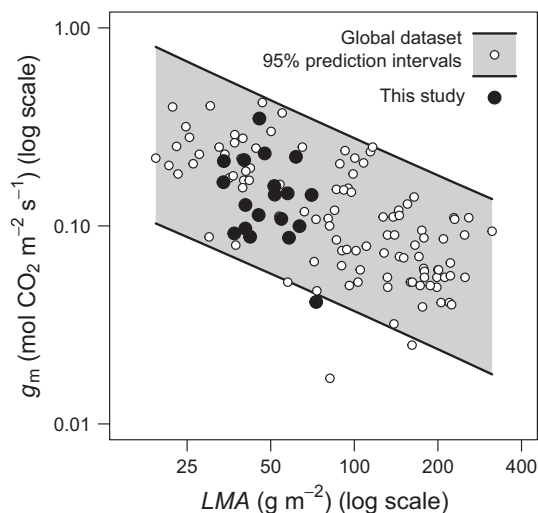
Biologists seek to distill organismal variation down to a manageable number of key functional traits (e.g. Perez-Harguindeguy *et al.*, 2013). Leaf traits, in particular, which are necessarily constrained by the need to capture sunlight and  $\text{CO}_2$  and to manage nutrients/water, may fall along a single ‘fast–slow’ continuum (Reich, 2014 and references therein) associated with resource-acquisitive and resource-conservative traits (Mason & Donovan, 2015). Among the key assumptions are that leaf structural traits, such as  $LMA$ , strongly constrain photosynthesis by reducing  $\text{CO}_2$  diffusion, and that there is strong selection for tight coordination between  $\text{CO}_2$  diffusion and biochemical capacity for photosynthesis. These assumptions have been evaluated at broad phylogenetic scales, but rarely addressed within or among closely related species. This is important because variation among closely related taxa can identify the incremental evolutionary steps taken as organisms adapt to new environments, and can provide a baseline for predicting evolutionarily accessible phenotypes under climate change or in a breeding program.

In tomatoes, we found evidence for a ‘fast–slow’ spectrum associated with diffusional/biochemical constraints and leaf structure, but this spectrum explained a modest amount of total leaf





**Fig. 4** The resource-conservative to resource-acquisitive axis of leaf trait variation was uncorrelated with mean annual precipitation (a) and temperature (b). Each point shows the climate at the habitat of origin for a different wild tomato taxon and its position, averaged from multiple individuals in the experiment, along the resource-acquisitive to resource-conservative axis. There was a significant positive correlation between PC1 and precipitation, but this was driven by a single influential species, *Solanum juglandifolium*; the correlation was not significant once this species was removed. All *P*-values are from phylogenetic linear regression.



**Fig. 5** Leaf mass per area (*LMA*) weakly constrains mesophyll  $\text{CO}_2$  conductance ( $g_m$ ) among tomato species (closed circles, this study) and a globally distributed sample of species (open circles, see the Materials and Methods section). Data from tomatoes fall within the 95% prediction intervals (shaded gray area) estimated from the global dataset, indicating that the relationship between *LMA* and  $g_m$  in tomatoes is consistent with the broader dataset.

trait variation. There is no precise prediction for exactly how much coordination we *should* have seen, but variation was well distributed across the first four components, indicating multiple important axes. Furthermore, although thicker leaves with greater *LMA* were associated with reduced  $\text{CO}_2$  diffusion, the relationships were weak (Fig. S6), demonstrating ample scope for thick and/or dense leaves to have relatively high diffusion, and vice versa. ‘Weak’ and ‘strong’ are subjective terms, but here we mean that coordination is weak in the sense that, for a given *LMA* or *LT*, tomato species vary widely in terms of  $\text{CO}_2$  diffusion, area-based photosynthetic rate and *iWUE*. This indicates that certain trade-offs (e.g. between *LMA* and  $g_m$ ) and/or selection for phenotypic integration do not strongly constrain the evolution of leaf

form and function among closely related species. As we explain below, weak coordination between structure and function helps to explain why trait axes, such as the leaf economics spectrum, can vary in direction and magnitude in different species or genera.

We interpret these results as evidence that robust leaf structure does not strongly constrain area-based photosynthetic function, but merely sets an upper bound on traits such as mesophyll  $\text{CO}_2$  diffusion. Grubb (2015) distinguished between ‘true trade-offs’ (e.g. if *X* goes up, *Y* must go down) and ‘boundary line’ trade-offs (e.g. *X* sets upper bound on *Y*). A true trade-off imposes hard constraints on evolution which should manifest very strong relationships between traits. Conversely, ‘boundary line’ trade-offs set soft ceilings on evolutionary divergence, and so correlations should generally be weak, consistent with what we found here. However, true trade-offs may appear weaker than they really are if measurements are error prone, inflating the noise relative to the signal. The signal-to-noise ratio alone cannot explain why we found weak coordination between leaf structure and function. Despite sharing a recent common ancestor, tomatoes exhibit a dramatic range in traits, such as  $A_N$  ( $6.4\text{--}33.0\ \mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$ ), that can be measured precisely under controlled conditions. However, tomato species are on the tender-leaved end of the spectrum (*c.*  $30\text{--}80\ \text{g m}^{-2}$ ; for comparison, the average *LMA* in global comparisons is *c.*  $120\ \text{g m}^{-2}$ ; Wright *et al.*, 2004). Among tender-leaved species, traits such as *LMA* may impose weak limitations, but become more of a constraint with larger divergence (e.g. herbs vs. sclerophylls). However, most closely related species, with the possible exception of dramatic island radiations (Givnish *et al.*, 2004; Dunbar-Co *et al.*, 2009), are not so disparate. Hence, the low amount of constraint and coordination in tomatoes may be typical of many clades. Indeed, a similar study of rice species also found separate, mostly uncorrelated, modules of structural and physiological traits (Giuliani *et al.*, 2013). In other ways, our experimental design should have been especially able to detect coordination, if it existed, because we measured plants with similar growth form and functional type in a common garden at the



same age, eliminating sources of variation common in other studies. The lack of tight coordination between leaf structure, CO<sub>2</sub> diffusion and photosynthetic biochemistry means that there may be multiple, loosely coordinated axes of leaf trait variation that provide a substrate for labile, unconstrained evolution in response to novel selective pressures from climate change and crop breeders.

Indeed, many combinations of stomatal conductance ( $g_s$ ), mesophyll conductance ( $g_m$ ) and  $V_{\text{cmax}}$  resulted in similar photosynthetic rates, but very different water-use efficiencies (Fig. 2). Typically, we expect that, to increase the photosynthetic rate, plants must increase stomatal conductance, increasing transpirational loss and decreasing water-use efficiency. If mesophyll conductance and biochemical capacity were closely coordinated with stomatal conductance, there would be limited opportunity to increase water-use efficiency without sacrificing the photosynthetic rate. However, we found that there may be substantial scope to increase photosynthetic rate whilst maintaining high water-use efficiency. Indeed, photosynthetic rate and water-use efficiency were essentially uncorrelated. This axis of variation was evident in a second principal component that loaded with  $iWUE$ , but was orthogonal to  $A_N$  (Fig. 3). Why don't all species have high photosynthetic rate and water-use efficiency? In nature, there are other trade-offs, especially nitrogen limitation, that prevent species from having greater mesophyll conductance and/or  $V_{\text{cmax}}$ . For example, environments with lots of water, but little nitrogen, might favor leaves with intrinsically high stomatal density, but low biochemical investment (Bloom *et al.*, 1985; Wright *et al.*, 2001, 2003) without necessarily changing selection on overall leaf structure. Our data suggest that, for tomato species, the fine tuning of optimal water, nitrogen and other resource-use efficiencies is nearly as important as selection for overall coordination along the resource acquisitive–conservative spectrum.

Photosynthetic variation in tomatoes is primarily mediated by leaf anatomical differences and  $V_{\text{cmax}}$  rather than differences in Rubisco kinetics. In contrast with other clades of C<sub>3</sub> plants that have undergone rapid diversification into different environments, evolutionary changes in Rubisco kinetics (e.g. faster rates of carboxylation or greater affinity for CO<sub>2</sub> over O<sub>2</sub>) do not appear to have played a major role in the evolution of tomatoes. This contrasts with other plant groups, such as *Limonium* (Galmés *et al.*, 2014), which underwent adaptive evolution of Rubisco kinetics during their radiations into novel environments. Future work is needed to understand why some clades respond to selection through changes in protein biochemistry, whereas other groups, such as tomato, seem to primarily differ in anatomical traits.

We also found no evidence that drier or hotter environments favor robust leaf structure or 'slow', resource-conservative strategies (low conductance, low  $V_{\text{cmax}}$ ) in wild tomatoes (Fig. 4). Obviously, our study does not have the statistical power of broad comparative analyses (e.g. Wright *et al.*, 2005), but the trends in the data were not even in the predicted direction. If anything, there was a tendency for species from the driest habitats to be on the 'fast' end of the leaf trait spectrum (see also Easlon & Richards, 2009), but this was largely influenced by a single species, *S. juglandifolium*. Thus, tomato species in dry habitats

may rely primarily on a form of drought tolerance, growing rapidly when water is available and dying back or going dormant during drought. We do not yet know enough about the microclimate or natural patterns of resource acquisition of these species to draw more definitive conclusions about how these species cope with arid climates. Alternatively, leaf traits may be decoupled from other traits (e.g. root : shoot ratios) that confer alternative drought avoidance or tolerance mechanisms. However, for herbaceous species, such as wild tomatoes, resource-acquisitive strategies are probably quite common in warm, dry environments (Mooney *et al.*, 1981; Gibson, 1996). Depending on the strategy of a species for coping with stress, apparently resource-acquisitive traits can be associated with stressful environments (Grady *et al.*, 2013; Brouillette *et al.*, 2014). Finally, we caution that, if species reaction norms differ, trait–environment relationships in nature might be quite different than in a common garden (Givnish & Montgomery, 2014). For example, fertilization may release some species from constraints that enforce resource-conservative trait combinations in nature.

The modest coordination observed here between leaf structure and physiological function indicates that bulk structural traits, such as  $LMA$ , are probably insufficient to identify the most important axes of trait variation in studies within species or genera (Fig. S6). Traits such as leaf thickness and  $LMA$  set upper bounds on maximum potential CO<sub>2</sub> diffusion and area-based photosynthetic rate (Flexas *et al.*, 2008), but realized values depend on the precise anatomical and biochemical features of the leaf that are not well captured by bulk structural traits (Tosens *et al.*, 2012; Tomás *et al.*, 2013). This suggests the possibility that there are many unique ways to vary leaf anatomy and photosynthesis without large effects on traits such as  $LMA$ . Each of these axes may be a different trade-off that could be evolutionarily important in some taxa, but not others. Hence, for axes such as the LES, there is lots of room beneath the soft ceiling imposed by  $LMA$  to modulate photosynthetic function and resource-use efficiencies. For example, in tomatoes, trait evolution orthogonal to the  $LMA$ – $g_m$  trade-off is as important as evolution along this trade-off (Fig. 5). However, different clades will experience different selection pressures and developmental constraints that will ultimately lead to divergent trait spectra. These 'boundary line' trade-offs (Grubb, 2015) only become major constraints at macroevolutionary rather than microevolutionary scales. We analyzed traits on an area basis, but note that mass-based traits are apparently more coordinated (analysis not shown). Although this has important consequences for whole-plant growth (Poorter *et al.*, 2014), apparent coordination among mass-based traits does not necessarily indicate functional constraints, because all area-based traits are multiplied by  $LMA$  (Lloyd *et al.*, 2013; Osnas *et al.*, 2013).

To address the questions posed in the Introduction, we draw four major conclusions.

- (1) Area-based photosynthesis and water-use efficiency are strongly affected by CO<sub>2</sub> diffusion and  $V_{\text{cmax}}$ , but there is little variation in Rubisco kinetics.
- (2) Although tomatoes vary widely in diffusional, biochemical and structural traits, there is little co-variation between bulk

structure and function, despite the fact that there is an overall resource-acquisitive–resource-conservative axis. Hence, broad-scale patterns of functional trait co-variation may not be useful in predicting adaptive evolution in the recent past or in response to climate change.

(3) Nor should we expect that the relationship between traits and environments observed at broad phylogenetic scales (e.g. thick leaves in hot, dry environments) will hold at smaller scales. If anything, we find that low-*LMA*, resource-acquisitive traits are associated with dry environments, the opposite of what is commonly predicted (see also Mason & Donovan, 2015).

(4) One of the primary reasons for limited coordination is that the bulk leaf structural traits, such as *LMA*, set a soft ceiling, but not a hard constraint, on  $\text{CO}_2$  diffusion. With the possible exception of dramatic radiation, most evolutionary differences in leaf structure among closely related species should not strongly constrain variation in photosynthetic function. This does not refute that intimate structure–function relationships exist. Rather, detailed anatomical traits, such as guard cell dimensions, mesophyll structure and photochemical enzyme concentrations, are needed to identify the most important trade-offs defining leaf anatomy and physiology.

The adaptive radiation of tomatoes and other plant groups undoubtedly requires evolutionary diversification in morphological and physiological traits, but this study suggests that we do not yet have a general explanation for variation in the most important leaf traits affecting photosynthesis.

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## Author contributions

C.D.M., M.A.C. and J.G. designed the research. C.D.M., M.A.C., E.J.R., A.M. and J.G. performed the research and collected the data. C.D.M., M.A.C. and J.G. analyzed the data. C.D.M. wrote the paper with input from M.A.C. and J.G.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Fig. S1** Leaf CO<sub>2</sub> diffusion and biochemistry limit net CO<sub>2</sub> assimilation rates ( $A_N$ ) and alter intrinsic water-use efficiency ( $iWUE$ ).

**Fig. S2** CO<sub>2</sub> drawdown from the leaf interior ( $C_i$ ) to the chloroplast ( $C_c$ ) is negatively associated with the intrinsic water-use efficiency ( $iWUE$ ) in tomatoes, indicating that reduced diffusional constraints (lower  $C_i - C_c$ ) lead to increased  $iWUE$ .

**Fig. S3** Evolutionary patterns of the Rubisco large subunit (LSu) among wild and domesticated tomato species (GenBank accession numbers for the *rbcL* sequences: KX086685–KX086703).

**Fig. S4** Position of species in principal component space of leaf trait variation in tomato.

**Fig. S5** Leaf mass per area ( $LMA$ ) is positively correlated with mean annual precipitation in wild tomatoes.

**Fig. S6** Bulk leaf structure (leaf mass per area ( $LMA$ ) and leaf thickness ( $LT$ )) weakly constrain leaf CO<sub>2</sub> diffusive conductance through stomata ( $g_s$ ) and mesophyll ( $g_m$ ).

**Table S1** Key to species name, geographical origin and germplasm accession data

**Table S2** Eigenvalues and eigenvectors associated with principal components of leaf trait variation in tomato

**Table S3** Global dataset of mesophyll conductance to CO<sub>2</sub> and leaf mass per area

**Methods S1** Details of gas exchange, sequencing of the Rubisco large subunit, Rubisco catalytic characterization and comparison of tomatoes with a global dataset of leaf mass per area and mesophyll conductance.

**Notes S1** Sequence comparisons reveal two Rubisco large subunit (LSu) types in tomatoes.

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