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Research

More cells, bigger cells or simply reorganization? Alternative mechanisms leading to changed internode architecture under contrasting stress regimes

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Summary

- Shading and mechanical stress (MS) modulate plant architecture by inducing different developmental pathways. Shading results in increased stem elongation, often reducing whole-plant mechanical stability, while MS inhibits elongation, with a concomitant increase in stability.
- Here, we examined how these organ-level responses are related to patterns and processes at the cellular level by exposing *Impatiens capensis* to shading and MS.
- Shading led to the production of narrower cells along the vertical axis. By contrast, MS led to the production of fewer, smaller and broader cells. These responses to treatments were largely in line with genetic differences found among plants from open and closed canopy sites. Shading- and MS-induced plastic responses in cellular characteristics were negatively correlated: genotypes that were more responsive to shading were less responsive to MS and vice versa. This negative correlation, however, did not scale to mechanical and architectural traits.
- Our data show how environmental conditions elicit distinctly different associations between characteristics at the cellular level, plant morphology and biomechanics. The evolution of optimal response to different environmental cues may be limited by negative correlations of stress-induced responses at the cellular level.

Introduction

In their natural habitat, plants are subjected to a plethora of different environmental stimuli which have their own specific effects on developmental pathways. In concert, these cues modulate the architectural blueprint of a given plant. Plastic responses to one environmental cue can limit or alter the response to other environmental cues (Cipollini, 1997, 2004; Sultan, 2000; Weinig & Delph, 2001; Bruce *et al.*, 2007; Anten *et al.*, 2009; Li & Gong, 2011; Huber *et al.*, 2012; von Wettberg *et al.*, 2012). However, there may be a negative correlation between abillities to show plastic responses to cues eliciting contrasting phenotypic responses (Henry & Thomas, 2002), thereby preventing plants from producing responses to multiple distinct cues. Shading and mechanical stress (MS) are two commonly occurring environmental factors which select for opposite phenotypic responses. Responses to MS (thigmomorphogenesis) generally involve the production of short and thick stems, while shading induces longer and more slender stems (Schmitt *et al.*, 1999; Pruyn *et al.*, 2000; Henry & Thomas, 2002; Liu *et al.*, 2007; Anten *et al.*, 2009; Chehab *et al.*, 2009). However, in plants subjected to both shading and MS, the shade-induced stem elongation was reduced, indicating that the integrated phenotype is a compromise among the responses to the individual cues (Anten *et al.*, 2009). Yet, it is still largely unknown how developmental mechanisms at the cellular level such as cell division, cell growth and spatial arrangement of cells affect and, at the same time, are affected by changes in plant architecture. We expect that the primary developmental processes operate at the cellular level, and that the changes at the organ level, such as changes in internode length, are a consequence of these cellular changes. Yet, the cues leading to modifications in processes at the cellular level may be detected in other parts of the plant. Furthermore, natural selection acts on integrated whole-organism phenotypes and selection on the specific responses at the cellular level will be determined by the relative costs and benefits of this response in terms of plant performance. Thus, bottom-up and top-down processes probably interact in determining the basic architectural blueprint of plants and environmentally induced changes therein. Here, we investigated to what extent changes at the cellular level contribute to

plant architectural changes in response to shading and/or MS. Better knowledge of these patterns will allow us to understand both the evolutionary constraints and also the opportunities shaping architectural plasticity in response to multiple environmental cues.

Differences in plant architecture are basically achieved by variation in the number and size of units of which plants are composed. Organ size and shape are regulated by a combination of cell proliferation and cell growth. Although cell proliferation and enlargement are two fundamentally different processes, governed by different genetic mechanisms (Potters *et al.*, 2007, 2009), they are likely to be coordinated and controlled at the level of the organ (Leevers & McNeill, 2005; Tsukaya, 2005; Weijschede *et al.*, 2008; Malinowski *et al.*, 2011). Previous research has revealed an apparent trade-off between cell number and cell size in leaf tissue and petioles, with plants being able to buffer variation in cell proliferation to some extent by alteration of cell size (Tsukaya, 2005; Weijschede *et al.*, 2008). However, it has been shown for *Trifolium repens* that changes in cell number and size have different effects on biomechanical characteristics; genotypes that elongated their petioles mainly by cell division had less flexible tissue than genotypes that elongated preferentially by producing longer cells (Huber *et al.*, 2008). This indicates that, even if variation in cell size is compensated by variation in cell proliferation, the emergent, integrated phenotype may have different characteristics (Huber *et al.*, 2008). In addition, there may also be physiological constraints on cell size; for example, the efficiency of intracellular passive diffusion processes may be constrained by the cell volume to cell area relationship (Jorgensen & Tyers, 2004).

A possible alternative but largely overlooked mechanism that could underlie organ size changes is alterations of the shape of cells and their spatial arrangement or organization. For example, the production of longer but narrower cells and the alignment of a greater proportion of the cells vertically along the internodes can contribute to increased internode length. From an economic perspective, changes in cell shape and alignment may have lower production costs, as they require fewer additional structures, but may result in costs in terms of reduced stem thickness. Whether selection acts by favoring increased cell number, cell size, cell shape or spatial alignment of cells will depend on the structural costs of a given response and the fitness consequences associated with the resulting biomechanical characteristics in a given environmental setting (Callahan *et al.*, 2008). Detailed knowledge of the developmental pattern at the level of individual cells may provide further insight into the mechanisms and consequences of architectural changes in response to environmental cues.

In this paper, we build on data presented in a previous paper on *Impatiens capensis* (Anten *et al.*, 2009), where we showed the effects of contrasting environmental stimuli (light and MS) on plant architecture and biomechanical characteristics. In the current paper, we describe the cellular mechanisms determining internode architecture and investigate to what extent patterns at the cellular level affect biomechanical characteristics. To the best of our knowledge, this is the first study that disentangles environmental effects on size and number of cells from the spatial alignment and shape of cells and which relates habitat-specific variation in internode architecture to patterns at the cellular level. Our experiment aimed to answer the following questions. How do spectral shading, MS and habitat of origin affect the basic traits determining plant architecture, namely cell size, cell number, cell shape and spatial cell alignment? How are these cellular characteristics related to plant architecture and performance in terms of mechanical stability? Is there a negative correlation between shading- and MS-induced plasticities of cellular, architectural or biomechanical characteristics?

Materials and Methods

Plant material

Impatiens capensis Meerb. (jewelweed) is an annual herb that occurs over a wide range of habitats in North America (Tabak & von Wettberg, 2008). The species is characterized by hollow stem centers and can grow to be over 1.5 m in high-light field sites, but is typically only 30–50 cm high in low-light forest understories. It has a mixed mating system, producing both self-fertilized cleistogamous flowers early and outcrossed chasmogamous flowers later in the season (Waller, 1979). Seeds usually disperse < 1.5 m from parent plants (Schmitt *et al.*, 1985). Consequently, substantial micro-geographic genetic differentiation exists in morphological and life history traits both within and among natural populations, and genetically and phenotypically differentiated open and closed canopy forms have been observed (Simpson *et al.*, 1985; Argyres & Schmitt, 1991; Dudley & Schmitt, 1995; Donohue *et al.*, 2000; von Wettberg & Schmitt, 2005; von Wettberg *et al.*, 2008). Genetic differentiation in this species has been demonstrated between populations growing as close as a few meters apart (Lechowicz & Bell, 1991).

In the spring of 2003, seedlings were collected from grassland and forest populations at Weetomo Woods Tiverton Park in Rhode Island, USA (41.5°N, 71.2°W), as described in more detail in von Wettberg *et al.* (2008). The forest population grew in a mixed *Acer rubrum– Fagus americana* deciduous forest understory where canopy openness of the trees was *c.* 6% (von Wettberg *et al.*, 2008). The grassland population grew in a large *Carex* meadow, at least 50 m from the forest edge, where canopy openness was *c.* 80%. The two populations have been shown to be morphologically and genetically differentiated despite the short geographic distance between them (von Wettberg *et al.*, 2008). For the current experiment, we used the fifth generation of inbred seeds from 10 families (hereafter genotypes) from each of the two habitats.

Experimental set-up

The experiment was conducted in the glasshouse facility of Utrecht University, Utrecht, the Netherlands. On 28 August 2006, 24 seeds of each genotype were sown in 1.3-l pots, filled with a 1 : 1 mixture of sand and potting soil and 3 g of slow-release fertilizer (Osmocote, Scotts Co. LLC, Marysville, OH, USA; 10% N + 10% P + 10% K + 3% Mg + trace elements). On

19 September, 16 similar plants of each genotype, consisting of the still elongating hypocotyl and first internode, the cotyledons and the first leaf pair, were selected and randomly assigned to each of two shading and two MS treatments, for a total of four replicates per treatment combination. Individual pots were placed 0.35 m apart. The experiment was laid out in four blocks across the glasshouse, with each block containing one replicate 4×5 grid of each treatment combination. Grids were placed 1 m apart to minimize shading effects. Positions of plants within grids and grids within blocks were changed randomly every week to further minimize possible effects of position in the glasshouse.

Two levels of shading were applied by exposing plants to either 15% daylight with a red-to-far red ratio (R : FR) of 0.3 (denoted 'shade') or 50% daylight and R:FR = 1.2 (denoted 'high light') (Anten *et al.*, 2009). The 'shade' treatment was meant to simulate the light conditions that plants experience under a forest canopy and was applied by creating cages covered with one layer of a plastic film (no. 122; Lee Colortran International, Andover, UK). As the plants grew taller, the heights of the cages were increased from 0.7 to 1.5 m. We left 0.2 m of open space below the film to allow free air movement; microclimatic measurements revealed no differences in air temperature and relative humidity between the two shading treatments. Mechanical treatments were applied by flexing plants either 0 (no MS) or 40 times once per day (MS). This was done by gently grasping the stem at *c.* 80% of its height and by bending it back and forth no further than 30° from the vertical direction. We chose this type of flexing because it simulates the mechanical effect of wind on plants (swaying of the stem) without affecting their microclimate (Smith & Ennos, 2003; Anten *et al.*, 2010).

Measurements

Between 9 and 12 October, a destructive harvest was conducted. On each day, one complete block was harvested. Total plant height and the length and diameter of the second internode were measured. The shoots were then destructively separated into stems, branches and petioles and the fresh mass of all aboveground plant parts was immediately determined. Stems were immediately packed in wet tissue paper to avoid loss of turgor and stored at 5°C for mechanical measurements and measurements of cell length and diameter. These measurements were performed on the second internode, which is situated between the first and the second leaf pairs.

We investigated the mechanisms of internode elongation based on the cells in the epidermal layer. Even though the contribution of the epidermis to the cross-section of stems is small, its contribution to bending resistance of herbaceous stems might be significant. In turgid herbaceous stems, the epidermis (and probably some cell layers under it) form a kind of 'skin' that is held in a state of tension by the hydrostatically inflated inner part of the stem (in much the same way as the interaction between the protoplast and the cell wall in turgid cells). A study by Niklas & Paolillo (1997) showed that the epidermis may thus contribute a significant fraction of stem flexural rigidity. In addition, the epidermis plays an important mechanical role in stem elongation (Schopfer, 2006 and citations therein). Therefore, while the epidermis only represents a small fraction of the stem cross-section, we believe that it can serve as a useful proxy for mechanical stem traits. The epidermal layer has previously been used to demonstrate that cell proliferation and extension can differ among environments and genotypes (Allard *et al.*, 1991; Huber *et al.*, 2008; Weijschede *et al.*, 2008) and to determine the extent to which these cellular patterns at the epidermal layer are correlated with biomechanical characteristics of the whole organ (Ridge & Amarasinghe, 1984; Loodts *et al.*, 2006). For the measurement of cell length and width, a thin peel of the outer epidermal layer was taken at harvest and immediately preserved in a solution of 50% formaldehyde. Photographs of these peels were taken under a light microscope with a digital camera attached to the microscope, and the length and width of 10 randomly chosen cells were measured to the nearest 0.01 mm in three areas. We measured 30 cells per internode, because cells can vary greatly in size and shape within tissue type. The values presented in this paper are the mean values of these measurements. We defined the growth direction of the plant (i.e. along the vertical stem axis) as the main axis of cell alignment, and care was taken that the epidermal peels were positioned in the correct direction when measuring. Cell length refers to the vertical cell dimension and cell width to the horizontal cell dimension (Fig. 1). These data were used to further calculate cell area (cell length $\times$ cell width), number of cells vertically along and horizontally around the perimeter of the internode, total number of epidermal cells on the internode, cell shape (cell length/cell width) and cell alignment (number of cells along the internode/number of cells around the internode) (Fig. 1). Cell area will be used as a measure for cell size. Cell shape values > 1 indicate that cells are relatively narrow and long, and cell shape values < 1 indicate the reverse. Differences in cell alignment may point at different patterns of cell division, namely cell division predominately taking place either in a vertical plane (high values of cell alignment) or in a horizontal direction (lower values of cell alignment). Alternatively, cell arrangement may have been determined after cell division in the early stages of internode growth. Our data do not allow us to differentiate between the effects of early cell division and later cell positioning on the relative alignment of cells in the epidermal layer.

Two stem mechanical characteristics were measured on the second internode: the Young's elastic modulus (E; MN m^{-2}), a measure of the tissue rigidity, and the breaking stress (σ_b; MN m^{-2}), a measure of tissue strength (Niklas, 1992; Gere & Timoshenko, 1999). High values for E and σ_b indicate that the tissue is stiff and resistant to rupture, respectively. The stem-level traits were used to calculate two measures of mechanical stability at the whole-plant level. The first measure is the buckling safety factor (BSF), which indicates the ability of plants to carry their own weight. BSF < 1 indicates that the plant stem is too long or too thin to carry the aboveground plant structures, even in the absence of lateral forces, and will thus buckle globally (Gere & Timoshenko, 1999). The second measure is the maximum lateral force that plants resist before breaking (F_{max}) and is a measure of the ability to resist external (wind) forces. Specifically, it indicates the minimum lateral force required to induce an amount of stress

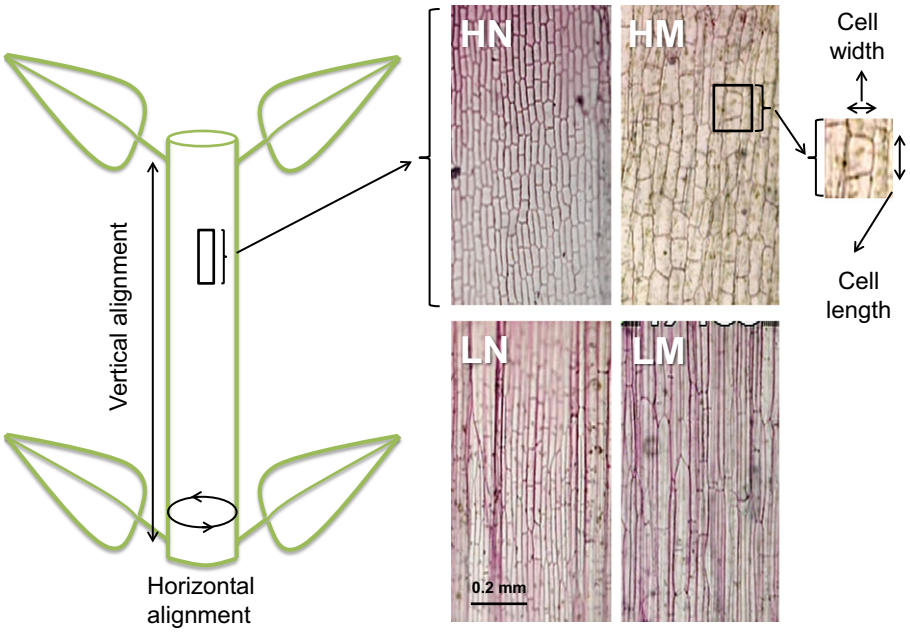

Fig. 1 Schematic drawing of a stem internode, indicating how we defined vertical and horizontal alignments. The number of cells aligned along the internode was estimated as internode length divided by average cell length; the number of cells aligned around the internode was estimated by dividing internode circumference by average cell width. On the left hand side, images of sample epidermal peels of *Impatiens capensis* show the cells. HN, high light, no mechanical stress (MS); HM, high light, MS; LN, low light, no MS; LM, low light, MS. The epidermal peels were aligned in the orientation of the stems.

at the stem base that exceeds σ_b, causing rupture (Anten *et al.*, 2005). Measurements were performed with a universal testing machine (Instron Model 5542; Instron, Canton, OH, USA) using a three point bending technique. Further details of measurements, including equations and the assumption underlying the calculation of E, σ_b, F_{max}, and BSF, are given by Anten *et al.* (2005, 2009).

Statistical analysis

The responses of plant traits to treatments were analyzed with mixed model nested ANOVA (Proc GLM, SAS 9.1; SAS Institute, Cary, NC, USA). Habitat, light and MS were treated as fixed effects and the random factor genotype was nested within habitats. Two- and three-way interactions were also included in the model. Block was included in the model to account for spatial variation in the glasshouse conditions and for the difference in harvest times among blocks. Data were transformed when necessary to improve normality and homogeneity of the residual variances.

Shade-induced plasticity was calculated as the percentage change of a trait in shaded, no-MS conditions as compared with high-light, no-MS conditions (Weijschede *et al.*, 2006; Huber *et al.*, 2008). MS-induced plasticity was calculated as the percentage change of traits in high-light, MS conditions as compared with high-light, no-MS conditions. This implies that the percentage response was scaled to the conditions where the specific stress did not occur. Consequently, a positive plastic response to MS indicates that the respective trait had a larger value in high-light, MS conditions compared with high-light, no-MS conditions, whereas a negative value for plasticity indicates the opposite. A value of 0 indicates that the respective trait value was not affected by MS. The calculations were performed using the within-treatment genotypic means. Genotypes of both habitats were pooled for the following analyses to increase the strength of the analyses. As there was a strong overlap in the relative responses of genotypes from forest and grassland habitats (see e.g. Fig. 6), we believe that the resulting correlations were not driven by habitat divergence.

The interrelationship among whole-organ traits and the effects of the environment on cellular traits, internode architecture and biomechanical characteristics were analyzed with a genotypic path analysis. Path analytical models can be used to explore and quantify patterns of variation in character correlations (Pigliucci & Kolodynska, 2006). Specifically, we were interested in how cellular characteristics (cell area, cell number, cell shape and cell alignment) contribute to internode architecture and to what extent biomechanical characteristics can be explained by traits at the cellular level. The four cellular traits were entered as exogenous traits. We calculated the correlation among these cellular traits and calculated how they were associated with the architectural traits internode length and internode thickness and the biomechanical traits internode flexibility and BSF (see Fig. 4). Genotypic means were used for all analyses. We performed three sets of analyses. In the first set, we investigated how cellular traits are associated with architectural and biomechanical traits under high-light, no-MS conditions. In the second set, we calculated how shading-induced changes of cellular characteristics are associated with shading-induced changes of architectural and biomechanical traits, and in the third set of analyses we performed the same analysis for MS-induced changes. The program AMOS (Arbuckle & Wothke, 1999) was used for these analyses.

We performed correlation analyses to test the hypothesis that there is a negative correlation between the expression of shade avoidance and the expression of thigmomorphogenesis (i.e. mechanical-induced changes of traits) by calculating Pearson's correlation coefficients of the percentage response to shading and to MS for cellular, architectural and biomechanical characteristics. Similar to the path analyses, these analyses were performed at the genotypic level, with plants from the two habitats being pooled.

Results

Plant response to shading, MS and habitat of origin

Plants responded to shading by producing significantly longer, thinner internodes and stiffer tissue and having a lower BSF (Fig. 2; Table 1). These internodes were composed of longer and narrower cells (Fig. 3a,b,d; Table 2). Shading did not significantly affect the mean individual cell area and the total number of epidermal cells, but it did affect the alignment of cells; while on average shading reduced the total number of epidermal cells on the second internode by only 4.4%, it significantly increased the relative alignment of cells in the vertical direction (Fig. 3e–h; Table 2). MS-induced responses were largely opposite to those induced by shading. Plants subjected to MS produced shorter and slightly thicker internodes and had a higher BSF (Fig. 2a,b,f; Table 1). Their internodes consisted of shorter and slightly smaller cells (Fig. 3a,c; Table 2). MS reduced cell area by up to 30% (Fig. 3c; Table 2). MS tended to reduce the total number of epidermal cells (5.5%; $P = 0.052$; Fig. 3g; Table 2). In contrast to the effect of shading, MS reduced the relative allocation of cells in the vertical direction (Fig. 3h; Table 2). Shade had a smaller stimulatory effect on cell elongation in plants subjected to MS than in unflexed individuals, as indicated by a significant MS × shade interactive effect on these traits (Fig. 3a; Table 2).

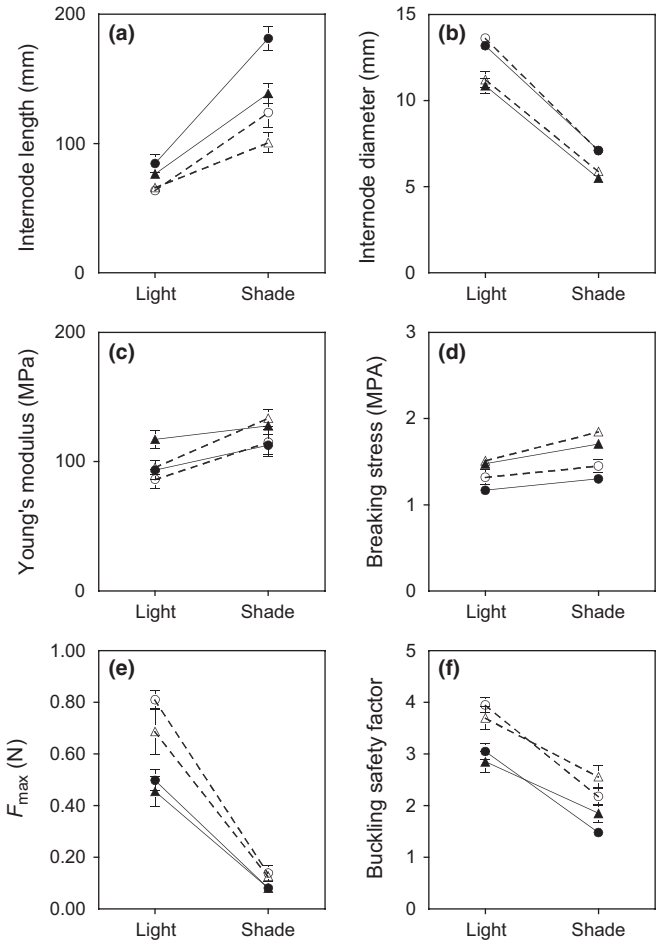

Fig. 2 Response (mean ± 1 SE) of internode architecture (a, b) and biomechanical traits (c–f) of plants originating from grassland (circles) and forest (triangles) habitats to light availability and mechanical stress (MS) (no MS, solid line and closed symbols; MS, dashed line and open symbols) in *Impatiens capensis*. (c) Young's modulus, representing the stem tissue rigidity; (d) breaking stresses, indicating the tissue strength; (e) F_{max}, the lateral force plants can resist before breaking, and (f) the buckling safety factor (BSF), the ability of plants to carry their own weight. For the significances of the results, see Table 1.

Plants originating from forest understory habitats produced significantly thinner internodes composed of fewer and narrower but on average longer cells than those from the grassland habitat (Figs 2b, 3a,c,d,g; Tables 1, 2). The total number of epidermal cells was on average 42% lower in forest compared with grassland plants (Fig. 3g; Table 2). This could be attributed to a significant reduction in both the number of cells aligned vertically along and the number of cells aligned horizontally around an internode (Fig. 3e,f; Table 2). Plants originating from the two habitats responded similarly to MS with respect to the cellular characteristics. However, habitat did affect the potential to respond to shading, as the cell shape of forest plants responded more strongly to shading than that of grassland plants, while under shaded conditions cell area was more similar between grassland and forest plants than under high-light conditions (Fig. 3c,d; Table 2).

Effects of cellular characteristics on plant architecture and biomechanical traits

The genotypic path analyses show the extent to which traits at the cellular level affect architectural traits and biomechanical characteristics, and whether cellular traits are interrelated with each other. Under high-light, no-MS conditions, there was a positive correlation between cell shape and cell area as well as between cell alignment and total cell number (Fig. 4a). That is, genotypes that aligned more cells in the vertical direction also had more cells overall, and genotypes with longer cells also had larger mean cell areas (Fig. 4a). Total cell number was negatively correlated with cell area, and genotypes that aligned a larger proportion of their epidermal cells vertically had on average smaller cells (Fig. 4a) under high-light, no-MS conditions.

Internodes were consistently longer in genotypes with a larger cell area or a larger number of cells (Fig. 4). Shading- or MS-induced changes of cell area or total cell number had a similar effect on internode length plasticity, as genotypes that produced more or larger cells in response to shading also produced longer internodes, while genotypes that produced shorter or fewer cells in response to MS also decreased the internode length (Fig. 4b,c). Interestingly, under high-light, no-MS conditions, genetic variation in internode diameter was not correlated with any of the cellular characteristics (Fig. 4a). However, shading- and MS-induced changes in internode diameter were positively associated with environmentally induced increases in total cell number and cell area, but negatively associated with cell width (Fig. 4b,c).

The association between environmentally induced changes of cellular characteristics and environmentally induced changes of biomechanical traits strongly differed for the different

Table 1 Result of mixed model ANOVA testing for the effects of habitat of origin (H), light availability (L) and mechanical stress (MS) on responses of morphological and biomechanical traits in *Impatiens capensis*

	df	Internode length	Internode diameter	Young's modulus	Breaking stress	F_{max}	Buckling safety factor
H	1	2.85 ns	**34.57** ***	**5.59** *	**26.05** ***	0.58 ns	0.11 ns
L	1	**245.58** ***	**611.14** ***	**22.47** ***	**16.39** ***	**196.62** ***	**394.00** ***
MS	1	**245.66** ***	**17.07** ***	2.81 ns	**11.78** **	**138.91** ***	**151.43** ***
H × L	1	**18.43** ***	*4.24* $	0.05 ns	2.72 ns	1.40 ns	**18.95** ***
H × MS	1	**5.76** *	1.24 ns	0.81 ns	0.85 ns	1.08 ns	0.05 ns
L × MS	1	**38.19** ***	1.0 ns	**7.61** *	0.66 ns	**132.54** ***	*3.14* $
H × L × MS	1	*3.19* $	1.13 ns	1.87 ns	1.09 ns	*3.70* $	0.07 ns
Genotype(habitat) (G(H))	18	**13.39** ***	**8.29** ***	**5.52** ***	**2.84** ***	**8.83** ***	**14.43** ***
L × G(H)	18	**2.41** **	**4.5** ***	**2.50** ***	**1.71** *	**6.01** ***	1.33 ns
MS × G(H)	18	0.73 ns	0.42 ns	1.05 ns	0.75 ns	0.80 ns	1.16 ns
L × MS × G(H)	18	0.82 ns	1.02 ns	1.06 ns	0.45 ns	0.48 ns	0.64 ns
Block	3	*2.28*$	**6.71** ***	**75.44** ***	**48.57** ***	**16.44** ***	*2.40* $

F-values and their significance are given. Significance levels: ns, $P > 0.1$; $, $0.1 \geq P > 0.05$; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \leq 0.001$. Significant values are presented in bold, and marginally significant values in italics.

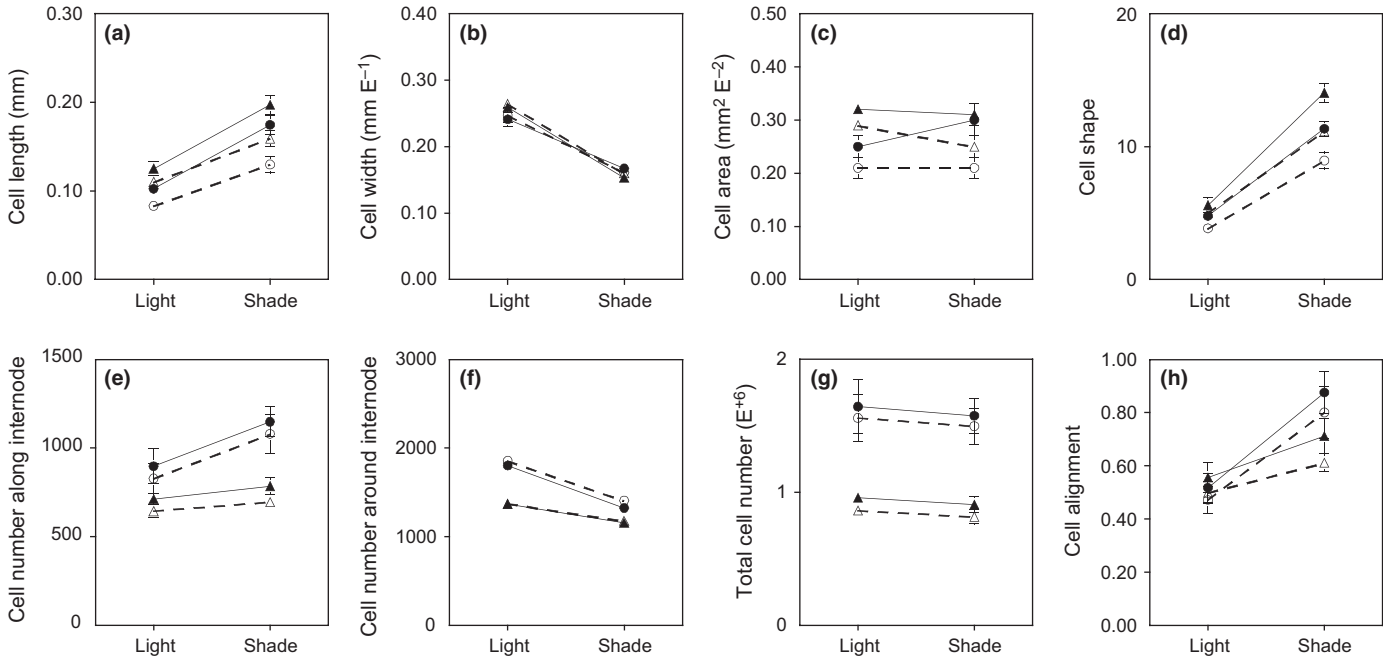

Fig. 3 Response (mean ± 1SE) of cellular characteristics of *Impatiens capensis* plants originating from grassland (circles) and forest (triangles) habitats to light availability and mechanical stress (MS) (no MS, solid line and closed symbols; MS, dashed line and open symbols). In the first row dimensions of individual cells (a: cell length, b: cell width, c: cell area, d: cell shape), and in the second row data describing the number and distribution of cells (e: cell number aligned vertically along the internode, f: cell number aligned horizontally around the internode, g: total cell number, h: relative cell alignment) are given. Data are based on measurements performed on the second internode (between the first and second leaf pairs). For significances of the results, see Table 2 .

environmental conditions and was characterized by only a few significant correlations (Fig. 4). It should be noted, however, that MS-induced reductions of cell area and cell number were associated with a significant increase in tissue stiffness, as indicated by larger values of Young's modulus (Fig. 4c). Aligning more cells in the vertical direction in response to MS, however, was associated with the production of internodes with more flexible tissue (Fig. 4c). In contrast to these strong effects of MS-induced changes in cellular characteristics on internode tissue stiffness, there was no direct association with MS-induced changes in whole-plant stability (BSF, Fig. 4c).

Correlation between responses to shade and MS

We found a positive correlation of plastic responses to shading and to MS for cell shape, number of cells around the internode, total cell number, cell area and internode diameter (Figs 5, 6). For example, genotypes that increased their internode diameter more strongly in response to MS exhibited a smaller reduction in internode diameter in response to shading and vice versa (Fig. 5b). It has to be noted that this apparent positive correlation actually describes a negative correlation between the abilities to respond to shading and MS: as shade avoidance and MS have

Table 2 Result of mixed model ANOVA testing for the effects of habitat of origin (H), light availability (L) and mechanical stress (MS) on responses of cellular characteristics in *Impatiens capensis*

	df	Cell length	Cell width	Cell number along internode	Cell number around internode	Total cell number	Cell area	Cell shape	Cell alignment
H	1	**8.90** **	0.69 ns	**9.26** **	**33.26** ***	**20.46** ***	**5.82** *	**13.46** **	0.99 ns
L	1	**156.88** ***	**314.35** ***	15.77 ns	**90.57** ***	1.06 ns	0.12 ns	**593.42** ***	**45.13** ***
MS	1	**57.21** ***	0.31 ns	**9.08** **	0.74 ns	*4.31* $	**35.4** ***	**46.91** ***	**4.53** *
H × L	1	0.17 ns	**5.44** *	*4.40* $	**13.09** **	0.22 ns	**6.23** *	**4.61** *	**7.26** *
H × MS	1	0.76 ns	0.29 ns	0.25 ns	0.28 ns	0.18 ns	1.2 ns	0.00 ns	0.21 ns
L × MS	1	*4.38* $	0.23 ns	0.16 ns	0.00 ns	0.03 ns	1.72 ns	**6.44** *	0.46 ns
H × L × MS	1	0.02 ns	0.28 ns	0.16 ns	0.01 ns	0.07 ns	0.41 ns	0.23 ns	0.04 ns
Genotype(habitat) (G(H))	18	**2.67** ***	1.32 ns	**8.54** ***	**2.18** **	**6.15** ***	**2.58** ***	**1.60** $	**6.05** ***
L × G(H)	18	0.9 ns	0.99 ns	*1.54* $	0.75 ns	1.25 ns	0.82 ns	0.59 ns	*1.56* $
MS × G(H)	18	0.58 ns	1.13 ns	0.57 ns	0.86 ns	0.44 ns	0.54 ns	0.51 ns	1.24 ns
L × MS × G(H)	18	1.34 ns	0.97 ns	0.88 ns	0.50 ns	0.54 ns	1.07 ns	1.07 ns	1.17 ns
Block	3	**22.68** ***	**5.67** ***	**23.41** ***	**4.29** **	**18.72** ***	**12.3** ***	**22.05** ***	**12.72** ***

F-values and their significance are given. Significance levels: ns, $P > 0.1$; $, $0.1 \geq P > 0.05$; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \leq 0.001$. Significant values are presented in bold, and marginally significant values in italics.

opposite effects on internode diameter, a positive correlation means that genotypes that elicited the expected response to one environmental cue inevitably expressed a reduced response to the other environmental cue.

In contrast to the cellular traits, shade- and MS-induced responses of biomechanical traits and internode length were not positively correlated. Shade- and MS-induced changes in resistance to rupture (breaking stress, σ_b) tended to be negatively correlated, indicating that genotypes that exhibited stronger increases in response to MS hardly responded to shading with respect to σ_b, while genotypes that increased σ_b in response to shading hardly responded to MS for this trait (Fig. 5d).

The correlation analyses also revealed that different groups of traits responded to either shading or MS (Figs 5, 6). Genotypes varied very little in the anatomy and morphology of internodes in the vertical direction (cell length, cell shape, number of cells vertically along internodes and internode length) in response to MS, but they showed a large variation in the response to shading for these cellular traits. The opposite was true for traits characterizing the plasticity of biomechanical stability and in terms of the maximum lateral force that plants can resist (F_{max}), as genotypes varied much more in their response of F_{max} to MS than to shading (Fig. 5e).

Discussion

Variation in plant architecture is linked to processes at the cellular level

Individuals of the same species are often phenotypically variable. Variation in plant growth and development can be related to cell structural attributes – their size, number and geometry – as cells form the basic architectural units of plants. Yet, the degree to which plasticity in these cellular traits in response to different environmental cues scales to responses at the organ level and how this relates to the habitat in which plants evolved have been rarely investigated. We found that two common environmental cues, shading and MS, had significant effects on the balance between horizontal and vertical cell divisions in *I. capensis*, which contributed to organ-level variation in internode length and thickness. The specific patterns of these responses depended largely on the cellular traits in question and the type of environmental stimuli. Only cell length, shape and alignment responded to both shading and MS. Other traits, such as total cell number, number of cells aligned in the vertical direction and area of individual cells, remained relatively constant across light environments but responded to MS, while cell width and the number of cells around the internode were not affected by MS but responded to shading.

While cell number is widely regarded to be the major determinant of organ size (Johnson & Lenhard, 2011), cell size can theoretically also play an important role. This latter notion is supported by our results. Furthermore, we found a significant negative correlation between cell area and total cell number across genotypes, indicating that variation in internode length is not solely determined by cell number variation. This trade-off proved to be consistent over a wide range of conditions, as it was apparent not only in terms of genotypic variation in characteristics but also in terms of the phenotypic plasticity in these characteristics. That is, genotypes that were highly responsive to environmental stress by changing cell area had a more constant cell number and vice versa. Plants have only a limited amount of resources available to invest in growth, which can be invested in the production of either more or larger structures, leading to a trade-off between cell size and number (Huber *et al.*, 2008; Weijschede *et al.*, 2008; Fujikura *et al.*, 2009; Kawade *et al.*, 2010). Such a trade-off may have important evolutionary consequences, as high levels of phenotypic integration (Murren, 2012), especially negative correlations, have been argued to limit the evolution of plastic responses (Pigliucci, 2003; Gianoli & Palacio-Lopez, 2009).

Different environmental conditions select for different plant morphologies, requiring large flexibility at the level of the basic units. Thus, under shaded conditions, the production of longer stem internodes has been frequently shown to be advantageous (Dudley & Schmitt, 1996; Griffith & Sultan, 2006; Bell & Galloway, 2008; von Wettberg *et al.*, 2008; Huber *et al.*, 2011), while the negative effect of MS can be alleviated by the

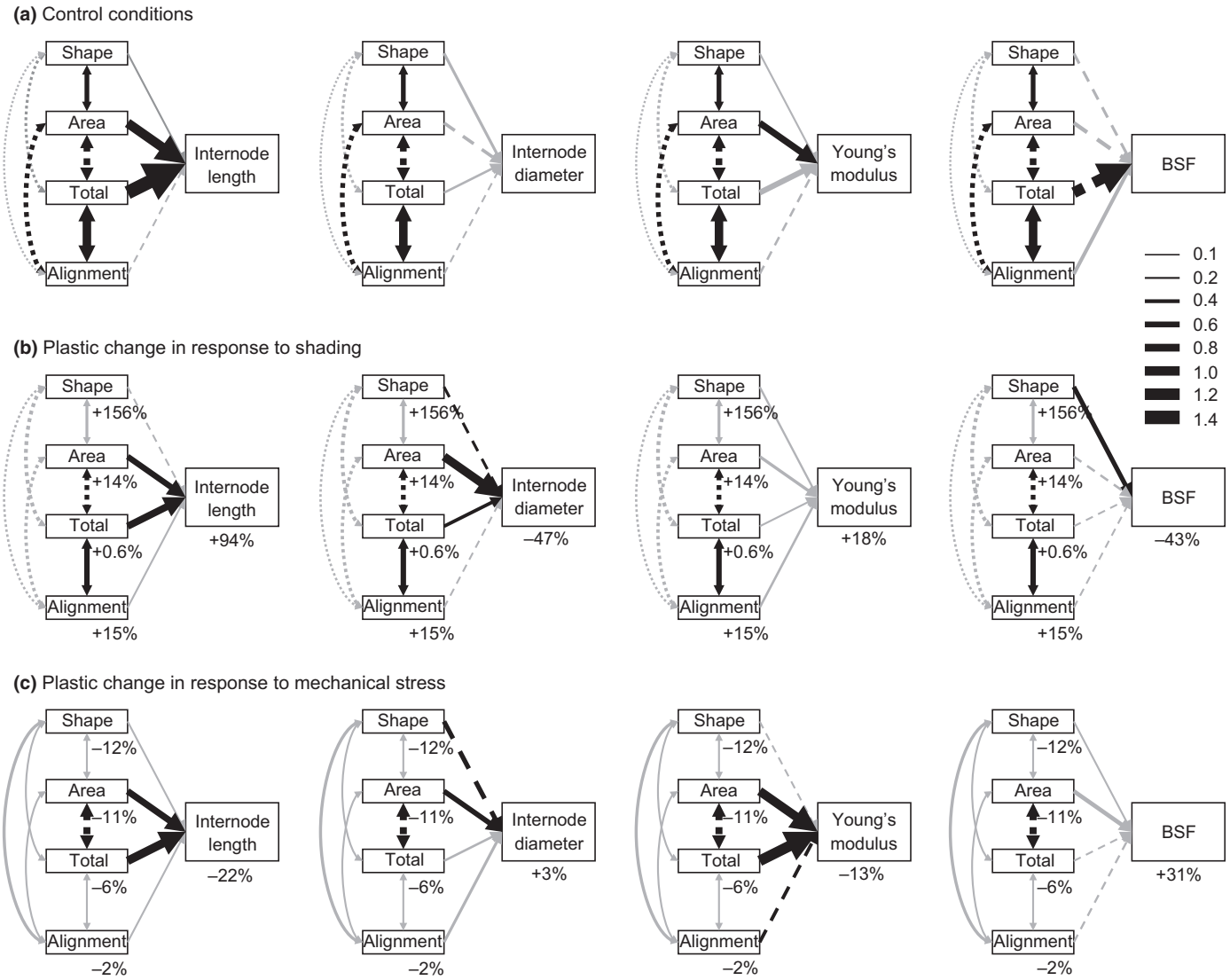

Fig. 4 Effects of (a) cellular characteristics on plant architecture and biomechanical traits under high-light, no mechanical stress (MS) conditions, and (b) shade-induced and (c) MS-induced changes in cellular characteristics on shade- and MS-induced changes of architectural and biomechanical traits in *Impatiens capensis*. We chose Young's modulus (i.e. tissue rigidity) as an organ-level trait and the buckling safety factor (BSF) to represent mechanical stability of the whole plant. Genotypes from open and closed canopy sites were pooled for the analyses to increase the phenotypic space. Double-headed arrows show the strength of correlation between cellular characteristics. Solid lines indicate positive relationships and dashed lines negative relationships. The thickness of the lines is proportional to the strength of the relationship, as indicated by standardized regression coefficients obtained by the path analyses conducted in AMOS. The key shows which line thickness relates to a given regression coefficient (for single-headed arrows) or correlation coefficient (double-headed arrows). Black lines, significant and marginally significant relationships ($P < 0.1$); gray lines, nonsignificant relationships ($P \geq 0.1$). The values below the traits indicate the overall plastic change (direction and magnitude) of the respective trait in response to shading or MS.

production of shorter and thicker internodes (Telewski, 1990; Telewski & Pruyn, 1998; Henry & Thomas, 2002). This indicates that plants have evolved a means to change their architecture in response to the environment despite the above-mentioned limitations on the number and size of the cells. In our experiment, internode length was one of the few characteristics that consistently responded plastically to both shading and MS.

Genetic variation in internode length under unstressed (high-light and no-MS) conditions was correlated with both cell number and cell area. We also found that both cellular traits can respond to environmental variation and in turn lead to environmentally induced plastic changes of internode length, a rather plastic trait in *Impatiens*. The limited response to the environmental manipulations of total cell number seemed to have mainly been compensated by the higher responsiveness of cell area. Theoretically, changes in cell alignment and cell shape may have provided an additional mechanism to further extend internodes either by producing narrower but longer cells or by aligning a greater proportion of cells in the vertical direction. However, though responsive to environmental conditions, cell shape and alignment were not directly associated with internode length or internode length plasticity.

While shading and MS had opposite effects on internode length and diameter and affected the individual cellular traits

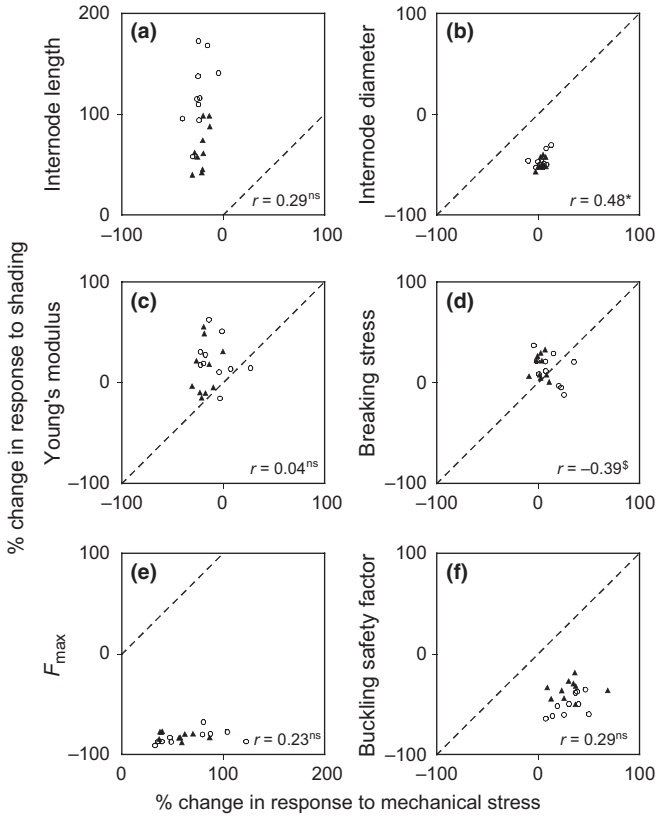

Fig. 5 Interrelationship between plastic responses to shading and plastic responses to mechanical stress (MS) for plant architecture (a: internode length, b: internode diameter) and biomechanical traits (c: Young's modulus representing the stem tissue rigidity, d: breaking stress, describing the tissue strength, e: F_{max}, the maximal lateral force plants can resist before breaking, f: BSF, describing the ability of plants to carry their own weight) in *Impatiens capensis*. Plasticity was calculated as the percentage change of trait values in either MS- or shading-conditions compared with plants grown in high-light, no-MS conditions. Each symbol represents the response of a single genotype to shading and MS (circles, grassland; triangles, forest). Genotypes from open and closed canopy sites were pooled for the analysis. The hatched diagonal line indicates where shading- and MS-induced plasticity would be exactly the same; symbols below the line indicate higher values for MS-induced plasticity and symbols above the line higher values for shading-induced plasticity. As shading and MS select for opposite morphological responses (see also overall responses in Fig. 2), a negative relationship would indicate that genotypes can respond appropriately to both MS and shading. The Pearson's correlation coefficient and its significance are given in each graph. Significance levels: ^{ns}, $P > 0.1$; $^{\$}$, $0.1 \geq P > 0.05$; *, $0.05 \geq P > 0.01$.

differently, we found very similar correlation patterns between plasticity of cellular patterns and changes of internode architecture in response to these two stimuli. Independent of the type of stimulus, environmentally induced changes in internode length were correlated with changes in cell area and total cell number, while changes in internode diameter could be related to changes in cell shape and area. This shows that, despite the different natures of these two environmental stimuli (shading but not MS entails an overall reduction of assimilates available to the plant), changes at the cellular level had similar effects on internode architecture, indicating that response patterns were canalized to some extent.

Patterns at the cellular level influence biomechanical characteristics to some extent

The biomechanical properties of an organ can depend on the characteristics of the cells comprising the tissues (Huber *et al.*, 2008; Onoda *et al.*, 2008; Kitajima & Poorter, 2010). In *I. capensis*, cellular characteristics and shade-induced changes at the cellular level had surprisingly few direct effects on the tissue flexibility. In contrast to the more canalized pattern found for internode architecture, the effects of plasticity of cellular traits on biomechanical characteristics differed between shaded and MS conditions. Plasticity of cellular traits had the strongest effects on tissue flexibility in plants subjected to MS, but these differed between cellular traits. In genotypes with a greater MS-induced reduction in cell area and cell number, there was also a larger increase in tissue flexibility (i.e. lower stem Young's modulus, *E*). Conversely, MS-induced alignment of more cells in the vertical direction along the internode resulted in reduced tissue flexibility. MS-induced increases in tissue flexibility have been observed in other studies (Anten *et al.*, 2005; Liu *et al.*, 2007). Stem flexibility may enable plants to more easily reconfigure under wind-loading, thus reducing the magnitude of the force to which they are exposed (Puijalon *et al.*, 2008, 2011). Selection can directly act on the underlying cellular traits that facilitate this form of stress avoidance. In contrast to the results for MS-induced plasticity of cellular traits, we did not find a concomitant change of shading-induced plasticity of cellular traits and mechanical stability of plants. This may be explained by the fact that under shaded conditions stem stiffness is of less importance. Under natural conditions, high levels of shading are often caused by conspecific competitors which also act as wind shields, resulting in lower MS (Nagashima & Hikosaka, 2011, 2012). The relatively low variation in the plasticity of whole-plant biomechanical characteristics in response to shading may also indicate that selection has led to canalized responses by minimizing investment in biomechanical stability in shaded plants.

Under high-light, no-MS conditions, total cell number was closely associated with mechanical stability at the whole-plant level, while cell area affected tissue flexibility. Genotypes that produced more cells had a lower BSF (i.e. a reduced ability to carry their own weight) under high-light, no-MS conditions. As increased cell proliferation was mainly invested in the production of longer, but not thicker internodes, internodes of the same diameter had to support longer stems, and thus were associated with a higher risk of mechanical failure. This increased mechanical risk may have selected against genotypes investing preferentially in height growth. However, plants may have also evolved other mechanisms, such as a changed internode structure and efficient use of stem cavity, to gain the required internode strength (Anten *et al.*, 2009).

Cellular characteristics strongly differ in plants originating from different habitats

Differential selection on the response pattern of the integrated phenotype may occur between habitats and populations; these differences can be interpreted as a signature of past evolutionary

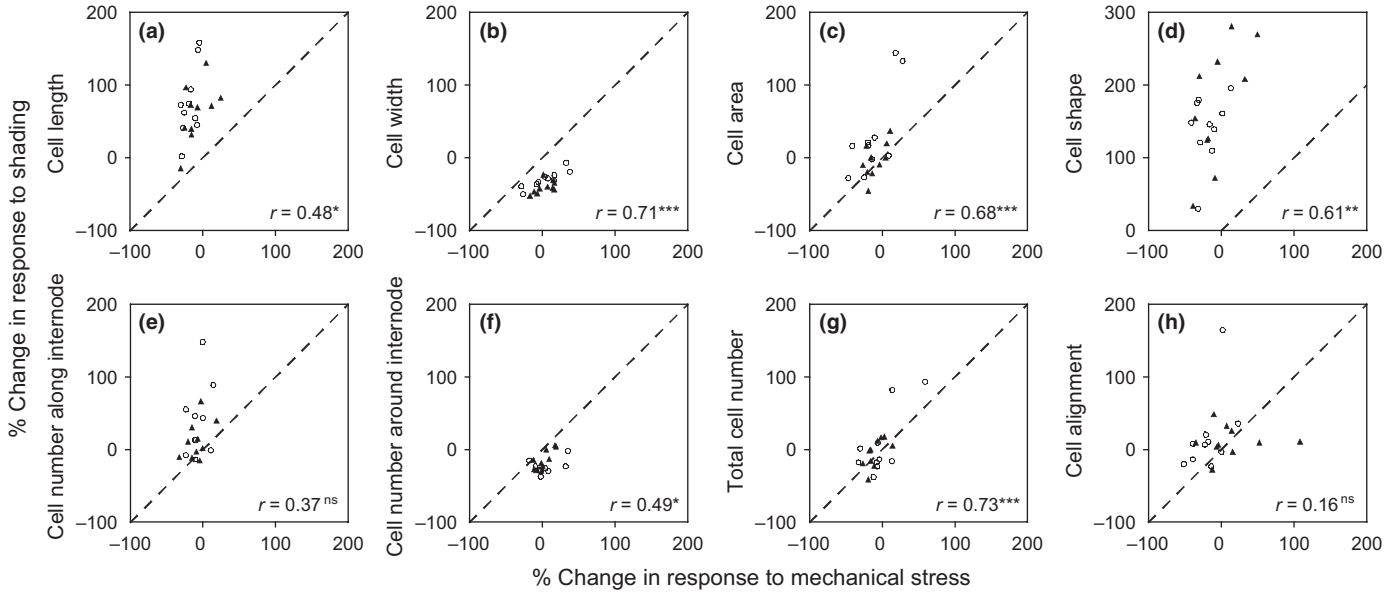

Fig. 6 Interrelationship between plastic responses to shading and plastic responses to mechanical stress (MS) for cellular characteristics in *Impatiens capensis*. Plasticity was calculated as the percentage change of trait values under either MS- or shading-conditions compared with plants grown in control conditions (high light, no MS). In the first row dimensions of individual cells (a: cell length, b: cell width, c: cell area, d: cell shape), and in the second row data describing the number and distribution of cells (e: cell number aligned vertically along the internode, f: cell number aligned horizontally around the internode, g: total cell number, h: relative cell alignment) are given. Each symbol represents the response of a single genotype to shading and MS (circles, grassland; triangles, forest). Genotypes from open and closed canopy sites were pooled for the analysis. The hatched diagonal line indicates where shading- and MS-induced plasticity would be exactly the same; symbols below the line indicate higher values for MS-induced plasticity and symbols above the line higher values for shading-induced plasticity. As shading and MS select for opposite responses for cellular traits (see also overall responses in Fig. 3), a negative relationship would indicate that genotypes can respond appropriately to both MS and shading. The Pearson's correlation coefficient and its significance are given in each graph. Significance levels: ns, $P > 0.1$; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \leq 0.001$.

events leading to different developmental trajectories (Pigliucci, 2002). Plants in contrasting light environments, such as open habitats or forest understories, are typically also exposed to different levels of MS that impose different selective forces on their tissue and structural characteristics. In the forest understory, under both light-limited and wind-protected conditions, selection for stiffer stems provides sufficient self-support at a relatively small resource investment. Conversely, in more open habitats where plants are exposed to both high light and stronger wind forces, the production of more flexible shoots, which reconfigure more easily in the wind and thus reduce drag forces, might be more beneficial (Anten *et al.*, 2009; Puijalon *et al.*, 2011). Our results show that these contrasting selective forces have also led to genetic differences in cellular characteristics.

As discussed previously, both shading and mechanical stimulation of *I. capensis* had significant effects on cellular traits that contribute to internode length and thickness. However, the strength and direction of these treatment effects varied depending on the habitat from which plants originated. While the overall number of epidermal cells on the internode remained unaffected by treatments, plants originating from the forest understory produced fewer cells than plants originating from grassland sites. Plants originating from different habitats hardly differed with respect to the shape and alignment of cells under high-light conditions but showed distinctly different response patterns to shading. Plants originating from forest understory responded to shading primarily by producing longer cells, while plants originating from grassland responded mainly by aligning a greater proportion of the cells in the vertical direction along the internode.

Interestingly, the response to shading was in the same direction as the habitat differentiation: the forest habitat plants had on average longer cells than plants from grassland habitats. Similarly, plants exposed to MS and plants from the more open and wind-exposed grassland habitats both produced shorter and wider cells. This consistent response to long-term past evolutionary events and present environmental conditions indicates that under sheltered, light-limiting conditions the production of long and narrow cells is favored by selection, while under open, disturbed conditions the production of shorter and wider cells is selected for. The habitat-specific difference in cell number, however, was larger than the differences in cell number induced by the experimental treatments. This supports the notion that cell proliferation is a more stable trait that can be altered by selection processes but not by relatively short-term environmental fluctuations. As total cell number is the product of vertical and horizontal divisions, the relatively low plasticity of total cell number points at a tight control among these different processes. The variation in cell number across habitats may indicate that cell proliferation is associated with fitness consequences, but additional experiments under a more natural setting would be needed to test this hypothesis.

Trade-off in shading- and MS-induced plasticities

We tested the hypothesis that genotypes cannot optimally respond to different cues inducing opposite responses (Henry & Thomas, 2002), leading to a negative correlation in the responses to MS and shading. Our results show a positive correlation for cellular traits in the responses to shading and to MS. Genotypes that reduced their cell area or total cell number in response to MS also produced fewer cells in response to shading (Fig. 6c,g), while genotypes that were more responsive to MS, producing wider and shorter cells, were less able to produce long and narrow cells in response to shading. Interestingly, this apparent positive correlation actually points at a negative functional relationship between the abilities to respond adequately to shading and MS. Previous research has shown that shade avoidance and MS select for opposite morphological responses, that is, the production of thinner internodes under shaded conditions and sturdier internodes under MS. A positive correlation between shade- and MS-induced plasticities in internode thickness thus means that plants that display an adequate response to one environmental cue (i.e. improving the ability to deal with the environmental effect, for example by increasing internode thickness in response to MS) actually displayed a reduced or a 'wrong' response to the other environmental cue (e.g. retaining relatively thicker internodes under shaded conditions). The same reasoning can be applied to the various cellular traits. This apparent trade-off may be explained by functional arguments. While strong responses to shade entail mechanical risks and would be detrimental under MS, similarly strong responses to MS come at the cost of competitive ability (Henry & Thomas, 2002). The presence of neighboring plants may also explain this apparent trade-off in the ability to respond to shade or MS. In a dense stand, neighboring plants negatively affect light availability but they also provide wind shielding (Speck, 2003), which may have resulted in a negative correlation in the strength of these contrasting selective forces, thereby impairing the ability of plants to elicit optimal responses to both, MS and shading. Our results not only reveal that different environmental cues select for opposite morphological and cellular responses, they also indicate that at the genotypic level the ability to respond adequately to different cues can be limited as a result of trade-offs in the responsiveness of cellular characteristics to MS or shading. This in turn may constrain genotypes in the responses to a wide range of environmental cues, such as shading and MS, if they select for opposite response patterns.

Conclusions

Plants have only a limited repertoire of possible responses at the cellular level – for example, faster or slower division, elongation in one or more directions, or thicker or thinner walls. Using these responses they have to deal with a variety of environmental changes and with simultaneously acting cues that can elicit opposite responses. With this limited repertoire, using differences in meristem activation patterns, plants can produce a remarkably large range of architectures. Differences in responses between forest and grassland genotypes suggest that there is strong selection pressure on this program of cellular adjustments to varying stress factors, reflecting the large risks involved – for example, being overtopped by neighbors, or succumbing to a sudden MS event.

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References

Allard G, Nelson CJ, Pallardy SG. 1991. Shade effects on growth of tall fescue. 1. Leaf anatomy and dry matter partitioning. *Crop Science* **31**: 163–167.

Anten NPR, Alcala-Herrera R, Schieving F, Onoda Y. 2010. Wind and mechanical stimuli differentially affect leaf traits in *Plantago major*. *New Phytologist* **188**: 554–564.

Anten NPR, Casado-Garcia R, Nagashima H. 2005. Effects of mechanical stress and plant density on mechanical characteristics, growth, and lifetime reproduction of tobacco plants. *American Naturalist* **166**: 650–660.

Anten NPR, von Wettberg EJ, Pawlowski M, Huber H. 2009. Interactive effects of spectral shading and mechanical mtress on the expression and costs of shade avoidance. *American Naturalist* **173**: 241–255.

Arbuckle JL, Wothke W. 1999. *Amos 4.0 users guide*. Chicago, IL, USA: SPSS.

Argyres AZ, Schmitt J. 1991. Microgeographic genetic-structure of morphological and life-history traits in a natural population of *Impatiens capensis*. *Evolution* **45**: 178–189.

Bell DL, Galloway LF. 2008. Population differentiation for plasticity to light in an annual herb: adaptation and cost. *American Journal of Botany* **95**: 59–65.

Bruce TJA, Matthes MC, Napier JA, Pickett JA. 2007. Stressful "memories" of plants: evidence and possible mechanisms. *Plant Science* **173**: 603–608.

Callahan HS, Maughan H, Steiner UK. 2008. Phenotypic plasticity, costs of phenotypes, and costs of plasticity - toward an integrative view. In: Schlichting CD, Mousseau TA, eds. *Year in evolutionary biology 2008*. New York, NY, USA: Wiley-Blackwell, 44–66.

Chehab EW, Eich E, Braam J. 2009. Thigmomorphogenesis: a complex plant response to mechano-stimulation. *Journal of Experimental Botany* **60**: 43–56.

Cipollini D. 2004. Stretching the limits of plasticity: can a plant defend against both competitors and herbivores? *Ecology* **85**: 28–37.

Cipollini DF. 1997. Wind-induced mechanical stimulation increases pest resistance in common bean. *Oecologia* **111**: 84–90.

Donohue K, Pyle EH, Messiqua D, Heschel MS, Schmitt J. 2000. Density dependence and population differentiation of genetic architecture in *Impatiens capensis* in natural environments. *Evolution* **54**: 1969–1981.

Dudley SA, Schmitt J. 1995. Genetic differentiation in morphological responses to simulated foliage shade between populations of *Impatiens capensis* from open and woodland sites. *Functional Ecology* **9**: 655–666.

Dudley SA, Schmitt J. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *American Naturalist* **147**: 445–465.

Fujikura U, Horiguchi G, Ponce MR, Micol JL, Tsukaya H. 2009. Coordination of cell proliferation and cell expansion mediated by ribosome-related processes in the leaves of *Arabidopsis thaliana*. *Plant Journal* **59**: 499–508.

Gere JM, Timoshenko SP. 1999. *Mechanics of materials*. Cheltenham, UK: Stanley Thornes Ltd.

Gianoli E, Palacio-Lopez K. 2009. Phenotypic integration may constrain phenotypic plasticity in plants. *Oikos* **118**: 1924–1928.

Griffith TM, Sultan SE. 2006. Plastic and constant developmental traits contribute to adaptive differences in co-occurring *Polygonum* species. *Oikos* **114**: 5–14.

Henry HAL, Thomas SC. 2002. Interactive effects of lateral shade and wind on stem allometry, biomass allocation, and mechanical stability in *Abutilon theophrasti* (Malvaceae). *American Journal of Botany* **89**: 1609–1615.

Huber H, Chen X, Hendriks M, Keijsers D, Voesenek L, Pierik R, Poorter H, de Kroon H, Visser EJW. 2012. Plasticity as a plastic response: how submergence-induced leaf elongation in *Rumex palustris* depends on light and nutrient availability in its early life stage. *New Phytologist* **194**: 572–582.

Huber H, de Brouwer J, de Caluwe H, Weijschede J, Anten NPR. 2008. Shade induced changes in biomechanical petiole properties in the stoloniferous herb *Trifolium repens*. *Evolutionary Ecology* **22**: 399–417.

Huber H, von Wettberg EJ, Aguilera A, Schmitt J. 2011. Testing mechanisms and context dependence of costs of plastic shade avoidance responses in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany* **98**: 1602–1612.

Johnson K, Lenhard M. 2011. Genetic control of plant organ growth. *New Phytologist* **191**: 319–333.

Jorgensen P, Tyers M. 2004. How cells coordinate growth and division. *Current Biology* **14**: R1014–R1027.

Kawade K, Horiguchi G, Tsukaya H. 2010. Non-cell-autonomously coordinated organ size regulation in leaf development. *Development* **137**: 4221–4227.

Kitajima K, Poorter L. 2010. Tissue-level leaf toughness, but not lamina thickness, predicts sapling leaf lifespan and shade tolerance of tropical tree species. *New Phytologist* **186**: 708–721.

Lechowicz MJ, Bell G. 1991. The ecology and genetics of fitness in forest plants. 2. Microspatial heterogeneity of the edaphic environment. *Journal of Ecology* **79**: 687–696.

Leevers SJ, McNeill H. 2005. Controlling the size of organs and organisms. *Current Opinion in Cell Biology* **17**: 604–609.

Li ZG, Gong M. 2011. Mechanical stimulation-induced cross-adaptation in plants: an overview. *Journal of Plant Biology* **54**: 358–364.

Liu Y, Schieving F, Stuefer JF, Anten NPR. 2007. The effects of mechanical stress and spectral shading on the growth and allocation of ten genotypes of a stoloniferous plant. *Annals of Botany* **99**: 121–130.

Loodts J, Tijskens E, Wei CF, Vanstreels E, Nicolai B, Ramon H. 2006. Micromechanics: simulating the elastic behavior of onion epidermis tissue. *Journal of Texture Studies* **37**: 16–34.

Malinowski R, Kasprzewska A, Fleming AJ. 2011. Targeted manipulation of leaf form via local growth repression. *Plant Journal* **66**: 941–952.

Murren CJ. 2012. The integrated phenotype. *Integrative and Comparative Biology* **52**: 64–76.

Nagashima H, Hikosaka K. 2011. Plants in a crowded stand regulate their height growth so as to maintain similar heights to neighbours even when they have potential advantages in height growth. *Annals of Botany* **108**: 207–214.

Nagashima H, Hikosaka K. 2012. Not only light quality but also mechanical stimuli are involved in height convergence in crowded *Chenopodium album* stands. *New Phytologist* **195**: 803–811.

Niklas KJ. 1992. *Plant biomechanics: an engineering approach to plant form and function*. Chicago, IL, USA: University of Chicago Press.

Niklas KJ, Paolillo DJ Jr. 1997. The role of the epidermis as a stiffening agent in *Tulipa* (Liliaceae) stems. *American Journal of Botany* **84**: 735–744.

Onoda Y, Schieving F, Anten NPR. 2008. Effects of light and nutrient availability on leaf mechanical properties of *Plantago major*: a conceptual approach. *Annals of Botany* **101**: 727–736.

Pigliucci M. 2002. Touchy and bushy: phenotypic plasticity and integration in response to wind stimulation in *Arabidopsis thaliana*. *International Journal of Plant Sciences* **163**: 399–408.

Pigliucci M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecology Letters* **6**: 265–272.

Pigliucci M, Kolodynska A. 2006. Phenotypic integration and response to stress in *Arabidopsis thaliana*: a path analytical approach. *Evolutionary Ecology Research* **8**: 415–433.

Potters G, Pasternak TP, Guisez Y, Jansen MAK. 2009. Different stresses, similar morphogenic responses: integrating a plethora of pathways. *Plant, Cell & Environment* **32**: 158–169.

Potters G, Pasternak TP, Guisez Y, Palme KJ, Jansen MAK. 2007. Stress-induced morphogenic responses: growing out of trouble? *Trends in Plant Science* **12**: 98–105.

Pruyn ML, Ewers BJ, Telewski FW. 2000. Thigmomorphogenesis: changes in the morphology and mechanical properties of two *Populus* hybrids in response to mechanical perturbation. *Tree Physiology* **20**: 535–540.

Puijalon S, Bouma TJ, Douady CJ, van Groenendael J, Anten NPR, Martel E, Bornette G. 2011. Plant resistance to mechanical stress: evidence of an avoidance-tolerance trade-off. *New Phytologist* **191**: 1141–1149.

Puijalon S, Bouma TJ, Van Groenendael J, Bornette G. 2008. Clonal plasticity of aquatic plant species submitted to mechanical stress: escape versus resistance strategy. *Annals of Botany* **102**: 989–996.

Ridge I, Amarasinghe I. 1984. Ethylene and growth-control in the fringed waterlily (*Nympheoides peltata*) - stimulation of cell-division and interaction with buoyant tension in petioles. *Plant Growth Regulation* **2**: 235–249.

Schmitt J, Dudley SA, Pigliucci M. 1999. Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants. *American Naturalist* **154**: S43–S54.

Schmitt J, Ehrhardt D, Swartz D. 1985. Differential dispersal of self-fertilized and outcorssed progeny in jewelweed (*Impatiens capensis*). *American Naturalist* **126**: 570–575.

Schopfer P. 2006. Biomechanics of plant growth. *American Journal of Botany* **93**: 1415–1425.

Simpson RL, Leck MA, Parker VT. 1985. The comparative ecology of *Impatiens capensis* Meerb (Balsaminaceae) in central New-Jersey. *Bulletin of the Torrey Botanical Club* **112**: 295–311.

Smith VC, Ennos AR. 2003. The effects of air flow and stem flexure on the mechanical and hydraulic properties of the stems of sunflowers *Helianthus annuus* L. *Journal of Experimental Botany* **54**: 845–849.

Speck O. 2003. Field measurements of wind speed and reconfiguration in *Arundo donax* (Poaceae) with estimates of drag forces. *American Journal of Botany* **90**: 1253–1256.

Sultan SE. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* **5**: 537–542.

Tabak NM, von Wettberg E. 2008. Native and introduced jewelweeds of the Northeast. *Northeastern Naturalist* **15**: 159–176.

Telewski FW. 1990. Growth, wood density, and ethylen production in response to mechanical perturbation in *Pinus taeda*. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **20**: 1277–1282.

Telewski FW, Pruyn ML. 1998. Thigmomorphogenesis: a dose response to flexing in *Ulmus americana* seedlings. *Tree Physiology* **18**: 65–68.

Tsukaya H. 2005. Leaf shape: genetic controls and environmental factors. *International Journal of Developmental Biology* **49**: 547–555.

Waller DM. 1979. Relative costs of self-fertilized and cross-fertilized seeds in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany* **66**: 313–320.

Weijschede J, Antonise K, de Caluwe H, de Kroon H, Huber H. 2008. Effects of cell number and cell size on petiole length variation in a stoloniferous herb. *American Journal of Botany* **95**: 41–49.

Weijschede J, Martinkova J, de Kroon H, Huber H. 2006. Shade avoidance in *Trifolium repens*: costs and benefits of plasticity in petiole length and leaf size. *New Phytologist* **172**: 655–666.

Weinig C, Delph LF. 2001. Phenotypic plasticity early in life constrains developmental responses later. *Evolution* **55**: 930–936.

von Wettberg EJ, Remington DL, Schmitt J. 2008. Partitioning adaptive differentiation across a patchy landscape: shade avoidance traits in *Impatiens capensis*. *Evolution* **62**: 654–667.

von Wettberg EJ, Schmitt J. 2005. Physiological mechanism of population differentiation in shade-avoidance responses between woodland and clearing genotypes of *Impatiens capensis*. *American Journal of Botany* **92**: 868–874.

von Wettberg EJB, Stinchcombe JR, Schmitt J. 2012. Early developmental responses to seedling environment modulate later plasticity to light spectral quality. *PLoS ONE* **7**: e34121.