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Constraint around Quarter-Power Allometric Scaling in Wild Tomatoes (*Solanum* sect. *Lycopersicon*; Solanaceae)

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ABSTRACT: The West-Brown-Enquist (WBE) metabolic scaling theory posits that many organismal features scale predictably with body size because of selection to minimize transport costs in resource distribution networks. Many scaling exponents are quarter-powers, as predicted by WBE, but there are also biologically significant deviations that could reflect adaptation to different environments. A central but untested prediction of the WBE model is that wide deviation from optimal scaling is penalized, leading to a pattern of constraint on scaling exponents. Here, we demonstrate, using phylogenetic comparative methods, that variation in allometric scaling between mass and leaf area across 17 wild tomato taxa is constrained around a value indistinguishable from that predicted by WBE but significantly greater than 2/3 (geometric-similarity model). The allometric-scaling exponent was highly correlated with fecundity, water use, and drought response, suggesting that it is functionally significant and therefore could be under selective constraints. However, scaling was not strictly log–log linear but rather declined during ontogeny in all species, as has been observed in many plant species. We caution that although our results supported one prediction of the WBE model, it did not strongly test the model in other important respects. Nevertheless, phylogenetic comparative methods such as those used here are powerful but underutilized tools for metabolic ecology that complement existing methods to adjudicate between models.

Keywords: allometry, metabolic scaling theory, phylogenetic comparative methods, *Solanum*, Ornstein-Uhlenbeck.

Introduction

Organisms are functionally constrained by physical and chemical laws, yet natural selection has produced seemingly endless forms within these fundamental limits. To take one example, plant size varies over many orders of magnitude. The smallest angiosperms, duckweeds (Lemnaceae), can be less than 0.5 mm at maturity (Les et al. 2002), whereas the tallest redwood (*Sequoia sempervirens*) is 112.7 m (Koch et al. 2004). Despite vast discrepancies in overall size, allom-

etry between constituent parts is often conserved, suggesting the action of fundamental functional and selective constraints common to all species. Metabolic scaling theory (MST), as developed by West, Brown, and Enquist (the WBE model), predicts that selection on efficient metabolism will result in quarter-power growth allometry between body mass, metabolic rate, and area of metabolic organs such as leaves (West et al. 1997; Enquist et al. 1999; West et al. 1999; Enquist and Niklas 2002). However, alternative models predict different scaling exponents (e.g., 2/3; White and Seymour 2003), heterogeneous exponents (Coomes and Allen 2009), or exponents that vary significantly between species (Bokma 2004; Price et al. 2009) and with organismal size (Reich et al. 2006; Savage et al. 2008; Kolokotrones et al. 2010; Mori et al. 2010; Peng et al. 2010). It is not yet clear whether these are truly competing models or points along the continuum of a more general model (e.g., Price et al. 2007). Despite the attention given to metabolic and allometric scaling in ecology, we know little about how rapidly these scaling parameters evolve and how much they are constrained among closely related species. In this study, we quantify both lability and constraint on allometric scaling between biomass and leaf area across short evolutionary timescales (<10 million years) in wild tomatoes (*Solanum* sect. *Lycopersicon*; Solanaceae).

Power-law scaling between size and photosynthetic rate in plants is a central component of MST:

$$B = B_0 M^{\theta}. \tag{1}$$

In this equation, B is the metabolic rate (photosynthesis), M is the dry mass, B_0 is the allometric constant, and θ is the scaling exponent. We use whole-plant leaf area as a proxy for metabolic rate because metabolism in plants (photosynthetic rate [e.g., Muller-Landau et al. 2006] or respiration rate [Price et al. 2010]) is closely proportional to leaf area (Koyama and Kikuzawa 2009; Lloyd et al. 2013; Osnas et al. 2013); hence, this equation applies specifically to plant allometry (Niklas and Enquist 2001; Niklas 2006; Price et al.

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2010). For brevity, we hereafter use "leaf-area allometric scaling" and "metabolic scaling" interchangeably. The other parameter, B_0, is a taxon-specific allometric constant that can vary widely, depending on, for example, plant functional traits (Enquist et al. 2007*a*). In contrast, the WBE model predicts that the scaling exponent θ tends toward 3/4 across species. The "3/4-power" prediction arises from the assumption that natural selection shapes the geometry of resource distribution networks (e.g., vascular networks in the context of tracheophytes) in order to minimize water and nutrient transportation costs. Therefore, a key prediction of the WBE model is that θ should tend toward 3/4 within species because of stabilizing selection (Vasseur et al. 2012) and between species because of long-term constraint (Enquist et al. 1999, 2007*b*; West et al. 1999; Price et al. 2007). For example, transgressive segregation of θ in recombinant lines of *Arabidopsis* ecotypes suggests that stabilizing selection maintains $\theta \approx 3/4$ in natural populations (Vasseur et al. 2012). However, constant stabilizing selection within species generally cannot explain variation between species (Estes and Arnold 2007; Pennell and Harmon 2013). Rather, a trait such as θ may diverge little across species because the adaptive landscape relating traits to fitness is variable yet constrained (Hansen 2012). Specifically, MST predicts that peaks of high fitness in the adaptive landscape relating θ to fitness should be variable yet ultimately constrained around 3/4. Testing this second prediction would provide an independent line of evidence for or against quarter-power scaling, but to our knowledge no studies have done so.

Although allometric-scaling exponents vary between species (McCulloh et al. 2003; Bokma 2004; Price et al. 2007, 2012; Savage et al. 2008, 2010; Glazier 2010; Hudson et al. 2013), we do not know whether scaling evolves rapidly or contributes to adaptation over short evolutionary timescales or why selection might favor particular parameter values. Computer simulations indicate that plants with quarter-power scaling should outcompete alternate forms (Hammond and Niklas 2012), leaving open the question of why allometry varies widely. One possibility is that allometric-scaling parameters mediate a trade-off between fitness components, which might lead to divergent natural selection. For example, if the optimal balance between two fitness components (e.g., stress tolerance and fecundity) varies with ecological context, then natural selection should cause species to occupy different positions along this trade-off axis. Indeed, variation in metabolic scaling has been linked to life-history trade-offs (Rüger et al. 2011; Vasseur et al. 2012). Given large differences in native precipitation levels across wild tomatoes (see below for references), we tested whether allometry was responsible for interspecific differences in drought-response traits. Specifically, we predicted that accessions with greater scaling coefficients would grow faster and have greater fecundity but would lose more water and wilt

sooner because leaf area expanded more rapidly relative to total mass.

In much of the literature, scaling exponents are treated as a constant across organisms of different sizes. In other words, a straight line is fitted through data that might actually have subtle curvature. Indeed, metabolic scaling in plants is often nonlinear, declining throughout development and across organisms of different size (Reich et al. 2006; Enquist et al. 2007*b*; Mori et al. 2010; Peng et al. 2010), although the mechanistic basis of mass-specific scaling is unknown. Interestingly, the curvature seen in plants (declining mass-specific scaling) bends in the direction opposite that in mammals (Kolokotrones et al. 2010). The WBE model predicts that metabolic scaling should be isometric ($\theta \approx 1$) early in development and later converge on 3/4 in large organisms (West et al. 1999; Enquist 2002; but see Savage et al. 2008). Determining whether scaling is constant or, if not, how it changes with mass has important implications for applying metabolic scaling to higher-scale ecological phenomena, such as individual growth rates, maximum body size, demography, and population growth, that do not currently take into account mass-specific scaling (Enquist et al. 1998; Price et al. 2010; Enquist and Bentley 2012; but see Muller-Landau et al. 2006). In addition to phylogenetic and functional analyses on mass-independent scaling, we tested for curvature in allometric scaling and whether it evolves among closely related species.

We investigated these questions as part of ongoing research on ecological diversification in our focal group, wild tomatoes (*Solanum* sect. *Lycopersicon*), a small, monophyletic clade that descends from a common ancestor ~2.7 million years ago (Kamenetzky et al. 2010). Despite their recent diversification, ecological niches in wild tomatoes span deserts, tropical rain forests, and highlands (>3,600 m; Moyle 2008; Peralta et al. 2008). We also included one closely related outgroup species, *Solanum sitiens*, native to xeric, high-elevation habitats (Chetelat et al. 2009). Ecological-niche modeling indicates that climatic variables related to drought (temperature and precipitation) are the strongest contributors to interspecific niche divergence (Nakazato et al. 2010). However, the link between phenotypic variation and climatic-niche adaptation in these species is still largely unknown (Nakazato et al. 2008, 2012; Chitwood et al. 2012). The phylogeny is well resolved (Rodriguez et al. 2009; Haak et al. 2014), meaning that we can effectively disentangle phenotypic similarity caused by shared ancestry from selection and compare models of trait evolution. Since precipitation and temperature are major ecological factors contributing to species divergence in this group (Nakazato et al. 2010), we looked specifically at drought responses.

To reiterate, our primary questions are as follows. (1) Is there variation in allometric scaling among closely related species? (2) Is there evidence of clade-wide constraint on

scaling exponents around the theoretically predicted 3/4? (3) How does variation in allometric scaling affect fecundity and drought response? (4) Is allometric scaling constant, or does it change with mass?

Material and Methods

Plant Material and Cultivation

We obtained seeds of 22 accessions representing 15 wild species or varieties (see table S1 in Supporting Information, available online) from the Tomato Genetics Resource Center at the University of California, Davis (http://tgrc.ucdavis.edu). All recognized tomato species (*Solanum* sect. *Lycopersicon*) except the cultivated species *S. lycopersicum* were included, plus an outgroup, *S. sitiens* (*Solanum* sect. *Lycopersicoides*; Peralta et al. 2008). Two accessions of *S. neorickii* (LA1321 and LA1322) and five accessions of *S. sitiens* (LA4112–LA4116) were combined into a single "accession" per species because of the limited numbers of seeds. In both species, accessions originated from similar locations (see table S1) and were highly phenotypically similar in this experiment. Most other accessions are different species, but we treated some geographically and phenotypically divergent accessions of the same species (e.g., *S. huaylasense*, LA1360 and LA1983) as distinct accessions in the analysis. For simplicity, we hereafter use "accession" to denote the 17 groups that were analyzed separately, but most often these are different recognized species. All decisions about combining accessions were made before the start of this experiment without any knowledge of how they would affect the results. On May 9, 2012, seeds were soaked in 50% household bleach for 30 min, rinsed thoroughly, and placed on moist paper in plastic boxes to germinate in a growth chamber. Most seeds germinated on May 11, 2012. After one week, seedlings were transplanted to cell-pack flats containing Metro-Mix 360 (Sun Gro Horticulture, Vancouver, British Columbia, Canada). Some seedlings that germinated late were transplanted five days later (May 21), but we accounted for this delay when calculating growth rate. Two weeks after the initial transplant, seedlings were transplanted again, to either 3.30-L pots (experiment 1) or 3.78-L pots (experiment 2) containing a 1 : 1 mixture of compost soil and Metro-Mix 360. Plants were grown at the Indiana University greenhouse with supplemental lighting to maintain a constant 16L : 8D cycle. Unless otherwise noted, plants were irrigated to field capacity daily to prevent drought stress and were fertilized weekly. We conducted two experiments simultaneously to measure allometry and fecundity under nonstressed conditions (experiment 1) as well as water use and drought response (experiment 2) on all accessions. Plants were arrayed on greenhouse benches in a randomized block design with 20 blocks per experiment. There were 9–20 biological replicates per accession per experiment ($\bar{n}_{\text{expt1}} = 16.9$, median $= 18$; $\bar{n}_{\text{expt2}} = 16.4$, median $= 18$).

Experiment 1: Allometry and Fecundity

Plants were destructively harvested at 10 time points, twice per week between June 11 and July 15, 2012 (31 and 65 days, respectively, after germination). At each harvest, two blocks of plants were detached from the base of their roots. Shoots, leaves, and inflorescences were separated, and the whole-plant leaf area was scanned with an Epson GT-20000 flatbed scanner. From scanned leaves, we measured leaf area, using custom macros in ImageJ (Ambrámoff et al. 2004). After scanning, whole plants were immediately transferred to a cooler to halt biochemical processes and later that day transferred to a desiccating oven at 60°C. Once the plants were dry, we measured leaf, shoot, and total (leaf + shoot) dry mass (LM, SM, and *M*, respectively), using either a CP225D or a CP622 balance (Sartorius, Edgewood, NY).

Allometry between Leaf Area and Mass. Although we were principally testing predictions about biomass allometry, we checked that photosynthetic rate per area did not covary with plant mass, a key assumption of MST. Across accessions we detected no correlation between mass and photosynthetic rate at a single time point during the experiment (see app. A; apps. A–D available online). In using leaf area, we ignored stem and truss photosynthesis, which can account for up to 14% of photosynthetic activity in tomatoes (see table 4 in Hetherington et al. 1998; we excluded fruit photosynthesis because fruits were removed in this experiment). If the proportion of nonleaf photosynthetic area scales with overall size, omitting these areas could introduce apparent nonlinear scaling between size and photosynthetic area measured from leaves only. We do not have the data to estimate this bias, but we suspect that it is small, since most photosynthetic occurs in leaves. We estimated the allometric relationship between whole-plant leaf area (LA) and aboveground dry mass ($M = \text{LM} + \text{SM}$). Parameters of the allometric equation ($B = B_0 M^{\theta_{\text{LA}}}$) were estimated by means of linear regression on log-transformed variables (Kerkhoff and Enquist 2009), such that the slope of the regression is the scaling exponent θ_{LA} and the intercept is the allometric constant $\log B_0$. We used Type III ANOVA to test whether there was variation among accessions in the slope and intercept of allometric relationships. Rather than standard linear regression, which minimizes the variance in the *Y*-direction, we used standard major-axis (SMA) regression, which estimates coefficients by minimizing the residual variance in both *X*- and *Y*-axes. SMA is appropriate for estimating allometric coefficients, especially in comparative data (Nunn and Barton 2000), because there is no cause-and-effect relationship and measurement error occurs

for both area and mass (Niklas 1994; Warton et al. 2006). However, using SMA to estimate allometric relationships has been criticized for bias (Hansen and Bartoszek 2012). Our use of SMA to estimate within-accession allometries was not biased by shared phylogenetic history, and bias due to observational error was minimal. Biologically, SMA is more appropriate, since it directly estimates the relative growth of biomass versus leaf area. Nevertheless, we show in appendix B that ordinary least squares (OLS) and SMA yield very similar estimates and hence that the choice of method did not qualitatively alter our results. We used the "sma" function in the smatr package, version 3 (Warton et al. 2012), in R to fit a single curve for all data and then separate curves for each accession. We removed data from the two smallest plants ($M < 0.05$ g), where maternal provisioning could still be relevant. We used a Šidák correction to account for multiple comparisons.

Fecundity. Since most wild tomato species need pollination to set seed (allogamous; table S1) and plants were grown in a greenhouse without insect pollinators, we could not measure seed or fruit set. Instead, we used flower production as a proxy for fecundity. To meaningfully compare fecundity between allogamous and autogamous accessions, we regularly removed all fruit very early in development from autogamous plants. At each harvest, we counted the total number of open flowers per plant. At the end of the experiment, average fecundity per accession was calculated as the mean number of flowers per plant throughout the entire experiment. In this way, we penalized against both slow onset of flowering and slow increases in total flower number with time. More complex methods, such as fitting curves to a time series of flower number, gave highly similar results. To normalize residuals in statistical analyses, we log-transformed the mean flower number $+ 1$ (necessary because $\log 0 = -\infty$).

Phylogeny. We used a maximum likelihood phylogenetic tree inferred from 18 genes (Haak et al. 2014). Maximum likelihood (ML) analyses were conducted with RAxML Pthreads 7.0.0 (Stamatakis 2006) using one step (option code: -f a, 10,000 bootstrapping replicates) by applying ML tree search and rapid bootstrapping. The topology of the best tree agreed with previous Bayesian estimates (Rodriguez et al. 2009). We assigned small divergence between accessions of the same species in order to have a strictly bifurcating tree. Specifically, we used three distances that span a realistic parameter range: all intraspecific branch lengths were set to 1%, 10%, or 100% of the minimum sister-taxon divergence (*S. galapagense–S. cheesmaniae*). We refer to these as "low–," "mid–," and "high–" intraspecific divergence trees, respectively. We tested for constraint (see "Phylogenetic Analysis of Constraint on θ_{LA}") on allometric scaling, using all three trees to examine whether

our results were robust to uncertainty in intraspecific branch length, although a priori we expected that modest distances (10%) were likely, since population structure within wild tomato species is moderately high (Nakazato and Housworth 2011; Tellier et al. 2011; Nakazato et al. 2012). All other phylogenetic regressions (see below) were performed on the 10% (mid–intraspecific divergence) tree only.

Phylogenetic Analysis of Constraint on θ_{LA}

MST predicts a universal tendency toward 3/4-power scaling between metabolic surface area (leaves) and body mass. This implies constraint on the adaptive landscape. Specifically, optimal scaling may differ between species, but deviations from the central tendency are eventually selected against. The Ornstein-Uhlenbeck (OU) model is a stochastic model of trait evolution that includes a long-term average (i.e., central tendency, μ) and a variance (σ^2). The amount of deviation from the average is determined by the amount of constraint (α). We tested for constraint on allometry by comparing the fit of an OU model to that of two simpler models without constraint, Brownian motion (BM) and white noise (WN). The BM model assumes high phylogenetic signal, whereas the WN model is nonphylogenenetic. We tested for constraint only on the constant scaling coefficient, which may be thought of as an average of mass-specific scaling coefficients, because the empirical pattern we want to address is the purported similarity in mass-invariant scaling across species.

We tested whether the OU model was a significantly better fit than either unconstrained model by using a simulation-based phylogenetic Monte Carlo method (Boettiger et al. 2012). We conducted 10^3 Monte Carlo simulations to empirically estimate the null distribution of the likelihood ratio test statistic. Each data set was simulated under the simpler, unconstrained model (BM or WN) and then fitted under both constrained (OU) and unconstrained models, and the likelihood ratio statistic was calculated. For all models, the fitting used the "fitContinuous" function in the geiger package, version 2.0.3 (Pennell et al. 2014). The P value was the proportion of simulated model comparisons in which the OU model did not improve the fit (likelihood ratio) as much as it did in the actual data. We predicted that not only would the OU model outperform the BM and WN models but that if the West-Brown-Enquist model were correct, the confidence intervals around μ would encompass 3/4. We calculated 95% confidence intervals on parameter estimates from 10^3 phylogenetic Monte Carlo simulations as described in Boettiger et al. (2012).

Experiment 2: Drought Response and Whole-Plant Water Use

We measured drought response in terms of time to wilting after withholding water. Initially, we allowed soil to dry

gradually by adding 100 mL of water twice during the week before we stopped watering completely on June 30 (50 days after germination). We assayed predawn wilting daily until all plants had succumbed to the effects of drought. We calculated a parameter, T_{wilt}, as the number of days to predawn wilting, after statistically removing block and transplant date effects with linear regression. Although counts are normally modeled as Poisson, we found that residuals of log-transformed T_{wilt} were normal.

The drought experiment cannot distinguish whether plants wilt sooner because they use soil water faster or because they are less tolerant of low soil water potential. Therefore, we measured whole-plant water use to examine whether this trait underlies variation in time to wilting. Since we were comparing allometry and T_{wilt} measured on separate groups of plants, we checked that water use was similar within an accession across experiments. We measured water-use rate (mL day^{-1}) on all plants (experiment 1: June 19–20; experiment 2: June 18–19). On the first day, we filled pots to field capacity and filled a plate reservoir below each plant. On the second day, we measured the volume of water it took to refill pots and reservoirs to capacity and calculated the time since we had initially filled the pots. The average time was similar for both experiment 1 ($\bar{t} = 21.52$ h) and experiment 2 ($\bar{t} = 21.23$ h) plants. Water loss from several empty pots (containing soil but no plants) interspersed throughout greenhouse benches indicated that most (>90%) water was lost through plant transpiration rather than through soil evaporation. We estimated a water-use rate coefficient for each accession, using linear mixed models fitted by restricted maximum likelihood in the lmer package (Bates et al. 2013). In separate models for each experiment, accession was a fixed effect because we were interested in exact coefficients for each taxon, and block was a random effect. Since late-germinating plants, regardless of accession, were slightly smaller and therefore used less water, we also included germination date (early or late) as a fixed effect.

We also calculated a water-use index (WUI) for every individual in experiment 2 (drought). For this, we combined the first water-use measurement described above with a second measurement taken a week later, on June 26. The second measurement differed slightly from the first in that we filled pots in the morning and refilled them in the evening of the same day ($\bar{t} = 9.65$ h). The water-use rate on the first and second measurements of individual plants were positively correlated ($r^2 = 0.45$, $P = 3.50 \times 10^{-38}$), even after accession and block effects were removed (partial correlation: $\rho^2 = 0.23$, $P = 6.01 \times 10^{-18}$), so we used the first principal component between the two measurements, which accounted for 84% of the variance, as our WUI.

Incorporating Phylogenetic Correction

We statistically accounted for the fact that shared phylogenetic history can cause more closely related species to appear similar. Following Revell (2010), we used phylogenetic least squares (PGLS) regression as implemented in the R packages caper, version 0.5 (Orme et al. 2012), and phylolm, version 2.2 (Ho and Ané 2014). First, we estimated phylogenetic signal by maximum likelihood with Pagel's λ (Pagel 1999). When $\lambda = 0$, there is no phylogenetic signal and PGLS is identical to ordinary least squares (OLS) regression. Second, we estimated phylogenetic signal by maximum likelihood with the OU model, where α indicates phylogenetic signal. Note that we are using the OU process here to model residuals in phylogenetic regression, not to test for constraint, as before. These models are equivalent to traditional phylogenetically independent contrasts (Felsenstein 1985) when $\lambda = 1$ and α, respectively. In many cases, the ML estimates of λ and α in OLS residuals were 0 and ∞, respectively, indicating no phylogenetic signal and that phylogenetic correction is unnecessary (Revell 2010). However, in small data sets such as ours, parameter estimation can be inaccurate (Boettiger et al. 2012), so we tested whether our results were robust to assumptions about phylogenetic signal by repeating those regressions, using PGLS with λ fixed to 1 (maximum phylogenetic signal). If there was evidence of phylogenetic signal, we report results from OLS, PGLS(λ), and PGLS(α); otherwise, we report OLS and PGLS (i.e., PGLS with $\lambda = 1$). Because we lacked statistical power, we did not use the more appropriate SLOUCH regression models (Hansen et al. 2008; Bartoszek et al. 2012), although doing so should be considered in future work with larger data sets.

Mass-Specific Scaling

Although linear scaling fit the data well, the curves appear somewhat nonlinear (see "Results"). This could indicate that allometric scaling is not constant but rather changes with mass. Hence, we refer to these models as "constant scaling" (linear) and "mass-specific scaling" (nonlinear). We tested for mass-specific scaling (i.e., nonlinearity) by fitting all possible models that included accession crossed with second- and third-order polynomials of mass. Alternative nonlinear models, such as a mixed-power model (Mori et al. 2010), did not fit the data as well as polynomials and often gave unrealistic parameter estimates. We selected the best model by using the Akaike information criterion. For these model comparisons, we used standard multiple linear regression because there is no exact analog of SMA for polynomial regression. Instead, after we identified the best model by using standard linear regression, we estimated the parameters again, using a total least squares (TLS) approach (SMA is a

special case of TLS). A detailed description of how we used TLS to fit the model and custom R scripts are available in appendix B.

Results

Experiment 1: Significant Interspecific Variation in Allometry

Leaf area (LA) scaled strongly with aboveground biomass (M) across all accessions (fig. S1A–S1Q). A statistical model including accession-specific intercepts (log B_0) and slopes (θ_{LA}) explained 97.6% of the variance in LA and indicated that there was significant among-accession variation in both log B_0 and θ_{LA} (table 1). Despite variation between accessions, θ_{LA} estimated with SMA was always close to 3/4, as predicted by MST (table 2). However, three accessions were significantly different from 3/4, but only one of those results remained significant after adjustment for multiple comparisons (table 2). Scaling exponents estimated with OLS rather than SMA were very similar (table B1; tables A1, B1, C1, C2 available online). In most places throughout the remainder of the article, we use "θ_{LA}" to refer to accession-specific or "ontogenetic" allometries, since it is an estimate of the growth in leaf area versus biomass through ontogeny of a particular accession.

Constraint on θ_{LA}

An Ornstein-Uhlenbeck (OU) model that includes a term for clade-wide constraint on θ_{LA} generally explained the trait distribution across the tree better than models without constraint (table 3). There was one exception: when we used the high–intraspecific divergence tree, the OU model was not significantly better than the unconstrained, nonphylogenetic white noise (WN) model (table 3). Although θ_{LA} differs between accessions (see above), variation away from quarter-power θ_{LA} appears to be constrained by natural selection. However, constraint did not mean that the allometry was phylogenetically conserved. Rather, θ_{LA} is evolutionarily labile despite tending toward a long-term average (table 3; high α indicates low phylogenetic signal). Importantly, the long-term average value

of θ_{LA} according to the OU model is not significantly different from the theoretically predicted 3/4 (table 3).

Experiment 2: Fecundity Is Negatively Correlated with Time to Wilting

In this experiment, drought response, measured as time to wilting, is largely determined by intrinsic genetic differences between accessions. By "genetic" we mean that accession explained a large and statistically significant portion of the trait variation. Since individuals were randomized within our greenhouse, consistent trait differences between accessions almost certainly reflect genetic, as opposed to chance environmental, differences between accessions. There was significant variation in the number of days until predawn wilting (T_{wilt}) among accessions ($F_{16, 242} = 32.66$, $P = 2.41 \times 10^{-51}$), which accounted for 63% of the variance. Similarly, variation in water use had a strong genetic component. Within an accession, water use in the two experiments was very similar. The correlation between accession water-use rate (mL H_2O day^{-1}) coefficients was strongly positive ($r^2 = 0.74$, $P = 8.16 \times 10^{-6}$); accessions that transpired a lot of water in experiment 1 also transpired a lot in experiment 2. This indicates that water use, like T_{wilt}, largely reflects genetic differences between accessions. Unsurprisingly, there was a strong negative correlation between time to wilting and the water-use index, which we discuss in the next section.

Allometry Mediates Drought Response and Fecundity

We tested whether there was a negative correlation between fecundity (experiment 1) and time to predawn wilting following drought stress (experiment 2) caused by variation in allometric scaling between aboveground biomass and leaf area. Results from phylogenetically corrected regression are always preceded by "PGLS"; otherwise, results are from OLS. Fecundity and T_{wilt} are negatively correlated (fig. 1; OLS: $r^2 = 0.26$, $P = .035$; PGLS: $r^2 = 0.26$, $P = .036$). Ontogenetic allometry between LA and M was a strong predictor of an accession's T_{wilt} and fecundity, but in the direction opposite the one we initially predicted. Accessions with a higher scaling exponent (θ_{LA}) took longer to wilt after the

Table 1: ANOVA indicates significant variation in both intercept ("Accession") and scaling ("Accession $\times$ log M") between aboveground biomass (M) and leaf area

Source	df	SS	MS	F	P
Intercept	1	252	252	7,603	9.07×10^{-191}
Accession	16	2.11	.132	3.99	9.43×10^{-7}
Log M	1	14.1	14.1	426	3.71×10^{-56}
Accession $\times$ log M	16	1.33	.089	2.52	.0013
Residual	253	8.38	.03		

Table 2: Estimated scaling exponents (θ_{LA}) and 95% confidence intervals (CI) from standard major-axis regression for all 17 accessions of wild tomato studied

Accession	θ_{LA} (95% CI)	P (no correction)	P (Šidák)
S. arcanum (LA2172)	.70 (.64–.75)	.058	.57
S. cheesmaniae (LA0429)	.72 (.65–.80)	.44	.99
S. chilense (LA4339)	.75 (.67–.84)	.97	1
S. chilense (LA0458)	.76 (.72–.80)	.66	1
S. chmiewlewskii (LA3643)	.70 (.63–.77)	.17	.86
S. corneliomulleri (LA3219)	.62 (.55–.70)	**.0029**	**.049**
S. galapagense (LA0436)	.79 (.71–.88)	.25	.93
S. habrochaites (LA1777)	.74 (.69–.79)	.64	1
S. huaylasense (LA1360)	.67 (.60–.76)	.071	.61
S. huaylasense (LA1983)	.70 (.65–.76)	.093	.69
S. neorickii (LA1321, LA1322)	.73 (.65–.81)	.59	1
S. pennellii (LA3791)	.74 (.69–.80)	.80	1
S. pennellii (LA0716)	.75 (.71–.80)	.95	1
S. peruvianum (LA2744)	.70 (.63–.78)	.23	.93
S. pimpinellifolium (LA1269)	.67 (.61–.73)	**.019**	.26
S. pennellii var. puberulum (LA3778)	.75 (.69–.80)	.88	1
S. sitiens (LA4112–LA4116)	.84 (.76–.92)	**.032**	.38

Note: *Solanum* species names and Tomato Genetics Resource Center accession numbers (in parentheses) are given in the left column (see table S1 in Supporting Information, available online for accession details). All θ_{LA} were significantly different from 0 (not shown) and had a central tendency toward 3/4, as predicted by metabolic scaling theory. The P values are the probability that θ_{LA} for a given accession is different from 3/4, before and after a Šidák correction for multiple comparisons. Statistically significant ($P < .05$) results are in boldface.

start of drought stress ($r^2 = 0.70$, $P = 2.61 \times 10^{-5}$; fig. 2A) but had lower fecundity ($r^2 = 0.49$, $P = .002$; fig. 2B). Although there was no evidence for phylogenetic signal in OLS residuals, these results were robust to phylogenetic correction (θ_{LA} vs. T_{wilt}: PGLS(λ): $r^2 = 0.25$, $P = .042$; PGLS(α): $r^2 = 0.70$, $P = 3.79 \times 10^{-5}$; θ_{LA} vs. fecundity, PGLS: $r^2 = 0.36$, $P = .011$). In contrast to θ_{LA}, the allometric constant (B_0)

was not significantly correlated with T_{wilt} ($r^2 = 0.03$, $P = .48$) or fecundity ($r^2 = 0.00$, $P = .87$). The scaling exponent appears to play a dominant role in mediating the relationship between T_{wilt} and fecundity in these species; the partial correlation between T_{wilt} and fecundity is not significant after conditioning on θ_{LA} ($\rho = 0.18$, $P = .49$). Mechanistically, accessions with lower θ_{LA} transpired more water, causing them to dry soil faster and wilt sooner (higher WUI; fig. S2), although we cannot rule out differences in tolerance to low soil water potential. The OLS correlation between θ_{LA} and WUI was highly significant ($r^2 = 0.74$, $P = 8.43 \times 10^{-6}$). Although there was no evidence of phylogenetic signal in the OLS residuals, the PGLS coefficients were similar (see fig. S2), but the relationship became marginally significant ($r^2 = 0.19$, $P = .08$). The relationship between θ_{LA}, WUI, and T_{wilt} was not an indirect effect of accessions with lower θ_{LA} having greater leaf area or being larger (see app. C). Thus, we conclude that θ_{LA} per se is strongly associated with both flower production and whole-plant water use, manifested in a negative correlation between fecundity and time to wilting in response to drought across wild tomato species.

Allometry Depends on Mass

There was strong statistical support for mass-specific allometric scaling in all accessions, including accession-specific coefficients (table S2; fig. S1A–S1Q). From the best-fit polynomial model, we calculated the clade-wide median and 95% confidence intervals of mass-specific allometric scaling from the mixture distribution of accession-specific ontogenetic trajectories (see app. D for details). These analyses showed that leaf area increased almost isometrically with aboveground biomass early in development ($\theta_{LA} \approx 1$) and decreased later in development (fig. 3). However, there is no indication from these data that θ_{LA} was approaching 3/4 or any other value, since 95% confidence intervals span $\sim$0.34–0.77 in the largest plants. Hence, we cannot infer from this experiment what allometric scaling would have been had we allowed plants to grow indefinitely. Allometry clearly changes through development but otherwise does not strongly support or reject the WBE model (see "Discussion").

Discussion

Metabolic scaling theory predicts that scaling between metabolic rate and size should cluster around quarter-powers (West et al. 1997, 1999; Enquist et al. 1999). Significant variation in scaling exists between species, but frequently not in agreement with quarter-power scaling (McCulloh et al. 2003; Bokma 2004; Price et al. 2007, 2012; Savage et al. 2008, 2010; Glazier 2010; Hudson et al. 2013). Here, we examined variation and constraint in the allometry between leaf area, which is assumed to be proportional to

Table 3: The scaling exponent (θ_{LA}) between leaf area and above ground biomass is constrained

Model, tree, parameter	ML parameter estimates (95% CI)	ln L	Likelihood ratio
Brownian motion (unconstrained; phylogenetic signal):			
Low–intraspecific divergence tree		11.08	**38.91 ($P < .001$)**
X_0	.76 (.09–1.40)		
σ^2	8.35 (7.68–58.35)		
Mid–intraspecific divergence tree		21.80	**16.84 ($P < .001$)**
X_0	.76 (.51–1.01)		
σ^2	1.38 (.54–2.33)		
High–intraspecific divergence tree		23.22	**9.65 ($P < .001$)**
X_0	.76 (.58–.95)		
σ^2	.68 (.21–.94)		
White noise (unconstrained; no phylogenetic signal):			
Low–intraspecific divergence tree:		27.37	**6.35 ($P = .007$)**
X_0	.73 (.55–.98)		
σ^2	.002 (.012–.050)		
Mid–intraspecific divergence tree			**5.71 ($P = .007$)**
High–intraspecific divergence tree			1.37 ($P = .107$)
Ornstein-Uhlenbeck (constrained):			
Low–intraspecific divergence tree		30.54	
μ	.72 (.70–.75)		
σ^2	34.62 (4.26–115)		
α	6,296 (777–34,495)		
Mid–intraspecific divergence tree		30.22	
μ	.72 (.69–.75)		
σ^2	4.66 (.86–14.6)		
α	858 (180–4,559)		
High–intraspecific divergence tree		28.05	
μ	.72 (.69–.75)		
σ^2	2.54 (.58–3,188)		
α	497 (122–10^6)		

Note: Statistical comparison of unconstrained trait models (Brownian motion [BM] and white noise [WN]) with constrained models (Ornstein-Uhlenbeck [OU]) demonstrates that models including constraint generally outperform those without. In one case, the OU model did significantly improve the fit, compared to the WN model, when we assumed high intraspecific branch length (see "Material and Methods"). Unconstrained-model parameters: X_0 = average θ_{LA} (weighted by phylogeny in the BM model); σ^2 = variance in θ_{LA}. OU model parameters: μ = long-term average θ_{LA}; σ^2 = variance in θ_{LA}; α = strength of constraint around μ. Maximum likelihood (ML) parameter estimates, 95% confidence intervals (CI) estimated using phylogenetic Monte Carlo, and model log likelihood (ln$L$) for both models are given. Note that the parameter estimates and likelihood of the nonphylogenetic WN model are not affected by changes in branch length. The likelihood ratio (LR $= 2(\ln L_{OU} - \ln L_{BM/WN})$) and P value from parametric bootstrap indicate that the OU model is usually a significantly better fit than the unconstrained models.

photosynthetic rate, and aboveground biomass in wild tomatoes. Our data show that although allometric-scaling exponents evolve rapidly between closely related species (tables 1, 2), variation is ultimately constrained around a value close to 3/4 (table 3). The parameter estimates from the fitted Ornstein-Uhlenbeck (OU) models indicate that ~550–14,000 years are sufficient to erase half of the phylogenetic information (i.e., correlation between species descended from a common ancestor). We calculated these dates from the phylogenetic half-life (log $\alpha/2$ in the OU model), assuming that the most recent common ancestor of species used in this study (including *S. sitiens*) occurred ~5–10 million years ago (Särkinen et al. 2013). Although this amount of phylogenetic signal was sufficient to statistically distinguish

models with and without constraint, it indicates that evolution of allometric scaling is extremely fast on macroevolutionary timescales.

Despite rapid evolution, scaling exponents were constrained. Under an OU process, the stationary distribution of a trait is normal, with a mean equal to the long-term average (μ) and a variance equal to $\sigma^2/2\alpha$. Using estimates from analysis on the mid–intraspecific divergence tree (table 3; $\mu = 0.72$, $\sigma^2/2\alpha = 2.72 \times 10^{-3}$), our data predict that 95% of species should have ontogenetic allometric-scaling coefficients between 0.62 and 0.82. Thus, even among species evolving under a selective regime with a central tendency toward quarter-power scaling, a substantial fraction may fall significantly above or below the average. However,
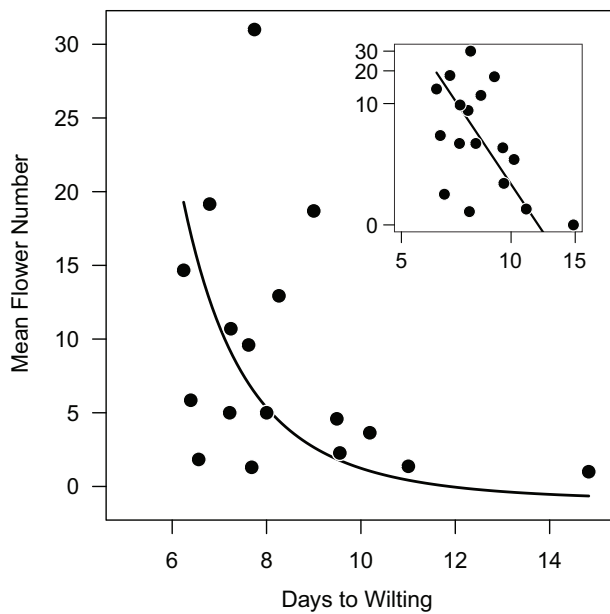
Figure 1: Fecund species wilted faster in response to drought. Fecundity (*Y*-axis) was measured as average flower number per plant in experiment 1. Drought response (*X*-axis) was measured as days to wilting after the start of drought stress in experiment 2. Each point represents an accession mean. The standard major-axis regression was fitted on a log-log scale (inset), but we plot data and best-fit curve on a nominal scale for reference.

constraint on metabolic scaling acting at both micro- and macroevolutionary scales.

The optimal ontogenetic allometry within a given species might evolve over time as the selective environment
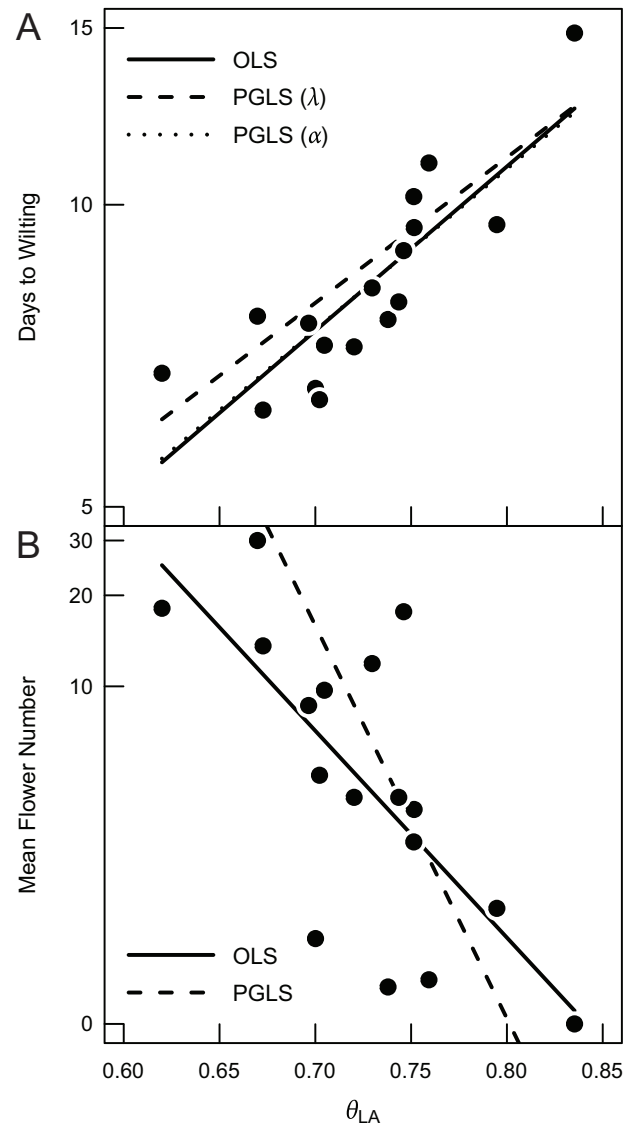
variation in allometric scaling between species is unlikely to be the result of stabilizing selection on a single adaptive landscape shared across species, as this would require unrealistically small population sizes and/or strength of stabilizing selection (Estes and Arnold 2007). Rather, our data are consistent with a model in which the adaptive landscape mapping θ_{LA} to fitness varies between species, but with peaks constrained around 3/4 (Hansen 2012; Pennell and Harmon 2013). Within a particular species, there may be a single fitness peak of optimal scaling that is set by environmental or other factors. The position of this peak evolves through time but does not stray too far from the long-term average. Extrapolated over long periods of time, rapid evolution bounded by constraint on the adaptive landscape leads to the pattern often observed in real data sets consisting of many, distantly related taxa: biologically significant variation around a central tendency toward quarter-power scaling. Therefore, our data do not support strictly universal allometry but rather that quarter-power scaling is a "basin of attraction" around which organisms cluster (Price et al. 2007). Our data bearing on macroevolutionary constraint offer a striking parallel to observations made by Vasseur et al. (2012) within species. Both studies evince constraint on allometric variation, possibly driven by fitness trade-offs. These studies point to general patterns of variation and

Figure 2: The scaling exponent (θ_{LA}) between leaf area (LA) and aboveground biomass mediates the trade-off between time to wilting and fecundity in wild tomatoes. Each point represents an accession mean. Accessions with higher θ_{LA} (*X*-axis) took longer to wilt after the onset of drought (*Y*-axis in *A*) but had lower fecundity (*Y*-axis in *B*). Note that both days to wilting and mean flower number are plotted on a log scale. Both relationships were similar and statistically significant whether we used standard ordinary least squares (OLS, solid lines) or phylogenetic least squares (PGLS, dashed or dotted lines). In *A*, we show two PGLS lines, one where phylogenetic signal was estimated with Pagel's λ (dashed line) and the other where it was estimated with the α parameter (dotted line nearly on top of the solid line) of an Ornstein-Uhlenbeck model.
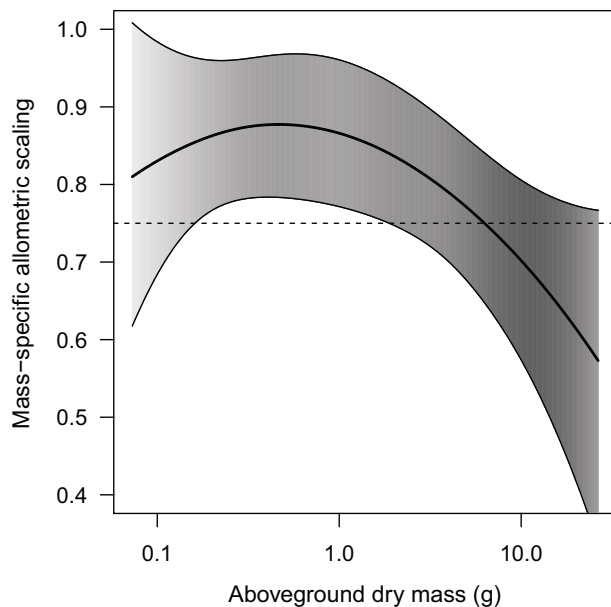
Figure 3: Clade-wide average allometric scaling decreases during ontogeny. Leaf area and plant dry mass are almost isometric (mass-specific allometric scaling near 1) early in development (aboveground dry mass < 1 g), but scaling decreases later in development. The thick line represents the median from the joint probability distribution of accession-specific ontogenetic curves (see app. C for detailed methods); thin lines represent the upper and lower 95% confidence intervals. Shading is proportional to the density of data used to estimate mass-specific scaling. For reference, the dashed line indicates the 3/4 predicted by the West-Brown-Enquist model.

changes, if allometry affects fitness. Indeed, we found that θ_{LA} was a very good predictor of traits that could affect fitness in nature: fecundity, whole-plant water use, and drought response (time to wilting). However, the data on drought response and fecundity must be treated with caution. The drought experiment, in particular, was highly artificial, in that there was no opportunity for drought hardening through osmotic adjustment, nor did we carefully control soil water content in a way that mimics natural soils. Because of experimental limitations, we could not measure fecundity directly and instead used flower number as a proxy. Keeping these caveats in mind, it is nevertheless striking that θ_{LA} was a better predictor of water use, time to wilting, and fecundity than other obvious predictors such as mass, leaf area, growth rate, or the allometric constant (app. C). These observations suggest that θ_{LA} is capturing, perhaps indirectly, an important component of plant growth that could be under selection in nature and demands both mechanistic and evolutionary explanations. We cannot definitively explain these unanticipated results with our current data set, but instead we offer ideas that we hope will stimulate new experiments.

There are two phenomena in particular that have to be explained. First, why do accessions with lower θ_{LA} use more water and therefore succumb to drought faster? Second, why do those same accessions have greater fecundity? We hypothesize that accessions with lower θ_{LA} have greater fecundity because they have more space to develop inflorescences but lose water faster. The exponent θ_{LA} is the ratio of growth rates for leaf area and stem mass (Poorter and Sack 2012); hence, accessions with lower θ_{LA} have "stemmier" growth (i.e., faster growth of stem mass relative to leaf area). Stemmier growth should facilitate greater fecundity in species, such as wild tomatoes, with indeterminate sympodial growth. During sympodial growth, the apical meristem differentiates into a determinate structure (leaf or inflorescence) while the shoot continues to grow from the lateral meristem. Wild tomatoes are typically 2- or 3-foliate, meaning that they produce 2 or 3 leaves between each inflorescence (Peralta et al. 2008). Greater allocation to stems consequently allows more sympodia to grow simultaneously and hence a greater number of inflorescences per unit time.

It is less clear, even counterintuitive, why accessions with greater stem mass growth relative to leaf area growth (lower θ_{LA}) use more water. In fact, we initially predicted that accessions with higher θ_{LA} (i.e., greater growth in leaf area relative to mass) would transpire more water, not less. However, this prediction was incorrect because leaf area in tomatoes was determined by both the allometric constant and the scaling coefficient. Furthermore, leaf area was not actually a strong predictor of whole-plant water use (table C2). This indicates that water loss was instead determined by hydraulic capacity and/or physiological regulation of leaf-level transpiration, independent of total leaf area. These hypotheses were not tested here but should be tested in future studies to identify the direct or indirect connection between allometric scaling and whole-plant water use.

The apparent tendency for scaling exponents to cluster around 3/4 must be qualified because scaling between mass and leaf area was not constant but changed during ontogeny within accessions (fig. 3). Hence, the constant scaling exponents we used to test for constraint are really integrated averages or approximations of mass-specific allometry. However, these log–log linear approximations are commonly used to evince quarter-power scaling at broad phylogenetic scales. Hence, it is still relevant to test whether mass-independent scaling exponents are phylogenetically constrained. The ontogenetic trajectory in allometry, averaged across accession-specific ontogenetic allometries, was consistent with the West-Brown-Enquist (WBE) model but also with many other models. The WBE model predicts that leaf area and mass should increase isometrically early in development (<1 g; Enquist et al. 2007*b*) and decline asymptotically to 3/4 as mass increases. Indeed, the best model

for our data indicates nearly isometric growth early in development (<1 g). Once plants reach ~1 g, mass-specific allometry declines rapidly in all accessions. However, the wide confidence intervals around mass-specific allometry in the largest plants preclude us from discriminating among competing theories (e.g., 3/4 vs. 2/3 scaling). Contrary to predictions of the WBE model, there is no evidence that allometric scaling is reaching a particular asymptote, although an asymptote may have become apparent if the experiment had been run longer. Our data are consistent with many other studies that find that mass-specific metabolism in plants declines during ontogeny (Reich et al. 2006; Mori et al. 2010; Peng et al. 2010) but do not strongly support any particular model. We need more-explicit quantitative predictions of mass-specific allometry (e.g., Savage et al. 2008) in order to rigorously infer what is driving allometric variation within and between species.

In conclusion, mass-invariant allometric scaling evolves rapidly in wild tomatoes but is nevertheless constrained around an "optimal" 3/4-power relationship. To our knowledge, this is the first study to use a phylogenetic approach to test for macroevolutionary constraint on allometric scaling. That allometry evolves rapidly yet is apparently constrained, according to the WBE model, by a combination of selective and functional constraints indicates that allometric variation may be adaptive rather than shaped by immutable developmental processes (Maynard Smith et al. 1985). Although we find suggestive evidence for constraint on quarter-power scaling when curvilinearity is ignored, we caution that it is premature to conclude that our results strongly support the WBE model in general. For example, further work is required to test whether the many assumptions (Savage et al. 2008; Price et al. 2012) underlying the WBE model are valid in these species. Furthermore, scaling changed during ontogeny in a pattern that did not clearly support or reject the WBE model. Nevertheless, we demonstrate that phylogenetic comparative methods are a complementary approach that should be used to test particular evolutionary predictions of metabolic scaling models with additional data sets. Finally, the fact that allometric-scaling exponents evolve rapidly, appear to be constrained across species, and are strongly correlated with fecundity, water transport, and drought response indicates that this trait could be an important component of local adaptation in wild tomatoes. Thus, our study opens up new testable hypotheses on how allometric variation affects fitness, driving variation and constraint on organismal growth and form over evolutionary time.

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A leaf of the wild tomato *Solanum chilense*. The leaf area of tomato plants closely follows a 3/4 power-law relationship with plant size. Photograph by Christopher D. Muir.