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Research

What determines organ size differences between species? A meta-analysis of the cellular basis

Ayelén Gázquez[1,2] and Gerrit T. S. Beemster[1]

[1]Laboratory for Integrated Molecular Plant Physiology Research (IMPRES), Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium; [2]Laboratorio de Fisiología de Estrés Abiótico en Plantas, Unidad de Biotecnología 1, IIB-INTECH – CONICET – UNSAM, Chascomús, Argentina

Author for correspondence:
Gerrit T. S. Beemster
Tel: +32 3 265 34 21
Email: gerrit.beemster@uantwerpen.be

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Summary

- Little is known about how the characteristic differences in organ size between species are regulated. At the cellular level, the size of an organ is strictly regulated by cell division and expansion during its development. We performed a meta-analysis of the growth parameters of roots, and *Graminae* and eudicotyledonous leaves, to address the question of how quantitative variation in these two processes contributes to size differences across a range of species.
- We extracted or derived cellular parameters from published kinematic growth analyses. These data were subjected to linear regression analyses to identify the parameters that determine differences in organ growth.
- Our results demonstrate that, across all species and organs, similar conclusions can be made: cell number rather than cell size determines the final size of plant organs; cell number is determined by meristem size rather than the rate at which cells divide; cells that are small when leaving the meristem compensate by expanding for longer; mature cell size is primarily determined by the duration of cell expansion.
- These results identify the regulation of the transition from cell division to expansion as the key cellular mechanism targeted by the evolution of organ size.

Introduction

How plants regulate growth is a central question in plant development. Differences in the size of organs between species are staggering: leaves of *Arabidopsis thaliana* are only 10–20 mm^2 (Kalve *et al.*, 2014), whereas those of *Helianthus annuus* reach 18 000–30 000 mm^2 (Granier & Tardieu, 1998), 1500 times larger. Superimposed on this, ontogenetic and environmental effects cause substantial differences, even in a single genotype: the fifth leaf of wheat seedlings is three times the length of the first (Beemster & Masle, 1996), and leaves of bonsai trees are up to 25 times smaller than those of the same species grown under normal conditions (Körner *et al.*, 1989).

Cells are the building blocks of the plant body, and therefore an obvious question to ask is 'which cellular process is primarily responsible and how is it modulated to cause differences in organ size?'. To address this question, it is crucial to examine cell division and expansion in parallel. Linear, steady-state growing organs, such as root tips and *Graminae* leaves, have been analyzed for decades (Goodwin & Stepka, 1945) using a rigorous quantitative framework (Silk & Erickson, 1979). In these linear systems, organ growth rate is a direct consequence of the number of cells produced per unit of time by cell division (cell production, P) and the length of cells entering the mature part of the organ (l_{mat}; Table 1; Eqns 2, 3). Moreover, P is determined by the number of cells in the division zone (N_{div}) and the average cell division rate (D; Table 1; Eqn 4), whereas l_{mat} is defined by the length of cells leaving the meristem (l_{div}), and the average rate (RER) and duration of cell expansion (T_{el}; Table 1; Eqn 5).

Although eudicotyledonous leaves also exhibit a spatial developmental gradient (Donnelly *et al.*, 1999; Kazama *et al.*, 2010; Andriankaja *et al.*, 2012), they do not grow at steady state and are more conveniently described on a temporal basis, considering the leaf as a whole, essentially ignoring variations in developmental patterns across the leaf (Das Gupta & Nath, 2015). The mature leaf blade area (LBA) is a function of the cell number (N_{mat}) and the cell area at maturity (A_{mat}; Table 1; Eqn 7). N_{mat}, in turn, is determined by the initial number of cells that are recruited in the primordium (N_{prim}), the duration of the division phase (T_{div}) and the average rate of cell division (D; Table 1; Eqn 8). Similarly, A_{mat} is a function of the area of cells exiting the division phase (A_{div}), the duration of the cell expansion phase (T_{exp}) and the average (relative) rate of cell expansion (RER; Table 1; Eqn 9).

The relationships between these cellular parameters and whole-organ growth have been used to study genetic differences, environmental conditions and physiological treatments in single species. Across these studies, no single parameter can be found

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Table 1 The quantitative relationships between cellular parameters and whole organ growth

	Symbol	Relationship between parameters	Equation no.
Roots and *Graminae* leaves			
Final leaf length (mm)	FLL	$= \text{LER} \times T_{LE}$	Eqn 1
Time of leaf elongation (h)	T_{LE}		
Leaf elongation rate (mm h^{-1})	LER	$= (P \times l_{mat})/1000$	Eqn 2
Overall root elongation rate (mm h^{-1})	ORER	$= (P \times l_{mat})/1000$	Eqn 3
Cell production rate (cells h^{-1})	P	$= N_{div} \times D$	Eqn 4
Number of cells in the division zone	N_{div}		
Relative division rate (cell per cell h^{-1})	D		
Mature cell length (μm)	l_{mat}	$= l_{div} \times e^{RLI}$	Eqn 5
Relative length increase	RLI	$= \text{RER} \times T_{el}$	Eqn 6
Relative expansion rate (μm per μm h^{-1})	RER		
Length of cells leaving the meristem (μm)	l_{div}		
Cell residence time in elongation zone (h)	T_{el}		
Eudicotyledonous leaves			
Leaf blade area (cm^2)	LBA	$= (N_{mat} \times A_{mat})/1e^8$	Eqn 7
Mature cell number	N_{mat}	$= N_{prim} \times e^{T div \times D}$	Eqn 8
Number of cells in the primordium	N_{prim}		
Time that cells expend dividing (h)	T_{div}		
Relative division rate (cell per cell h^{-1})	D		
Mature cell area (μm^2)	A_{mat}	$= A_{div} \times e^{RLA}$	Eqn 9
Relative area increase	RLA	$= \text{RER} \times T_{exp}$	
Area of cells in division (μm^2)	A_{div}		
Time that cells expend expanding (h)	T_{exp}		
Relative expansion rate (μm^2 per μm^2 h^{-1})	RER		

that is responsible for the observed differences in organ size, indicating that the treatments and genotypic differences differentially affect the growth process. However, these studies have allowed us to identify the main parameters that determine differences between species, across a wider range of organ sizes than the individual studies. Therefore, we extracted the data from all kinematic studies we could find for three types of organ (root tips, and *Graminae* and eudicotyledonous leaves) and performed a meta-analysis to determine the cellular mechanisms controlling the characteristic differences in organ size between plant species.

Materials and Methods

Data extraction

We extracted quantitative cellular parameters (Table 1) from the main texts, tables and figures of the published kinematic studies. If they were not explicitly given, we calculated them from kinematic equations and data in the same article (shown in red in Supporting Information Tables S1–S3). In total, 21 manuscripts were analyzed for roots (Table S1), 20 for *Graminae* leaves (Table S2) and 17 for eudicotyledonous leaves (Table S3).

Statistical analysis

We performed multiple linear regression analyses to investigate the relationships between cellular and whole-organ growth parameters (Table 1) using SPSS statistical software for Windows (v22.0, SPSS Inc., Chicago, IL, USA). Equations 1–9, described in Table 1, were linearized by log-transformation, constituting Eqns 10–16, that were fitted to the data obtained or derived from the literature.

For root tips (using the data in Table S1):

$$\log(\text{ORER}) = C_0 + C_1 \times \log(P) + C_2 \times \log(l_{mat}) \quad \text{Eqn 10}$$

$$\log(P) = C_0 + C_1 \times \log(N_{div}) + C_2 \times \log(D) \quad \text{Eqn 11}$$

$$\log(l_{mat}) = C_0 + C_1 \times \log(l_{div}) + C_2 \times \text{RLI} \quad \text{Eqn 12}$$

$$\log(\text{RLI}) = C_0 + C_1 \times \log(\text{RER}) + C_2 \times \log(T_{el}) \quad \text{Eqn 13}$$

For *Graminae* leaves (using the data in Table S2):

$$\log(\text{FLL}) = C_0 + C_1 \times \log(\text{LER}) + C_2 \times \log(T_{LE}) \quad \text{Eqn 14}$$

$$\log(\text{LER}) = C_0 + C_1 \times \log(P) + C_2 \times \log(l_{mat}) \quad \text{Eqn 15}$$

Equations 11, 12 and 13 were also used for *Graminae* leaves.

For eudicotyledonous leaves (using the data in Table S3):

$$\log(\text{LBA}) = C_0 + C_1 \times \log(N_{mat}) + C_2 \times \log(A_{mat}) \quad \text{Eqn 16}$$

The data and regressions were plotted using the OriginPro software package for Windows (v9.1.0, OriginLab Corporation, Northampton, MA, USA).

Results

Roots

The linear, indeterminate development of roots is ideally suitable for the study of the cellular basis of organ growth differences. Consequently, the earliest studies, as well as the largest total number of kinematic studies (21; see Table S1), have been performed on root tips. These have included six different species: *Allium cepa*, *A. thaliana*, *Solanum lycopersicum*, *Phleum pratense*, *Triticum aestivum* and *Zea mays*. Although data for metaxylem and epidermal cells have been reported in some studies, the majority of the data involve cortical cells.

We first determined the contributions of variations in mature cell length (l_{mat}) and cell production (P) to differences in the overall root elongation rates (ORER; Table 1; Eqn 3). As these two parameters directly determine the organ growth rate, variation in l_{mat} and P accounted for 100% of the variation across species and cell types (Eqn 10; Fig. S1a). Both parameters contributed significantly, but the standardized coefficients (Beta) suggested that the impact of P on ORER was larger than that of l_{mat}. To compare their contributions in more detail, we analyzed the models of ORER vs l_{mat} and P, separately. As different cell types have characteristic differences in cell length, they were analyzed separately. For all three cell types represented in the dataset, there was a strong positive correlation between P and ORER (R^2 between 0.78 and 0.90; Fig. 1a). By contrast, the correlation between l_{mat} and ORER was only significant for cortical cells (R^2 of 0.33; Fig. 1b). There was no correlation between P and l_{mat}, suggesting that cell production and expansion are independent and high cell production does not lead to smaller cells, or that both vary in parallel (data not shown). Thus, these results show that differences in root growth rate between species are primarily driven by variations in the number of cells produced in the meristem.

Cell production (P), in turn, is determined directly by the number of dividing cells (N_{div}) and the rate at which these cells are dividing (D), allowing us to perform a similar regression analysis (Table 1; Eqn 4). Because there were insufficient data for the other cell types, we restricted this analysis to cortical cells. As expected, N_{div} and D together explained 99% of the variation in P (Eqn 11; Fig. S1b). Both parameters contributed significantly and roughly equally. Consistently, N_{div} explained 73% and D explained 74% of the variation in P (Fig. 1c,d). As the number of cells in the meristem largely determines the length of the meristem (L_{mer}, the distance between the stem cells and the position at which cells of a given cell type stop dividing and start expanding), the length of the meristem was also positively correlated with P (R^2 of 0.67; inset in Fig. 1c). There was no correlation between the average cell division rate and the number of cells in the meristem (data not shown). These results indicate that variations in rates of cell division and number of dividing cells contribute equally and independently to differences in overall cell production.

Although not contributing significantly to the variation in ORER, there were significant differences in l_{mat}. Therefore, we explored the basis of this variation, focusing on cortical cells, using the relationship between l_{mat}, the length of the cells exiting the meristem (l_{div}) and the relative length increase in the elongation zone (RLI; Table 1; Eqn 5). Indeed, these two parameters accounted for 100% of the variance in mature cell length (Eqn 12; Fig. S1c). Both l_{div} and RLI contributed significantly, but RLI had a higher Beta coefficient. However, individually, the length of cells leaving the meristem did not show a significant correlation (Fig. 1e), whereas RLI was positively correlated and explained 39% of the variance in l_{mat} (Fig. 1f). Interestingly, l_{div} and RLI were negatively correlated ($R^2 = 0.80$; Fig. S1d). As most of the variation in l_{mat} was explained by RLI, which, in turn, is a function of RER and T_{el} (Table 1; Eqn 6), we further investigated the relationship between the last three parameters. As expected, RER and T_{el} explained 100% of the variance in RLI, and both parameters had a comparable impact (Eqn 13; Fig. S1e). However, only T_{el} showed a significant correlation with RLI, although the correlation was weak (R^2 of 0.21; Fig. 1g,h). Together, these results show that differences in mature cell size are mostly explained by the duration of cell expansion, and small cells typically expand for a longer period.

Graminae leaves

The growth of *Graminae* leaves shows strong similarities with that of root tips: it is linear and cells are organized files with a spatial gradient of proliferating, expanding and mature cells. Although the growth of these leaves, in contrast with roots, is determinate, after emergence, there is usually a phase of steady-state growth that has been extensively used for kinematic analyses. To study the differences in leaf length and leaf growth rates between *Graminae* species, we extracted data from 20 published kinematic studies, involving 12 different species: *Aegilops caudata*, *Aegilops tauschii*, *Festuca arundinacea*, *Lolium perenne*, *Lolium multiflorum*, *Oryza sativa*, *Poa annua*, *Poa trivialis*, *Poa compressa*, *Poa alpina*, *T. aestivum* and *Z. mays* (Table S2). Across these studies, two different types of cell were most frequently analyzed: epidermal cells in the file adjacent to stomata (sister cells); and cells located more centrally between stomatal files that are substantially larger (Beemster & Masle, 1996).

To explain differences in final leaf length (FLL), we first analyzed the contribution of leaf elongation rate (LER) and the duration of that elongation (T_{LE}; Table 1; Eqn 1). In accordance with Eqn 1, LER and T_{LE} together accounted for 100% of the variance in FLL (Eqn 14). Both parameters contributed to the variation in FLL, although the Beta coefficients suggested that the effect of LER was stronger (Fig. S2a). Consistently, LER alone explained 94% of the variation in FLL and T_{LE} only 40% (Fig. 2). This analysis suggests that differences in mature leaf size are primarily correlated with differences in leaf growth rate, with a minor contribution of duration of the growth process.

Based on the kinematic data, we then investigated the role of cell production and mature cell length in determining the variation in LER. Analogous to roots, cell production (P) and mature cell length (l_{mat}) together fully accounted for the variation in LER (Eqn 15; Fig. S2b). Although both parameters contributed

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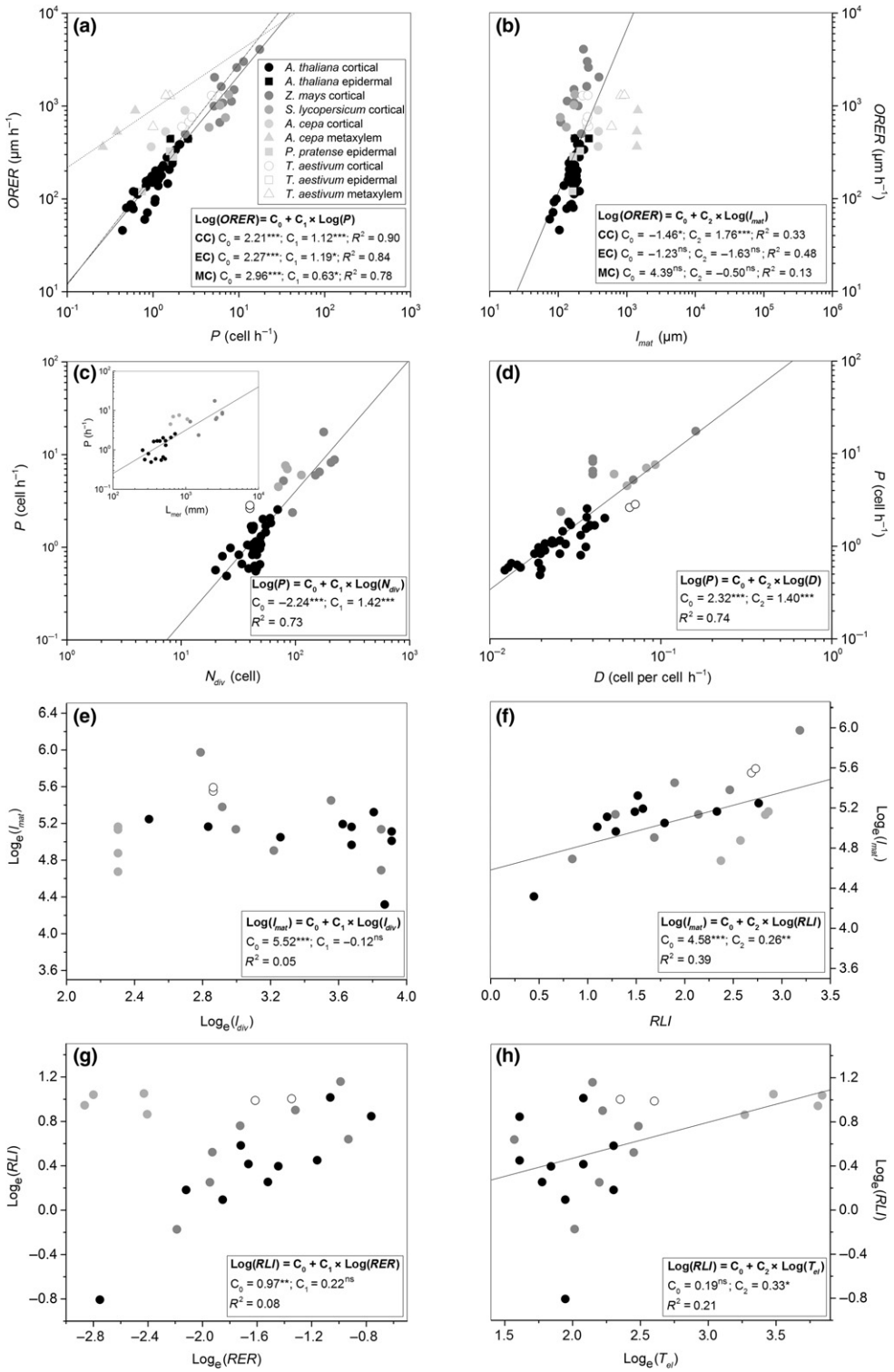

Fig. 1 The cellular basis of differences in root growth rate across a range of species. (a) The relationship between root elongation rate (ORER) and cell production (*P*). (b) The relationship between ORER and mature cell length (l_{mat}). (c) The relationship between *P* and number of cells in the meristem (N_{div}). (d) The relationship between *P* and average cell division rate (*D*). (e) The relationship between l_{mat} and length of cells at the end of the meristem (l_{div}). (f) The relationship between l_{mat} and relative length increase (RLI). (g) The relationship between RLI and relative elongation rate (RER). (h) The relationship between RLI and time of cells in the elongation zone (T_{el}). (a, b) Models for cortical cells (CC), epidermal cells (EC) and mesophyll cells (MC). Inset in (c) shows the correlation between *P* and length of the meristem (L_{mer}). Species and cell types include *Arabidopsis thaliana* (cortical and epidermal cells), *Zea mays* (cortical cells), *Solanum lycopersicon* (cortical cells), *Allium cepa* (cortical and metaxylem cells), *Phleum pratense* (epidermal cells) and *Triticum aestivum* (cortical, epidermal and metaxylem cells). Details of the regression parameters are shown in each figure: C_0, C_1 and C_2 are the partial coefficients; R^2 is the coefficient of correlation. $n = 63$ for CC and $n = 6$ for EC and MC in (a, b); $n = 47$ in (c, d); $n = 27$ in inset of (c); and $n = 23$ in (e–h). Significance (*t*-test): *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$; ns, *P* not significant.

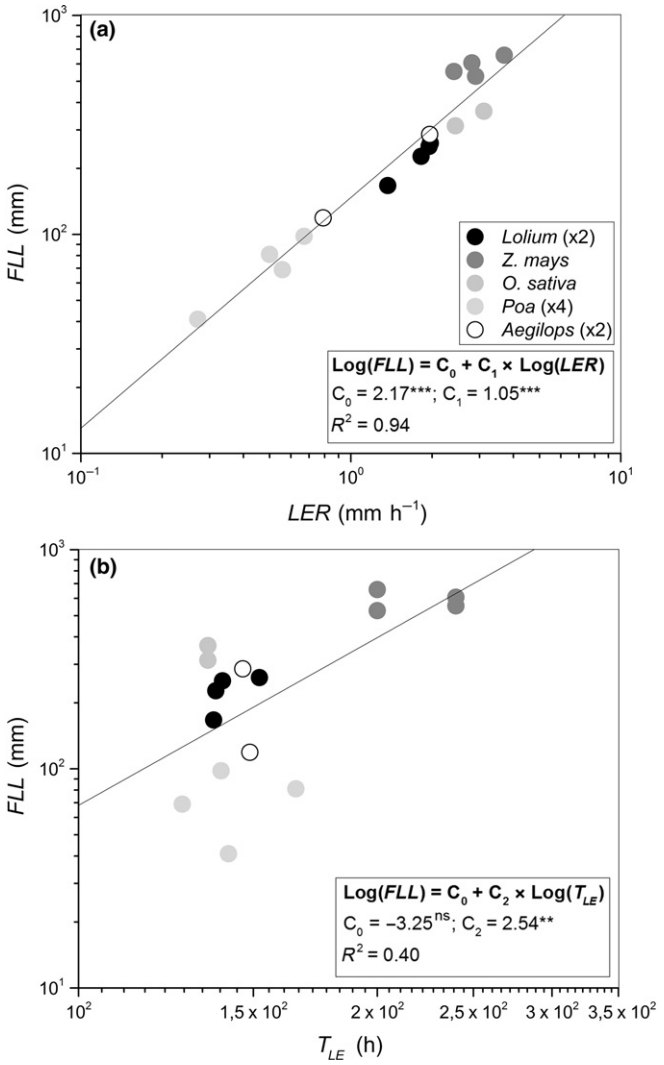

Fig. 2 The difference in growth pattern determining leaf size across a range of *Graminae*. (a) The relationship between final leaf length (FLL) and leaf elongation rate (LER). (b) The relationship between FLL and time of leaf elongation (T_{LE}). Species and cell types include *Lolium perenne* and *multiflorum*, *Zea mays*, *Oryza sativa*, *Poa annua*, *trivialis*, *compressa* and *alpina*, and *Aegilops caudata* and *tauschii*. Details of the regression parameters are shown in each figure: C_0, C_1 and C_2 are the partial coefficients; R^2, coefficient of correlation; $n = 16$. Significance (*t*-test): **, $0.001 < P < 0.01$; ***, $P < 0.001$; ns, *P* not significant.

significantly to the leaf elongation rate, the Beta coefficients suggested that the impact of cell production was larger. Indeed, cell production of sister cells and elongated cells between stomatal files by itself explained 89% and 47% of the variation in LER, respectively (Fig. 3a), whereas no significant correlation was found for mature cell length of either type (Fig. 3b). No evidence was found to suggest that l_{mat} and *P* were correlated (data not shown). Thus, analogous to the observations in the root tip, differences in leaf growth rate between species are largely controlled by cell production in the intercalary meristem.

To investigate whether the analogy between the two organs extends to the underlying mechanisms, we investigated the relationship between *P*, N_{div} and *D* across *Graminae* leaves (see Table 1), focusing on the sister cells for which sufficient data were available. As expected, N_{div} and *D* together fully accounted for the variation in cell production (Eqn 11; Fig. S2c). Although both parameters significantly contributed to cell production, N_{div} had a higher Beta coefficient than *D*. Consistently, N_{div} by itself accounted for 67% of the variance in cell production (Fig. 3c). In contrast with the observations for root tips, *D* was not significantly correlated with *P* in *Graminae* leaves (Fig. 3d). As the size of the meristem is related to the number of cells in it, L_{mer} explained 79% of the variation in *P* (inset in Fig. 3c). No correlation was found between N_{div} or L_{mer} and *D* (data not shown). Together, these data indicate that, in *Graminae* leaves, the size of the meristem (and hence the number of dividing cells) is the main determinant of leaf growth rate and, ultimately, leaf size.

Although variation in l_{mat} did not contribute significantly to LER, we analyzed the cell expansion parameters in detail to understand whether cell size variation in the leaf is achieved in a similar way as in the root. Similar to the root tip, l_{div} and RLI together accounted for 100% of the variation in l_{mat} (Table 1; Eqn 5) and, although both parameters had a significant effect, that of RLI was stronger than that of l_{div} (Eqn 12; Fig. S2d). This was confirmed with the partial models (Fig. 3e,f): although the correlation between l_{mat} and l_{div} was not significant, RLI explained 65% of the variation in l_{mat}. In analogy with roots, we found a negative correlation between l_{div} and RLI (Fig. S2e). Finally, as expected, the variation in RLI was fully explained by RER and T_{el} (Eqn 13; Fig. S2f). Similar to the situation in root tips, only T_{el} explained 64% of the variation in RLI, whereas RER did not correlate (Fig. 3g,h). These results show that, also in *Graminae* leaves, differences in mature cell size are caused by variations in the duration of the expansion phase, and cells that are small when leaving the meristem expand for a longer period to compensate.

Eudicotyledonous leaves

Kinematic analysis *sensu stricto* is not useful for the study of the cellular basis of the growth of eudicotyledonous leaves. Instead, measurement of the organ and cell size as a function of time is a more efficient way to achieve this (Fiorani & Beemster, 2006). We found 17 papers on four species, including *A. thaliana*, *H. annuus*, *Manihot esculenta* and the hybrid *Populus* × *canascens*, where such studies were performed (Table S3). We focused on epidermal cells, as palisade cells were only studied in one species (*A. thaliana*; Ferjani *et al.*, 2007; Hisanaga *et al.*, 2013).

We first analyzed the contribution of the number of cells (N_{mat}) and mature cell area (A_{mat}) to the variation in leaf blade area (LBA; Table 1; Eqn 7). Together, N_{mat} and A_{mat} accounted for 100% of the variance in LBA (Eqn 16; Fig. S3). Data points clustered into two groups: a group with low leaf area, corresponding to data from *A. thaliana*, and another group with high leaf area, including the other three species. Both N_{mat} and A_{mat} contributed to variations in leaf area as their partial coefficients were significant. However, the Beta coefficient of N_{mat} was higher than that of A_{mat}. Indeed, similar to the situation in roots and *Graminae* leaves, cell number was more important than cell size

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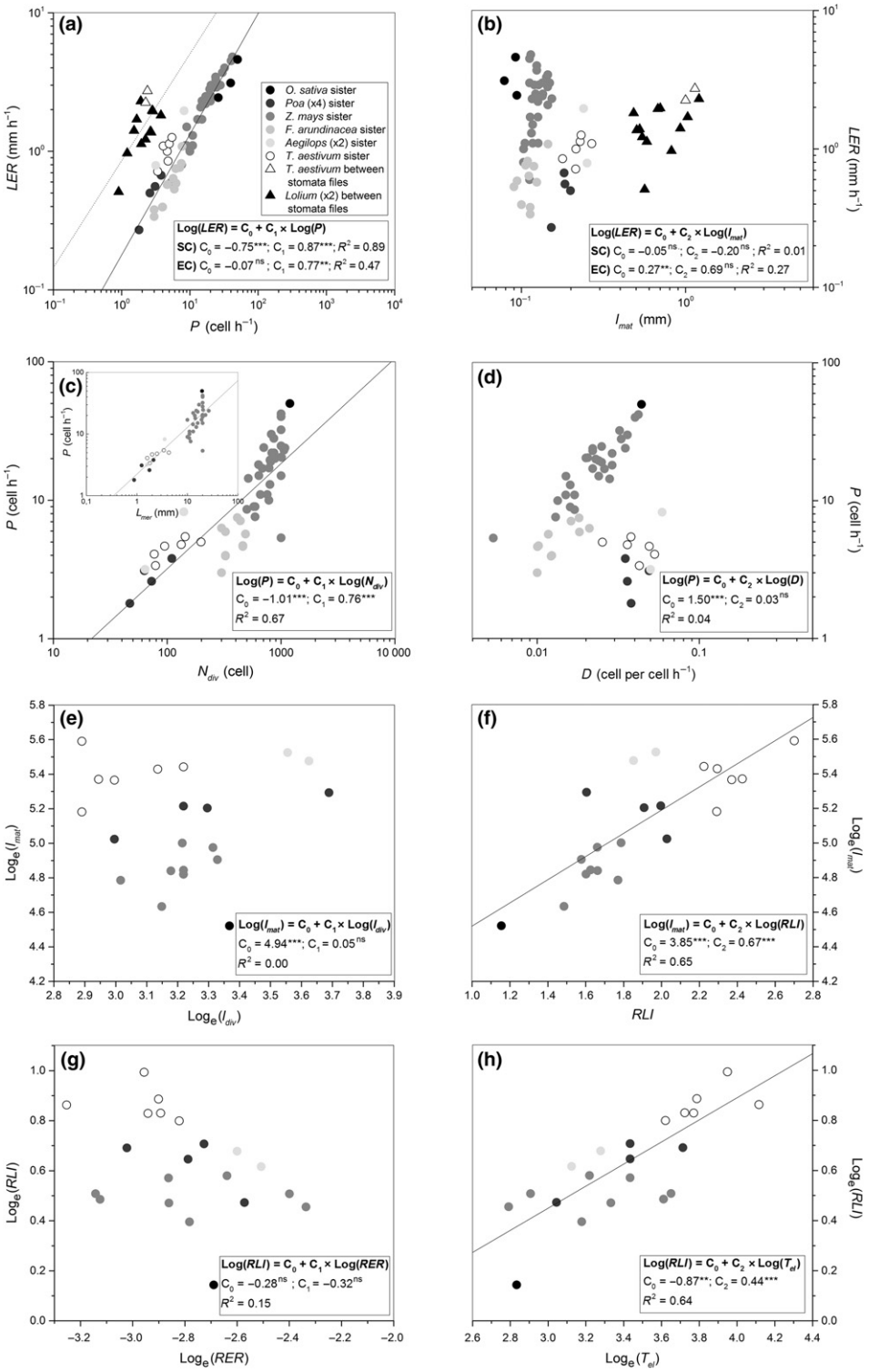

Fig. 3 The cellular basis of differences in leaf elongation rate across a range of *Graminae*. (a) The relationship between leaf elongation rate (LER) and cell production (*P*). (b) The relationship between LER and mature cell length (l_{mat}). (c) The relationship between *P* and number of cells in the meristem (N_{div}). (d) The relationship between *P* and average cell division rate (*D*). (e) The relationship between l_{mat} and length of cells at the end of the meristem (l_{div}). (f) The relationship between l_{mat} and relative length increase (RLI). (g) The relationship between RLI and relative elongation rate (RER). (h) The relationship between RLI and residence time of cells in the elongation zone (T_{el}). (a, b) Models for sister cells (SC) and elongated cells between stomata file cells (EC). Inset in (c) shows the correlation between *P* and length of the meristem (L_{mer}). Species and cell types include *Oryza sativa* (sister cells), *Poa annua*, *trivialis*, *compressa* and *alpina* (sister cells), *Zea mays* (sister cells), *Festuca arundica* (sister cells), *Aegilops caudata* and *tauschii* (sister cells), *Triticum aestivum* (sister cells and cells between stomatal files), *Lolium perenne* and *multiflorum* (cells between stomatal files). Details of the regression parameters are shown in each figure: C_0, C_1 and C_2 are the partial coefficients; R^2, coefficient of correlation. $n = 60$ for SC and $n = 14$ for EC in (a, b); $n = 56$ in (c, d); $n = 44$ in inset of (c); and $n = 21$ in (e–h). Significance (*t*-test): **, $0.001 < P < 0.01$; ***, $P < 0.001$; ns, *P* not significant.

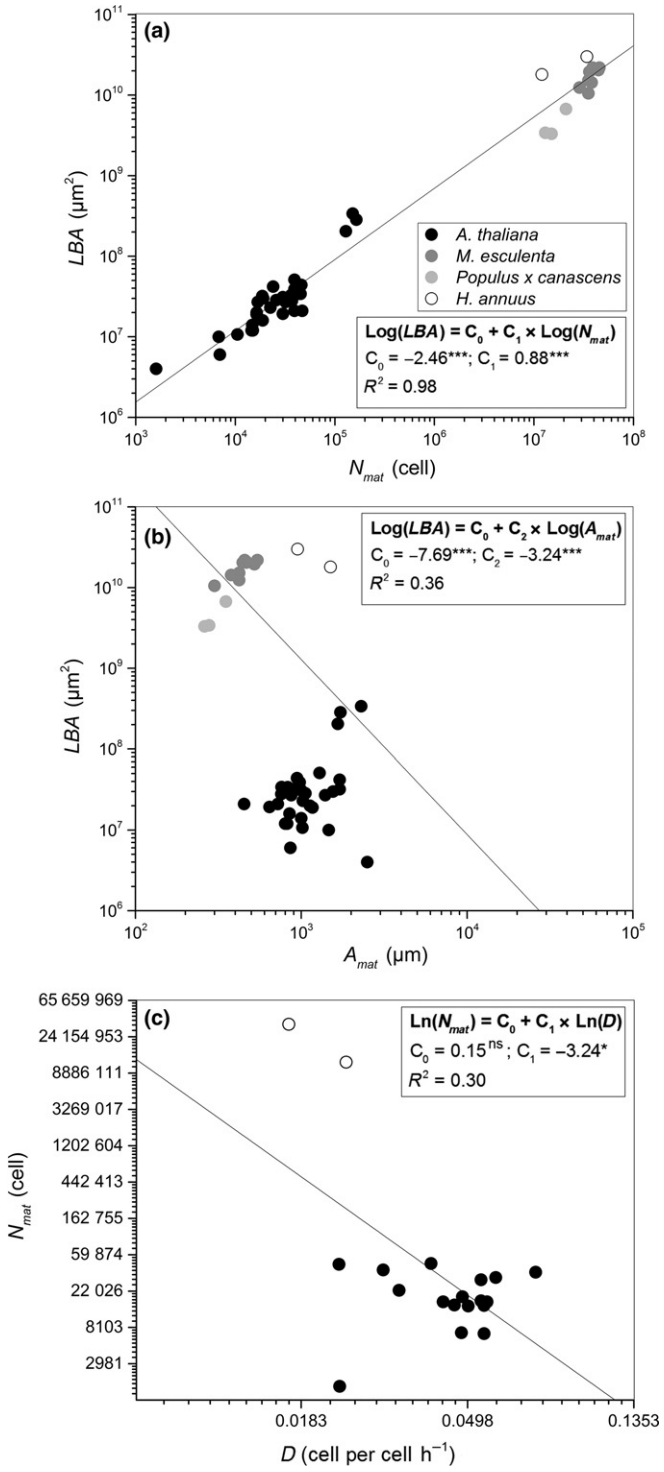

Fig. 4 The cellular basis of differences in leaf size across a range of eudicotyledonous species. (a) The relationship between leaf blade area (LBA) and number of cells in a mature leaf (N_{mat}). (b) The relationship between LBA and the area of mature cells (A_{mat}). (c) The relationship between N_{mat} and average cell division rate (D). Species include *Arabidopsis thaliana*, *Manihot esculenta*, *Populus* × *canascens* and *Helianthus annuus*. Details of the regression parameters are shown in each figure: C_0, C_1 and C_2 are the partial coefficients; R^2, coefficient of correlation. $n = 46$ in (a, b), $n = 19$ in (c). Significance (*t*-test): *, $0.01 < P < 0.05$; ***, $P < 0.001$; ns, P not significant.

in determining organ growth, as N_{mat} explained 98% of the variance of leaf area (Fig. 4a). Curiously, A_{mat} correlated negatively with LBA, accounting for 36% of the variation (Fig. 4b). No correlation was found between N_{mat} and A_{mat}, implying independence of the two parameters (data not shown). The relationships between N_{mat}, N_{prim}, T_{div} and D, and A_{mat}, A_{div}, T_{exp} and RER, could not be analyzed as these parameters were typically not determined. Only D for *A. thaliana* and *H. annuus* epidermal cells could be correlated with N_{mat}, showing a significant negative correlation, but with poor adjustment (Fig. 4c).

These data show that, consistent with the situation in roots and *Graminae* leaves, cell production rather than mature cell size determines variation in leaf size.

Discussion

We addressed the question of which cellular mechanisms determine the variation in organ size between plant species. To this end, we performed a quantitative meta-analysis of kinematic data obtained for root tips and *Graminae* leaves, and developmental studies of eudicotyledonous leaves, available in the literature. Although data from different laboratories and different conditions were compared, variation in organ size between species exceeded experimental variation, so that interspecies differences could effectively be studied based on the combined data. By analyzing the results from the three different organs across a wide range of species, a consistent picture emerges: first, cell proliferation (variation in cell number) rather than cell expansion (the size of mature cells) determines the final size of plant organs; second, the number of dividing cells (and meristem size) and not the rate at which cells divide determines cell production; third, cells that are small when leaving the meristem compensate by expanding for longer; fourth, variations in mature cell size are primarily determined by the duration of cell expansion.

Thus, the cellular basis of organ size variation between species is remarkably similar for the three different organs. The investigation of the underlying molecular regulation of these parameters by the comparison of species, to our knowledge, has not been performed to date. Nevertheless, findings within a single species provide us with a good basis. First, such studies largely confirm the importance of the size of the meristem as the crucial control point in organ growth regulation in both root tips (Baskin, 2000; West *et al.*, 2004) and *Graminae* leaves (Bultynck *et al.*, 2003; Barrôco *et al.*, 2006; Powell & Lenhard, 2012; Czesnick & Lenhard, 2015). The importance of this control mechanism has been widely recognized, and this has led to studies in which the size of the meristem was the only cellular parameter that was determined (Ioio *et al.*, 2008; Moubayidin *et al.*, 2010; Tsukagoshi *et al.*, 2010). Moreover, approaches to extract this parameter from cell length profile data (French *et al.*, 2012; Voorend *et al.*, 2014; Bizet *et al.*, 2015), velocity profiles (van der Weele *et al.*, 2003), the expression of cell cycle markers (Ferreira *et al.*, 1994; Donnelly *et al.*, 1999; West *et al.*, 2004) and infrared images (Bizet *et al.*, 2015) have been developed. In dicotyledonous leaves, it is virtually impossible to use meristem size as a basis to determine

overall cell production, as the group of cells recruited at the shoot apical meristem (SAM) to form the leaf exponentially grows during the proliferative development of the leaf. Therefore, the equivalent to meristem size in linear systems is the duration of the proliferative phase, as both parameters essentially represent the transition from proliferation to expansion. Indeed, kinematic studies in *A. thaliana* have shown that, across a range of genetic perturbations that cause variations in leaf size, the duration of cell proliferation is most frequently involved (Gonzalez *et al.*, 2012). This has facilitated the discovery of a hormonal network that controls the position at which cells exit from proliferation. Interestingly, not only is the cellular basis of organ growth very similar in different organs, as shown here, but also the molecular regulation shows strong similarities (Nelissen *et al.*, 2016).

A recent modeling study has demonstrated that cell autonomous regulation cannot account for the observed growth patterns in root tips and that spatial control by hormones is required (De Vos *et al.*, 2014). Auxin accumulates in proliferating tissues in root tips (Grieneisen *et al.*, 2007; Brunoud *et al.*, 2012) and in leaves of *A. thaliana* and *Z. mays* (Nelissen *et al.*, 2012), quickly dropping to low levels in expanding cells. Indeed, genetic perturbation of auxin signaling in root tips (Sabatini *et al.*, 1999; Swarup *et al.*, 2005), *Z. mays* (Tsiantis *et al.*, 1999; Guo *et al.*, 2014) and *A. thaliana* leaves (Schruff *et al.*, 2006; Bilsborough *et al.*, 2011; Kasprzewska *et al.*, 2015) affects the transition from proliferation to cell expansion.

In the growth zone of *Z. mays*, gibberellins show a striking accumulation pattern at the position at which cells exit the proliferation zone (Nelissen *et al.*, 2012). Indeed, altered levels of gibberellins result in marked differences in organ size, linked to meristem size (Nelissen *et al.*, 2012). Again, in *A. thaliana* leaves, the overproduction of gibberellins results in increased leaf size (Huang *et al.*, 1998), which is related to increased cell production (although cell size is also enhanced; Gonzalez *et al.*, 2010), and also, in the root tip, gibberellin directly controls the transition from meristem to elongation zones (Ubeda-Tomás *et al.*, 2008; Achard *et al.*, 2009; Perilli *et al.*, 2012). Thus, it appears that the molecular signaling network controlling organ growth has a similar architecture in different organs. Therefore, an obvious question to address is whether and how hormonal regulation is involved in the regulation of differences in organ size between species. Our results clearly demonstrate that such investigations should focus on the regulation of the exit from proliferation.

An interesting and unexpected finding was a strong negative correlation between the size of cells leaving the meristem and the duration of the subsequent phase of cell expansion. In theory, this could be a result of a technical artifact related to the determination of the position of the end of the proliferation zone. In *A. thaliana* root tips with very different sizes of the expansion zone, the duration is remarkably stable (Beemster & Baskin, 1998). However, the different methods employed to determine meristem size in the studies used could easily have resulted in the over- or underestimation of this boundary. This would have led to an over- or underestimation of the cells that exit, as the cell size increases quickly in this region (Beemster & Baskin, 1998), but also would have inversely affected the estimated size of the elongation zone and, consequently, the residence time within it. However, it is also plausible that the mechanisms that control exit from cell proliferation and exit from expansion are completely independent. This implies that, in some genotypes/species, cells exit mitosis at a smaller size, but as the size of mature cells is independently regulated and the rates of cell expansion do not contribute to size increase differences (Fig. 1e,g), smaller cells would, on average, expand for a longer period.

In contrast with the transition between cell proliferation and expansion, very little is known about the regulation of the transition from expansion to maturity. One hypothesis could be that cell size itself is sensed and a trigger for the termination of cell expansion. However, perturbations of cell division frequently lead to the adjustment of mature cell size that counteract the effects on mature cell number, a phenomenon called compensation (Tsukaya, 2002; Beemster *et al.*, 2003; Ferjani *et al.*, 2007; Horiguchi & Tsukaya, 2011). Therefore, a second, as yet unknown, spatial signal appears to be a more likely explanation.

Studies of the response to abiotic stresses suggest that the mechanism by which growth responds to abiotic stress depends on the duration of the exposure. In root tips of *A. thaliana* transplanted to 0.5% NaCl, the adaptation involved two phases: first, a rapid transient inhibition of the cell cycle during which cyclin-dependent kinase (CDK) activity and CYCB1;2 promoter activity were reduced, resulting in fewer cells remaining in the meristem. However, after meristem size had adjusted, the cell cycle duration returned to control values (West *et al.*, 2004). Remarkably, a similar response was found in wheat seedlings responding to soil compaction. In the first leaf that was exposed, cell production was reduced as a result of cells in a normal size meristem dividing more slowly. However, in subsequent leaves, a similar reduction in cell production was caused by a reduced meristem size and cells divided at similar rates to control plants (Beemster *et al.*, 1996). These findings indicate that short-term responses are primarily mediated by changes in the rate of division, whereas, in the longer term, the more structural response involves the adjustment of meristem size, allowing a similar reduction in cell production with cells proliferating at control rates. These examples of contrasting cellular responses to the same adverse condition, which nevertheless result in similar overall growth responses, indicate the flexibility of the cellular growth parameters within a single plant. Conversely, increased cell expansion in response to mutations that inhibit cell division demonstrates that these parameters can be adjusted to limit effects on overall organ size (Ferjani *et al.*, 2007) under a given environmental condition. This flexibility within individual species highlights the importance of our study of variations occurring in the broader context of interspecies variation, where differences in growth are much larger than observed in a single species. Moreover, it allows the identification of the parameters preferentially selected by evolution to determine organ growth differences and speculation on why this may be the case.

It could be hypothesized that, at normal rates, the cell division process is most efficient and has optimal possibilities to be adjusted in response to fluctuations in the environment. To some

extent, the same argument would account for cell size differences. Although cell sizes are often reduced by stress conditions, these changes are limited. Our finding that mature cell size does not contribute to size differences in the three types of organ analyzed across a wide range of species also suggests that there is optimal cell size, probably for physiological functioning.

Another argument for the exit from proliferation being the primary control mechanism for organ size variation is that cell division is an exponential process. One additional round of cell division before expansion doubles cell production at the organ scale, potentially doubling the organ size.

For these reasons, the main outcome of this study, that meristem size/exit from proliferation is the main parameter controlling organ size, is a logical solution from an evolutionary perspective.

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Author contributions

A.G. performed the data extraction and analysis. A.G. and G.T.S.B. planned and designed the research and wrote the manuscript.

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Supporting Information

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

Fig. S1 Regressions with full models of the cellular basis of differences in root growth rate across a range of species.

Fig. S2 Quantitative analysis of the whole-organ growth pattern and the cellular basis of leaf elongation rate that determine leaf size across a range of *Graminae*.

Fig. S3 Regressions with the full model of the cellular basis of differences in leaf size across a range of eudicotyledonous species.

Table S1 Root growth parameters extracted from published data and used for meta-analysis

Table S2 *Graminae* leaf growth parameters extracted from published data and used for meta-analysis

Table S3 Eudicotyledonous leaf growth parameters extracted from published data and used for meta-analysis

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