

RESEARCH PAPER

Growing larger with domestication: a matter of physiology, morphology or allocation?

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Keywords

Cabbage; chard; functional trait; maize; plant size; sunflower; tomato; wheat.

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Editor

D. Byers

Received: 15 December 2016; Accepted: 9 January 2017

doi:10.1111/plb.12545

ABSTRACT

- Domestication might affect plant size. We investigated whether herbaceous crops are larger than their wild progenitors, and the traits that influence size variation.
- We grew six crop plants and their wild progenitors under common garden conditions. We measured the aboveground biomass gain by individual plants during the vegetative stage. We then tested whether photosynthesis rate, biomass allocation to leaves, leaf size and specific leaf area (SLA) accounted for variations in whole-plant photosynthesis, and ultimately in aboveground biomass.
- Despite variations among crops, domestication generally increased the aboveground biomass (average effect +1.38, Cohen's *d* effect size). Domesticated plants invested less in leaves and more in stems than their wild progenitors. Photosynthesis rates remained similar after domestication. Variations in whole-plant C gains could not be explained by changes in leaf photosynthesis. Leaves were larger after domestication, which provided the main contribution to increases in leaf area per plant and plant-level C gain, and ultimately to larger aboveground biomass.
- In general, cultivated plants have become larger since domestication. In our six crops, this occurred despite lower investment in leaves, comparable leaf-level photosynthesis and similar biomass costs of leaf area (*i.e.* SLA) than their wild progenitors. Increased leaf size was the main driver of increases in aboveground size. Thus, we suggest that large seeds, which are also typical of crops, might produce individuals with larger organs (*i.e.* leaves) *via* cascading effects throughout ontogeny. Larger leaves would then scale into larger whole plants, which might partly explain the increases in size that accompanied domestication.

INTRODUCTION

Domestication and later evolution under cultivation have profoundly affected the phenotypes of crop species. Among other phenotypic shifts (Hancock 2004), domestication is usually considered to promote increases in plant size (Milla *et al.* 2014; Vico *et al.* 2016). The size of an organism influences its physiology and ecological strategy (Niklas 1994; West *et al.* 1997; Arendt 2007), as well as its interactions with other species and with conspecifics (Kraft *et al.* 2015), and its impacts on ecosystem processes (Grime 2001). In addition, size is critical for reproductive output of crop plants because fecundity and reproductive allocation are correlated with plant size (Obeso 2004). Size affects resource acquisition and biomass build-up directly, as well as indirectly because the allocation of biomass to different plant parts and processes is size-dependent (Porter *et al.* 2015). Therefore, understanding the drivers and effects of domestication on plant size are important research topics.

Gigantism in the plant organs harvested for human use is a common outcome of domestication (Fuller 2007; Meyer *et al.* 2012). However, quantitative information about the response of whole-plant size to domestication is scarce. A re-analysis of data from recent studies where plant size was reported for a

wide diversity of wild progenitors and domesticates grown under comparable experimental setups showed that the effects of domestication processes on plant size might be more diverse than previously considered [Fig. 1, containing a re-analysis of data from Milla *et al.* (2014)]. Fourteen out of 27 herbaceous crops exhibited size increases with domestication, the size of ten crops remained the same, and the three others became smaller during evolution under cultivation (Fig. 1). Summary data obtained from two other multi-species studies using crops and wild progenitors suggest similar patterns (Turcotte *et al.* 2014 Turcotte *et al.* 2015). Thus, domestication increases plant size more frequently than it decreases size. However, the effects of domestication on size differ in their magnitude, as well as the direction of the size shift to a lesser extent. In this study, we investigated the plant traits that underlie variations in aboveground plant size among crops and their wild progenitors. We also explored whether traits that are functionally linked to aboveground plant size have changed during domestication, thereby accounting for the general but diverse increases in plant size with domestication.

The physiological, allocation or morphological shifts that have accompanied variations in plant size during crop evolution remain largely unknown. Indeed, there may be various

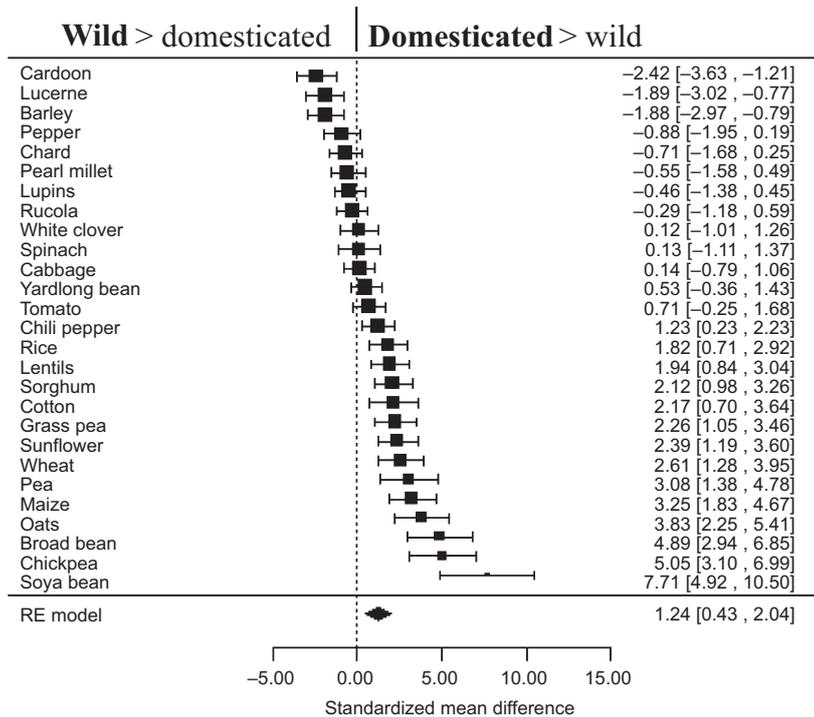


Fig. 1. Forest tree plot showing the effects of domestication (Cohen's d) on plant size (total plant dry mass, g) in a range of 27 herbaceous crops, where domesticated and wild progenitor accessions were grown under standard experimental set-ups. Wild and domesticated accessions differ if the confidence interval of their standardised mean difference does not overlap with zero. Data re-analysed from Milla *et al.* (2014) (Dryad repository: <http://datadryad.org/resource/doi:10.5061/dryad.g85v>). Data represent means \pm 95% confidence interval. RE model: overall mean of the random-effects model.

proximate drivers of plant size. For example, changes in the carbon uptake rate can boost the relative growth rate, thereby leading to increased plant size after growth for a comparable time period (Reich *et al.* 1997). The effects of domestication on photosynthetic performance are unclear (Cook & Evans 1983; Evans 1993), and thus it is not known whether changes in leaf physiology might account for variations in the size of crops. In terms of biomass allocation, larger plants require higher proportional investment in supporting tissue, even in herbaceous species (Poorter *et al.* 2015). Furthermore, there is evidence that crops have evolved into taller plants (Milla *et al.* 2014), which suggests that the allocation to stem tissues has increased. Therefore, shifts in biomass allocation during domestication might partly explain variations in plant size. The size and morphology of individual organs may also affect overall plant size, possibly due to allometric and biomechanical requirements (Price *et al.* 2014), and/or to the effects of size and shape of early developing organs on the subsequent build-up of biomass during growth (*i.e.* ontogenetic cascading effects; McKinney & McNamara 1991).

To understand how physiological, allocation and morphological traits affect the evolution of aboveground plant size under domestication, we addressed the following questions: (i) did the aboveground biomass of a set of six taxonomically diverse crops increase during domestication and, if so, was the increase in magnitude of the aboveground size similar in the six crops? (ii) do shifts in photosynthesis, allocation to leaf and stem tissues, leaf size and morphology explain the diverse range of biomass gains?

MATERIAL AND METHODS

We measured the aboveground biomass gained during the vegetative stage in chard (*Beta vulgaris* var. *cicla* L.), cabbage (*Brassica oleracea* var. *acephala* DC.), sunflower (*Helianthus*

annuus L.), tomato (*Solanum lycopersicum* L.), durum wheat *Triticum durum* (Desf.) and maize (*Zea mays* var. *mays* L.), and in each of their recognised wild progenitors. Using univariate and path analysis techniques, we tested whether the rates of C uptake, proportional allocation to leaf blades, leaf size and specific leaf area (*i.e.* SLA, the amount of light-capturing leaf area obtained with a unitary investment in leaf tissue) changed with domestication, and whether these changes explained the variations in whole-plant C uptake and, ultimately, in plant size.

Study system, experimental design and plant growth conditions

For each crop, we obtained seed lots of two accessions: the recognised wild progenitor and a cultivated variety of the crop (Table 1). Thirty seeds from each accession were germinated in sterilised germination paper set into Petri dishes soaked with distilled water. After the radicle emerged from the testa, 15 seedlings were transplanted into pots, with one germinated seed per pot. The pots (1.4 l; 10 × 10 × 17 cm) were filled with sand and supplemented with slow-release fertiliser (8 g l⁻¹; Basacote Plus 6M; Compo, Barcelona, Spain). The amount of fertiliser was set to grow plants under high-nutrient availability conditions (Day *et al.* 2003). All of the pots were arranged randomly on a greenhouse bench at Universidad Rey Juan Carlos (Móstoles-Madrid, central Spain). Watering was applied regularly with a sprinkler to maintain the pots at 90–100% of the field water-holding capacity.

Measurement of traits and plant harvest

The aboveground parts of the plants from a given accession were harvested on five consecutive days after 8 weeks of growth when flower buds or bolting were observed in the earliest

Table 1. Botanical and common names, domestication status and seed origin information for each accession used in this study. Domestication status (D: domesticated; W: wild ancestor).

common name	botanical name	domestication status	accesion identifier	seed donor	accesion country
chard	<i>Beta vulgaris</i> var. <i>cicla</i> L.	D	N.A.	Clausea	Commercial
	<i>Beta vulgaris</i> subsp. <i>maritima</i> (L.) Arcang.	W	1582	IPK	Italy
cabbage	<i>Brassica oleracea</i> var. <i>acephala</i> DC.	D	N.A.	Rocalba	Commercial
	<i>Brassica oleracea</i> L.	W	18947	CGN	Germany
sunflower	<i>Helianthus annuus</i> L.	D	HEL226	IPK	USA
	<i>Helianthus annuus</i> L.	W	PI413093	NPGS	USA
tomato	<i>Solanum lycopersicum</i> L.	D	N.A.	Clausea	Commercial
	<i>Solanum pimpinellifolium</i> L.	W	LA1383	NPGS	Peru
durum wheat	<i>Triticum durum</i> (Desf.)	D	BGE020911	CRF	Commercial
	<i>Triticum diccoides</i> (Korn. ex Asch. & Graebn.) Schweinf	W	352322	NPGS	Lebanon
maize	<i>Zea mays</i> subsp. <i>mays</i> L.	D	Ames26252	NPGS	Brazil
	<i>Zea mays</i> subsp. <i>mexicana</i> (Schrad.) Iltis	W	PI566674	NPGS	Mexico

Seed donor (IPK: Germplasm bank of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany; NPGS = National Plant Germplasm System-USDA, USA; CRF = Centro Nacional de Recursos Fitogenéticos-INIA, Spain; CGN = Center for Genetic Resources, The Netherlands; acommercial company). Accession identifier refers to the code assigned by each seed donor, excepting the commercial companies (N.A. = not applicable). Accession country refers to the country where the seeds were collected, if applicable.

flowering species (tomato and wheat in our experiment). We did not use strict phenological criteria (e.g. harvesting each plant at flower induction) to ensure comparability among all the accessions in the experiment. All the replicates for each domesticated–wild pair were harvested on the same day. Before harvesting, the following plant measurements were performed. Physiological measurements were obtained using a subsample of four to six (median five) individuals per accession ($N = 62$). Data were collected between 10:00–13:00 h on three homogeneously sunny days (28–30 April 2014). The *in situ* instantaneous photosynthetic rate (PHOTOSleaf, $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$) was measured using one fully expanded leaf per plant with a LiCor 6400 infrared gas analyser with a built-in LED light source and CO_2 mixer (LI-COR, Lincoln, NE, USA). Measurements were acquired using a reference $[\text{CO}_2]$ of 400 ppm, PFD of $1200 \mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ and gas flow of $500 \mu\text{mol} \cdot \text{s}^{-1}$. The relative humidity was kept constant and close to ambient conditions (range: 20–40%). The air temperature ranged from 20–30 °C. Measurements were logged only when the stability criteria were met (Licor 6400 User's Manual; Li-COR). If the leaf did not cover the chamber completely, leaf tracings were scanned and the leaf area was calculated using IMAGEJ software (<http://imagej.nih.gov/ij/>).

Leaf area (LEAF SIZE, dm^2) was measured as the area of one side of a fresh representative leaf lamina per individual, which was scanned at 400 dpi resolution and analysed using IMAGEJ software. Leaf laminae were then placed in a drying oven set at 65 °C for 3 days. SLA ($\text{dm}^2 \cdot \text{g}^{-1}$) was measured as the ratio of the area of one side of a fresh leaf lamina divided by its oven-dried mass, to the nearest mg (Pérez-Harguindeguy *et al.* 2013). The other leaf laminae, petioles and stem tissues were harvested from each plant and bagged separately, before oven-drying as described above and weighing. The ratio of the total leaf laminae mass relative to the mass of petioles plus stems (LEAF:STEM, unitless) was computed as a proxy measure for the proportional investment in leaf blades *versus* structural support. The total estimated leaf area (TELA, $\text{dm}^2 \cdot \text{plant}^{-1}$) per

individual plant was calculated by multiplying the total leaf lamina mass by the SLA. The plant-level instantaneous photosynthesis rate (PHOTOSplant, $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$) was computed by multiplying the arithmetic mean of the PHOTOSleaf by the TELA. Finally, the aboveground biomass per plant (ABOVEGROUND BIOMASS, g) was computed as the sum of all the harvested aerial parts, which was used as a proxy measure for the aboveground plant size. The sample size ranged between nine to 12 individuals per accession (median 11) for all traits, although the median was five for PHOTOSleaf.

Statistics

There were no missing data. Outliers were identified using box plots stratified per trait and accession if they exceeded generous lower ($Q1 - 3 \cdot \text{IQ}$ range) or upper ($Q3 + 3 \cdot \text{IQ}$ range) line bounds on the box plot. Five individual plants were detected as outliers for at least one trait, and thus were removed from further analyses.

Two levels of analyses were implemented. First, at the univariate level, we determined whether domestication and crop identity explained the variation in each of the measured traits independently of each other. The raw data for continuous traits were log-transformed prior to analyses in order to meet the normality and homoscedasticity criteria for the residuals. A series of linear models was then produced with each of the log-transformed traits as a response variable and domestication status (domesticated or wild) and crop identity (maize, wheat, tomato, sunflower, cabbage or chard), plus their interaction as fixed effects predictors. The statistical significance of each of the three terms in the model was assessed using Type III ANOVAs with the ANOVA function in the car package in R (Fox & Weisberg 2011). In addition, Cohen's d was computed using the ci.smd function in the MBESS 3.0 package in R (Kelley 2007) as a measure of the size of the domestication effect. The interaction term 'domestication status \times crop identity' was generally significant in the joint models, so the procedures described

above were repeated separately for each of the six crop species in the study. We applied *t*-tests that assumed unequal variances for the latter tests. The proportion of false discoveries due to multiple *t*-tests was corrected using the false discovery rate method (Benjamini & Hochberg 1995) with the `p.adjust` function in the stats package in R (R Core Team 2013). After running the models, the residuals obtained from the *t*-tests and linear models were tested for normality and homoscedasticity.

Second, at a multivariate level, confirmatory path analysis was performed to determine: (i) whether morphological (SLA and LEAF SIZE) and allocation (LEAF:STEM ratio) traits influenced the biomass gain directly; (ii) whether TELA and PHOTOSplant, as proximate components of ABOVEGROUND BIOMASS, determined the latter and how they were influenced by morphological and allocation traits; and (iii) the indirect (*via* the traits that we measured) and direct (*via* unknown mechanisms) effects of domestication status on the ABOVEGROUND BIOMASS. Leaf-level C gain (PHOTOSleaf, units) could not be incorporated in the multivariate analyses because we had insufficient replicates.

The *a priori* path model was specified to allow us to estimate all of the biologically plausible relationships based on points (i), (ii) and (iii) above. It should be noted that constraining a given path to zero (and thus not specifying it in the model) makes a stronger assumption than allowing it to be freely estimated, provided that it is a functionally plausible link between traits. The global model fit was assessed using a d-separation approach (Shipley 2009). We identified all of the links in the *a priori* model that were constrained to zero, and tested for each of their conditional independencies. The collection of *P*-values thus obtained was used, together with the number of conditional independencies, to calculate Fisher's *C* statistic as the goodness-of-fit for the proposed path model to the data, and its associated *P*-value (Shipley 2002). Estimates of the standardised path coefficients and their statistical significance were

computed for the predictors pointing to each endogenous variable in the model by running linear models based on z-standardised variables. Each individual model employed to assess independency or to estimate path coefficients included the structure of the data set (*i.e.* crop identity and domestication status; Shipley 2009). This was the case for all of the models, except for those in which domestication status was one of the predictors involved (*e.g.* the model used to estimate the path coefficient: Domestication → Leaf Size). In these models, only crop identity represented the structure of the data set. All of the univariate and multivariate analyses were performed in the R environment (R Core Team 2013).

RESULTS

The main response variable in our study was plant size, which we measured as the aboveground biomass of pre-reproductive individuals. Plant size varied by two orders of magnitude in our experiment, where it ranged from 0.88 g for the smallest wild cabbage plant to 19.9 g for the largest domesticated maize individual. The range of variation in morphological and allocation traits was also high (Table 2, S1, Fig. 2). However, leaf-level photosynthesis did not vary greatly among crop species or between domestication status within crops (Table 2, S1, Fig. 2). Overall, the coefficients of determination (R^2 in Table 2), Cohen's *d* scores (Fig. 2) and the strength of the path coefficients and Chi-square values of our path analysis (Fig. 3) all indicated the high explanatory power of the predictors used in the different models (domestication status, crop identity and/or trait scores) with respect to the variation in size and other traits, except for leaf-level photosynthesis.

Domestication generally increased plant size (ABOVEGROUND BIOMASS; Table 2) but in different ways for each crop. Domestication significantly increased the size of cabbage, sunflower, wheat and maize plants, whereas chard and tomato

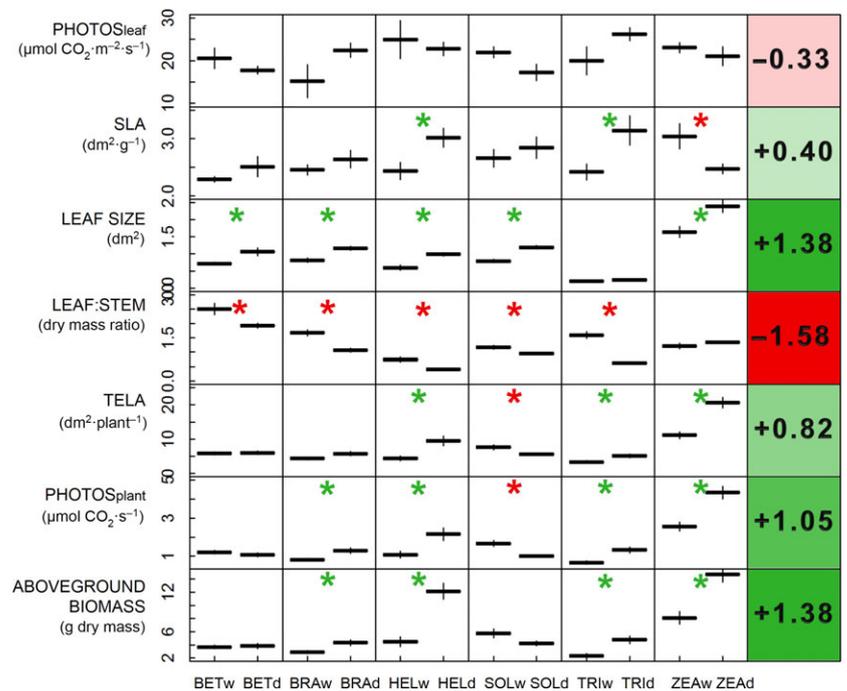
Table 2. Summary statistics of univariate analyses. Each model included log-transformed trait scores as the response variable, and Domestication status (domesticated or wild, 1 df) and Crop identity (maize, wheat, tomato, sunflower, cabbage or chard, 5 df), plus their interaction (5 df) as fixed-effects predictors.

fixed-effect predictor	PHOTOSleaf		SLA		leaf size		leaf:stem	
	F	P-value	F	P-value	F	P-value	F	P-value
domestication status	0.684	0.412	5.521	0.020	49.520	<0.000	72.352	<0.000
crop ID	1.189	0.329	1.588	0.169	130.127	<0.000		<0.000
domestication status*Crop ID	1.815	0.128	3.711	0.004	1.231	0.299	11.834	<0.000
size of domestication effect	−0.33		0.40		1.38		−1.58	
R2 of model	0.25		0.21		0.86		0.81	

	TELA		PHOTOSplant		ABOVEGROUND BIOMASS	
	F	P-value	F	P-value	F	P-value
domestication status	12.678	0.001	14.752	<0.000	35.278	<0.000
crop ID	25.663	<0.000	25.864	<0.000	28.506	<0.000
domestication status*Crop ID	3.411	0.006	6.453	<0.000	7.326	<0.000
size of domestication effect	0.82		1.05		1.38	
R2of model	0.58		0.61		0.65	

Statistical significance of each of the three terms in the model was assessed using Type III ANOVAS. Cohen's *d*, averaged across the six crops, was the metric of the size of the Domestication effect. The interaction term Domestication status × Crop identity was generally significant, thus species-wise analyses were carried out and their results are reported in Fig. 2. Bold values indicate significant differences.

Fig. 2. Effects of domestication on six physiological, morphological and allocation traits, and on the above-ground biomass gain. For each trait, each plot shows the mean (\pm SE) for the wild progenitor (w) and domesticated (d) accession of each of six crop species. Asterisks indicate significant differences ($P < 0.05$) between the domesticated and wild accessions for a given crop species based on t -tests adjusted for the false discovery rate due to multiple testing. The plots on the right of each row show the standardised mean effect (Cohen's d) of domestication averaged across all crops. BET, BRA, HEL, SOL, TRI and ZEA represent *Beta*, *Brassica*, *Helianthus*, *Solanum*, *Triticum* and *Zea*, respectively. The subscripts d and w indicate domesticated and wild accessions of each crop.



reached sizes that were statistically comparable to those of their wild progenitors (Fig. 2). The magnitude of domestication effects on plant biomass measured as the Cohen's d standardised mean difference ranged from +2.10 in sunflower to -0.66 in tomato (mean +1.38; Fig. 2). Leaf-level C gains (PHOTO-Sleaf) did not differ with domestication status in each crop (Table 2, Fig. 2). Similarly, domestication increased the SLA only in sunflower and wheat, whereas it decreased in maize, and there was no net effect in any other crops (Table 2, Fig. 2). Domestication led to larger leaves (LEAF SIZE) in all crops except wheat, and lower proportional investment in leaf laminae (LEAF:STEM ratio) in all crops except maize (Table 2, Fig. 2). The cultivated accessions of sunflower, wheat and maize had higher light-capturing leaf areas per individual plant (TELA) compared with their wild progenitors. In other crops, the response of the plant-level leaf area to domestication was either not significant, or the opposite in tomato (Fig. 2). Domestication increased plant-level C gains (PHOTOS_{plant}) in all crops except tomato and chard (Fig. 2).

Our *a priori* path model fitted the data adequately ($\chi^2 = 12.05$, $P = 0.28$). Among crop species, domestication generally increased the leaf size but decreased the biomass allocation to leaves (LEAF:STEM ratio; Fig. 3). Larger leaves yielded plants with a higher light-capturing total leaf area (TELA) irrespective of the proportional biomass allocation to leaves (LEAF:STEM ratio) and the biomass costs of leaf area investment (SLA; Fig. 3). Plants with a higher light-capturing leaf area (TELA) developed into larger plants overall, regardless of the instantaneous C uptake rate per unit leaf area (PHOTO-S_{leaf}) as integrated in PHOTOS_{plant}; Fig. 3). Interestingly, other effects of domestication that were not accounted for by our set of traits did not influence plant size (non-significant DOMESTICATION \rightarrow ABOVEGROUND BIOMASS direct path; Fig. 3). In addition to the overriding effect of TELA, other traits such as SLA, leaf size and LEAF:STEM had more modest

direct effects on the ABOVEGROUND BIOMASS. Thus, individuals with larger leaves, but lower SLA and LEAF:STEM ratio, tended to have a larger ABOVEGROUND BIOMASS (Fig. 3).

DISCUSSION

To the best of our knowledge, this is the first study to evaluate the roles of a diverse set of traits in the evolutionary response of aboveground plant size to cultivation. In general, we found that size increased after domestication, although to different extents in our six crop species. Interestingly, the leaf-level physiology was similar in the cultivated and wild progenitor accessions, and most cultivars allocated a lower proportion of the biomass to leaf tissues than their corresponding wild progenitors. In contrast, domestication consistently selected for larger individual leaves, which yielded larger plants because large-leaved individuals generally had a higher total leaf area per plant and they achieved higher plant-level C gains given that leaf-level photosynthesis varied little. Thus, increases in aboveground size occurred independently of the leaf-level C uptake rate and irrespective of reductions in the proportional biomass allocation to leaf tissues.

Aboveground plant size variation with domestication: a role for the size of individual organs

In our experiment, domestication increased the aboveground plant size in four out of six crops. In addition, the increases in size varied among crops, which ranged from large changes in sunflower to smaller ones in cabbage. Thus, domestication generally increased aboveground plant size but to different extents depending on the crop species, including crops where size was not affected by domestication. Our findings agree with the results of previous studies based on a wider range of species

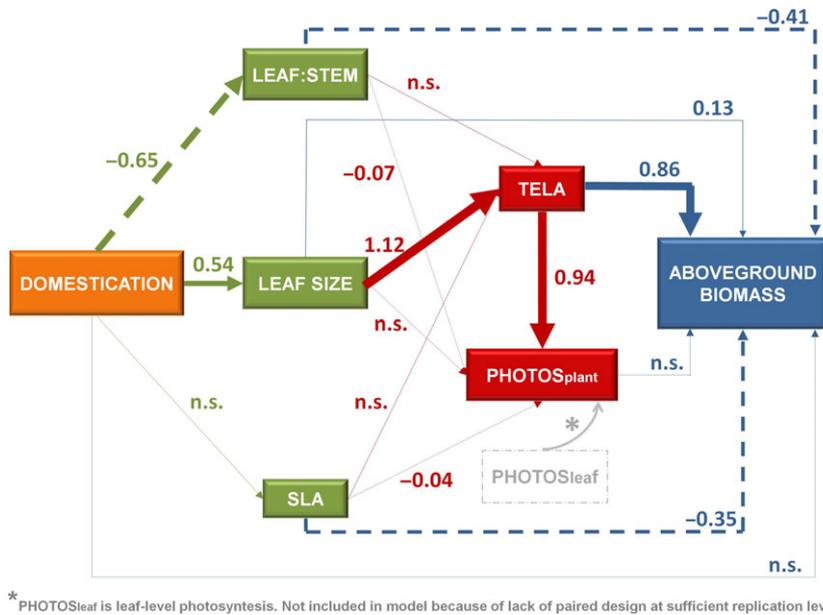


Fig. 3. Path model of domestication effects on traits and the cascading effects of traits on biomass gain for all six crop species combined. Each individual model in the path diagram is a general linear model, where the crop species identity is included as a fixed-effect predictor (see the Material and Methods for details). Numbers on each path are maximum likelihood estimates of the standardised path coefficients (thickness of the path is proportional to the coefficient size). n.s. = path not significantly different from zero at $P = 0.05$. Dashed line: negative effect; solid line: positive effect. DOMESTICATION: domestication effect (ordinal variable where wild = 0 and domesticated = 1); LEAF:STEM: leaf to stem dry mass ratio; PHOTOS_{plant}: plant level photosynthesis; TELA: total estimated plant leaf area; SLA: specific leaf area; LEAF SIZE: individual leaf lamina area; ABOVEGROUND BIOMASS: aboveground dry mass of individual plants at the end of the vegetative growth period. Fit of model: $\chi^2 = 12.05$; $P = 0.28$.

(Milla *et al.* 2014; Turcotte *et al.* 2014, 2015; Fig. 1). Given this overall pattern and the functional relevance of plant size (Niklas 1994), there is a strong case for investigating the drivers and effects of variation in size triggered by domestication.

The resource acquisition capacity required to increase plant size can be enhanced in at least two ways. First, acquisition processes can be optimised by either increasing the rate of resource acquisition (*i.e.* instantaneous rate of photosynthesis in leaves or rate of mineral nutrient uptake by roots) or by fine-tuning the allocation patterns to maximise investment in physiologically active tissues (Poorter & Remeks 1990; Poorter & Van Der Werf 1998). Root physiology was not examined here, which constitutes a limitation of the current study. To the best of our knowledge, no previous comparative studies have investigated the effects of domestication on nutrient uptake by roots. In terms of the C acquisition rate, similar to a previous study (Evans 1993), we did not find that crops consistently increased their photosynthetic performance after domestication (Fig. 2), thereby suggesting that its potential effects on plant size variation are limited. In general, the interspecific variation in leaf-level C gain and other leaf traits linked to the leaf economics spectrum were not related to variations in plant size (Price *et al.* 2014), which agrees with the predictions of metabolic scaling allometry (West *et al.* 1999; Brown *et al.* 2004). Furthermore, the increase in leaf size that accompanied domestication might be associated with lower physiological returns (Niklas *et al.* 2007; Milla & Reich 2007; analogous results for organ sizes in animals were reported by Maino & Kearney (2015). This is because large leaves invest proportionally less in photosynthetic tissues and require more structural support within the leaf tissues (Milla & Reich 2007), stems and other support structures, as found in the present study. In terms of allocation to productive tissues, we found that domesticates invested a higher proportion of biomass into support. Similarly, a previous study also failed to find evidence for preferential allocation to productive tissues with domestication (Milla & Morente-López 2014). Thus, the efficiency of C gain or allocation strategies appeared to contribute little to the increased aboveground plant size in our study.

Further mechanisms that might lead to large plants include organ-to-organism allometry (Price *et al.* 2014) and ontogenetic cascades (McKinney & McNamara 1991), which may fit our results better. Organ size affects plant size *via* biomechanics (Sperry *et al.* 2012); for example, larger leaves need larger petioles and thicker vascular strands require larger sustaining stems, thereby leading to larger overall plant sizes (it should be noted that this conflicts with the assumptions of invariance in metabolic scaling allometry theory; West *et al.* 1999). Indeed, previous studies have reported positive interspecific relationships between plant and leaf sizes (Fonseca *et al.* 2000; Price *et al.* 2014; but see Wright *et al.* 2007). Thus, allometric scaling promotes interdependency among the sizes of individual organs, which might ultimately affect plant size. In addition, the size of individual organs might influence plant size *via* cascading effects throughout ontogeny and through maternal effects. Larger organisms tend to produce larger offspring (Blueweiss *et al.* 1978; Moles *et al.* 2004). In plants, larger seeds frequently yield larger seedlings with bigger organs (Baraloto *et al.* 2005), which provide a head start in growth and in the competitive race for light (Paulsen & Högestedt 2002). An early advantage in terms of the size of leaves and fine roots means that productive organs are larger irrespective of the mass-based efficiency of the C or N uptake rates, and thus they acquire a higher proportion of the available resources on a per-individual basis regardless of the per-mass rate. If all other factors are equal and given ontogenetic time, the initial size advantage in terms of resource acquisition should scale up to larger leaves, stems or roots in the adult plant, and thus larger plants overall. In this study, we obtained evidence that larger leaves do lead to bigger plants. According to our path analysis, domestication selected for larger-leaved plants with a increased light-capturing leaf area per individual, and they grew into bigger organisms aboveground. Interestingly, other studies have reported that the size of seeds (Fuller 2007; Kluyver *et al.* 2013) or other plant organs (*e.g.* leaves; Baack *et al.* 2008; fruits Savage *et al.* 2015) has increased frequently during evolution under cultivation. Moreover, after re-analysing the data obtained from a previous multi-species study (Milla *et al.* 2014), we found that

the effect of domestication on plant size was positively correlated with the same effect on seed size (Fig. S1). Overall, these findings suggest that ontogenetic or organ-to-organism allometry might be a relevant driver of plant size evolution under domestication. Thus, future research should incorporate seed and plant size evolution under domestication into models of body size optimality (Allen *et al.* 2006). Adult and offspring sizes are subject to multiple life history trade-offs, so ever-increasing size is not optimal under natural selection (Kozłowski 1996). However, provided that selection and the nature of the constraints differ when plants evolve under cultivation (Litrico & Violle 2015; Milla *et al.* 2015), optimal size distributions may differ from those encountered in the wild for both the offspring (*i.e.* seeds) and adults (*i.e.* mature plants). A second research aim should be assessing the relative importance of the initial size (of seeds and seedlings), relative growth rate (RGR) and the dependence of RGR on organism size for generating variation in adult plant size, which can be achieved using modern plant growth analysis modelling (Paine *et al.* 2012). In addition, experimental limitations of the current study, like the size of our pots, which might have constrained root growth in the latest stages of the experiment, or the establishment of criteria for timing of plant harvesting, must be addressed.

Is high allocation to support structures in crops a cause or a consequence of increased plant size?

All of the domesticated accessions, except for maize, invested proportionally more biomass in support tissue than their wild progenitors, which agrees with a previous report of increased plant height with cultivation (Milla *et al.* 2014). Indeed, this might be a generalised pattern that accompanies domestication. Increased height usually evolves in response to intense competition, thereby driving competitive exclusion in the wild (Kraft *et al.* 2015). Importantly, competition for light is more intense among conspecifics (Johansson & Keddy 1991). Crop plants are raised and frequently selected in monocultures, which probably promotes natural selection for increased height. Over-investment in support tissue to achieve greater height can lead to a *tragedy of the commons* scenario (Falster & Westoby 2003), where trade-offs between competition for light, resistance to lodging and investment in harvested organs may decrease the crop yield (Denison 2011). To overcome a decrease in yield, recent scientific-oriented breeding efforts have aimed to reverse this trend, particularly by developing dwarf wheat and rice varieties during the Green Revolution (Khush 1999; Denison 2011). Nevertheless, given our results and those of previous studies (Milla *et al.* 2014), selection for short plants is not widespread among herbaceous crops.

However, we cannot exclude the possibility that investment in support structures by domesticates is equal to that in other wild plants of the same size. The root and stem biomass fractions increase disproportionately with plant size in wild plants (Poorter *et al.* 2015), where this pattern is widely acknowledged in woody plants but also holds in herbaceous species (Poorter *et al.* 2015). Thus, we did not consider whether increased investment in the stems of crops followed known plant allometry rules or if it was disproportional, even after accounting for plant size. The latter scenario would support a role for selective forces other than allometry (*e.g.* stronger natural selection for

tallness) in increasing the amount of support tissue with domestication. As a preliminary test of whether crops conform to the stem–leaf allometry that is generally observed in herbs, we plotted $\log(\text{stem dry mass})$ against $\log(\text{leaf dry mass})$ for the 402 herbaceous species compiled in Poorter *et al.* (2015), excluding crops (Fig. S2). We fitted a standardised major axis regression to this set of data, which confirmed the increased investment in support structures as plant size increased: [slope $\log(\text{leaf mass}) \sim \log(\text{leaf stem}) = 0.83$ (95% confidence interval = 0.80–0.85; Fig. S2)]. Next, we embedded our six crop species into the plot, which showed that they were within the range of variation for other herbaceous species of comparable size (Fig. S2). This indicates that crops might only exhibit allometric relationships that are appropriate to their size, which may exclude possible reactions to other selective forces such as intensified intraspecific competition in croplands. However, this result is only preliminary and it does not clarify the extent to which tallness is linked solely with allometry, as well as with increased competition or other selective factors and biophysical constraints that might be more relevant for crops than for other plants. For instance, increased investment in support structures might have evolved to suit the mechanical needs of individual leaves, which became larger in the domesticated species considered in this study, except in wheat (Fig. 2). Overall, the increasing need for support structures (*i.e.* reducing the LEAF:STEM ratio and producing larger leaves or taller plants) might meet the biomechanical requirements of large plants, but with only a modest impact on the resource acquisition capacity of crops. However, further research is required to determine the cost–benefit balance and the underlying selective pressure of increased investment in support structures that accompanied domestication.

CONCLUSIONS

Plants tend to become larger after domestication and during further evolution under cultivation. However, after re-analysing previous research, we showed that this trend is diverse in magnitude, where a few crops actually became smaller during cultivation. We found similar patterns in the six crops that we examined in detail in the present study, where four species exhibited increases in their aboveground size to different extents and the other two remained similar to their wild progenitors. Interestingly, the leaf-level C gain or proportional allocation to leaf tissues did not drive the variation in aboveground plant size during the domestication of our six crops. Instead, crops where the leaf size increased during domestication tended to have a larger leaf area per individual plant, which resulted in larger plants. This suggests that allometry and ontogenetic cascades were more important mechanisms than enhanced physiological performance or optimising the allocation to productive tissue for producing larger plants in our study. However, it is fair to constrain these conclusions to the species considered in our study. We only examined six crops and two individual accessions per crop, and thus there is a need for studies using more taxa to assess the roles of relevant explanatory factors (phylogenetic affinities, human use or geography of domestication) in accounting for the variation in the response of plant size to domestication. Moreover, more detailed analyses should be performed before ruling out a significant role for leaf physiology or allocation. Other processes

and study scales were not addressed here and must also be considered, including seasonality or circadian rhythms of C uptake, root traits, nutrient content and stoichiometry (Niklas 2005), growth trajectories based on either cell size or cell number stimulation (Arendt 2007) or genetic control of organ and body sizes (Mizukami 2001; Busov *et al.* 2008). Body size has a key role in all aspects of organism biology (Arendt 2007). In addition, plant size combined with the planting density directly impacts crop yields (Weiner & Freckleton 2010). Therefore, further research is needed to understand the causes and consequences of variations in plant size during the evolution of crops.

ACKNOWLEDGEMENTS

We thank Jose Luis Margalet, Marta Rodriguez, Irene Romero, Juan Ricardo, Irene Alcocer and Alicia Gómez-Fernández for assisting with data collection, and Duncan Jackson for editing the English language. The comments of Diane Byers and two anonymous reviewers greatly improved a previous version of

the manuscript. This study was supported by Madrid Regional Government (grant REMEDINAL-3), Ministerio de Economía y Competitividad of Spain (grants CGL2014-56567-R and PCIN-2014-053) and the European Union (Eco-serve project, Biodiversa-FACCE, Horizon 2020).

SUPPORTING INFORMATION

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

Fig. S1. Relationship between the effect of domestication on plant size and seed size measured across a range of 27 herbaceous crops.

Fig. S2. Relationship between (log)stem dry mass and (log) leaf dry mass in 402 herbaceous wild species and in the six crop species considered in this study.

Table S1. Summary trait data (arithmetic mean, SD and replication level) for the domesticated and wild progenitor accessions of each of the six crops studied.

REFERENCES

- Allen C., Garmestani A., Havlicek T., Marquet P., Peterson G., Restrepo C., Stow C., Weeks B. (2006) Patterns in body mass distributions: sifting among alternative hypotheses. *Ecology Letters*, **9**, 630–643.
- Arendt J. (2007) Ecological correlates of body size in relation to cell size and cell number: patterns in flies, fish, fruits and foliage. *Biological Reviews*, **82**, 241–256.
- Baack E.J., Sapir Y., Chapman M.A., Burke J.M., Rieseberg L.H. (2008) Selection on domestication traits and quantitative trait loci in crop–wild sunflower hybrids. *Molecular Ecology*, **17**, 666–677.
- Baraloto C., Forget P.-M., Goldberg D.E. (2005) Seed mass, seedling size and neotropical tree seedling establishment. *Journal of Ecology*, **93**, 1156–1166.
- Benjamini Y., Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*, **57**, 289–300.
- Blueweiss L., Fox H., Kudzma V., Nakashima D., Peters R., Sams S. (1978) Relationships between body size and some life history parameters. *Oecologia*, **37**, 257–272.
- Brown J.H., Gillooly J.F., Allen A.P., Savage V.M., West G.B. (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.
- Busov V., Brunner A., Strauss S. (2008) Genes for control of plant stature and form. *New Phytologist*, **177**, 589–607.
- Cook M., Evans L. (1983) Some physiological aspects of the domestication and improvement of rice (*Oryza* spp.). *Field Crops Research*, **6**, 219–238.
- Day K.J., Hutchings M.J., John E.A. (2003) The effects of spatial pattern of nutrient supply on the early stages of growth in plant populations. *Journal of Ecology*, **91**, 305–315.
- Denison R.F. (2011) Past evolutionary tradeoffs represent opportunities for crop genetic improvement and increased human lifespan. *Evolutionary Applications*, **4**, 216–224.
- Evans L.T. (1993) *Crop evolution, adaptation and yield*. Cambridge University Press, Cambridge, UK.
- Falster D.S., Westoby M. (2003) Plant height and evolutionary games. *Trends in Ecology & Evolution*, **18**, 337–343.
- Fonseca C.R., Overton J.M., Collins B., Westoby M. (2000) Shifts in trait-combinations along rainfall and phosphorus gradients. *Journal of Ecology*, **88**, 964–977.
- Fox J., Weisberg S. (2011) *An R companion to applied regression*, 2nd edn. Sage, Thousand Oaks, CA, USA.
- Fuller D.Q. (2007) Contrasting patterns in crop domestication and domestication rates: recent archaeobotanical insights from the Old World. *Annals of Botany*, **100**, 903–924.
- Grime J.P. (2001) *Plant strategies, vegetation processes and ecosystem properties*, 2nd edn. John Wiley & Sons, Chichester, UK.
- Hancock J. (2004) *Plant evolution and the origin of crop species*. CABI Publishing, Wallingford, UK.
- Johansson M., Keddy P. (1991) Intensity and asymmetry of competition between plant pairs of different degrees of similarity: an experimental study on two guilds of wetland plants. *Oikos*, **60**, 27–34.
- Kelley K. (2007) Confidence intervals for standardized effect sizes. *Journal of Statistical Software*, **20**, 1–24.
- Khush G.S. (1999) Green revolution: preparing for the 21st century. *Genome*, **42**, 646–655.
- Kluyver T.A., Charles M., Jones G., Rees M., Osborne C.P. (2013) Did greater burial depth increase the seed size of domesticated legumes? *Journal of Experimental Botany*, **64**, 4101–4108.
- Kozłowski J. (1996) Optimal initial size and adult size of animals: consequences for macroevolution and community structure. *The American Naturalist*, **147**, 101–114.
- Kraft N.J.B., Godoy O., Levine J.M. (2015) Plant functional traits and the multidimensional nature of species coexistence. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 797–802.
- Litrico I., Violle C. (2015) Diversity in plant breeding: a new conceptual framework. *Trends in Plant Science*, **20**, 604–613.
- Maino J.L., Kearney M.R. (2015) Ontogenetic and interspecific scaling of consumption in insects. *Oikos*, **124**, 1564–1570.
- McKinney M.L., McNamara K. (1991) *Heterochrony: The evolution of ontogeny*. Plenum Press, New York, NY, USA.
- Meyer R.S., DuVal A.E., Jensen H.R. (2012) Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New Phytologist*, **196**, 29–48.
- Milla R., Morente-López J. (2014) Limited evolutionary divergence of seedlings after the domestication of plant species. *Plant Biology*, **17**, 169–176.
- Milla R., Reich P.B. (2007) The scaling of leaf area and mass: the cost of light interception increases with leaf size. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **274**, 2109–2114.
- Milla R., Morente-Lopez J., Alonso-Rodrigo J., Martín-Robles N., Chapin F.S. III (2014) Shifts and disruptions in resource-use trait syndromes during the evolution of herbaceous crops. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **281**, 20141429.
- Milla R., Osborne C.P., Turcotte M.M., Violle C. (2015) Plant domestication through an ecological lens. *Trends in Ecology & Evolution*, **30**, 463–469.
- Mizukami Y. (2001) A matter of size: developmental control of organ size in plants. *Current Opinion in Plant Biology*, **4**, 533–539.
- Moles A.T., Falster D.S., Leishman M.R., Westoby M. (2004) Small-seeded species produce more seeds per square metre of canopy per year, but not per individual per lifetime. *Journal of Ecology*, **92**, 384–396.
- Niklas K.J. (1994) *Plant allometry: The scaling of form and process*. University of Chicago Press, Chicago, IL, USA.
- Niklas K.J. (2005) Plant allometry, leaf nitrogen and phosphorus stoichiometry, and interspecific trends in annual growth rates. *Annals of Botany*, **97**, 155–163.
- Niklas K.J., Cobb E.D., Niinemets U., Reich P.B., Sellin A., Shipley B., Wright I.J. (2007) “Diminishing returns” in the scaling of functional leaf traits across and within species groups. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 8891–8896.
- Obeso J.R. (2004) A hierarchical perspective in allocation to reproduction from whole plant to fruit and

- seed level. *Perspectives in Plant Ecology Evolution and Systematics*, **6**, 217–225.
- Paine C.E.T., Marthews T.R., Vogt D.R., Purves D., Rees M., Hector A., Turnbull L.A. (2012) How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. *Methods in Ecology and Evolution*, **3**, 245–256.
- Paulsen T.R., Högstedt G. (2002) Passage through bird guts increases germination rate and seedling growth in *Sorbus aucuparia*. *Functional Ecology*, **16**, 608–616.
- Pérez-Harguindeguy N., Díaz S., Garnier E., Lavorel S., Poorter H., Jaureguiberry P., Cornwell W.K., Craine J.M., Gurvich D.E., Urcelay C., Veneklaas E.J., Reich P.B., Poorter L., Wright I.J., Ray P., Enrico L., Pausas J.G., Vos A.C., De B., Funes G., Hodgson J.G., Thompson K., Morgan H.D., Steege H., Van Der Heijden M.G.A., Sack L., Blonder B., Poschlod P., Vaieretti M.V., Conti G., Staver A.C., Aquino S., Cornelissen J.H.C. (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, **61**, 167–234.
- Poorter H., Remeks C. (1990) Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia*, **83**, 553–559.
- Poorter H., Van Der Werf A. (1998) Is inherent variation in RGR determined by LAR at low light and by NAR at high light? In: Lambers H., Poorter H., Van Vuuren M.M.I. (Eds), *Inherent variation in plant growth: Physiological mechanisms and ecological consequences*. Backhuys, Leiden, The Netherlands, pp 309–336.
- Poorter H., Jagodzinski A.M., Ruiz-Peinado R., Kuyah S., Luo Y., Oleksyn J., Usoltsev V.A., Buckley T.N., Reich P.B., Sack L. (2015) How does biomass distribution change with size and differ among species? An analysis for 1200 plant species from five continents. *New Phytologist*, **208**, 736–749.
- Price C.A., Wright I.J., Ackerly D.D., Niinemets Ü., Reich P.B., Veneklaas E.J. (2014) Are leaf functional traits “invariant” with plant size and what is “invariance” anyway? *Functional Ecology*, **28**, 1330–1343.
- R Core Team (2013) *R: A language and environment for statistical computing*. R Core Team, Vienna, Austria. URL: <http://www.r-project.org>
- Reich P.B., Walters M.B., Ellsworth D.S. (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 13730–13734.
- Savage J., Haines D., Holbrook N. (2015) The making of giant pumpkins: how selective breeding changed the phloem of *Cucurbita maxima* from source to sink. *Plant, Cell and Environment*, **38**, 1543–1554.
- Shipley B. (2002) *Cause and correlation in Biology: A user's guide to path analysis, structural equations and causal inference*. Cambridge University Press, Cambridge, UK.
- Shipley B. (2009) Confirmatory path analysis in a generalized multilevel context. *Ecology*, **90**, 363–368.
- Sperry J.S., Smith D.D., Savage V.M., Enquist B.J., McCulloh K.A., Reich P.B., Bentley L.P., von Allmen E.I. (2012) A species-level model for metabolic scaling in trees I. Exploring boundaries to scaling space within and across species. *Functional Ecology*, **26**, 1054–1065.
- Turcotte M.M., Turley N.E., Johnson M.T.J. (2014) The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. *New Phytologist*, **204**, 671–681.
- Turcotte M.M., Lochab A.K., Turley N.E., Johnson M.T.J. (2015) Plant domestication slows pest evolution. *Ecology Letters*, **18**, 907–915.
- Vico G., Manzoni S., Murphy K., Weih M. (2016) Trade-offs between seed output and life span – a quantitative comparison of traits between annual and perennial congeneric species. *New Phytologist*, **209**, 104–114.
- Weiner J., Freckleton R.P. (2010) Constant final yield. *Annual Review of Ecology, Evolution, and Systematics*, **41**, 173–192.
- West G.B., Brown J.H., Enquist B.J. (1997) A general model for the origin of allometric scaling laws in biology. *Science*, **276**, 122–126.
- West G.B., Brown J.H., Enquist B.J. (1999) A general model for the structure and allometry of plant vascular systems. *Nature*, **400**, 664–667.
- Wright I.J., Ackerly D.D., Bongers F., Harms K.E., Ibarra-Manríquez G., Martínez-Ramos M., Mazer S.J., Müller-Landau H.C., Paz H., Pitman N.C.A., Poorter L., Silman M.R., Vriesendorp C.F., Webb C.O., Westoby M., Wright S.J. (2007) Relationships among ecologically important dimensions of plant trait variation in seven neotropical forests. *Annals of Botany*, **99**, 1003–1015.