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Research

Plant resistance to mechanical stress: evidence of an avoidance–tolerance trade-off

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Summary

- External mechanical forces resulting from the pressure exerted by wind or water movement are a major stress factor for plants and may cause regular disturbances in many ecosystems. A plant's ability to resist these forces relies either on minimizing the forces encountered by the plant (avoidance strategy), or on maximizing its resistance to breakage (tolerance strategy). We investigated plant resistance strategies using aquatic vegetation as a model, and examined whether avoidance and tolerance are negatively correlated.
- We tested the avoidance–tolerance correlation across 28 species using a phylogenetically corrected analysis, after construction of a molecular phylogeny for the species considered.
- Different species demonstrated contrasting avoidance and tolerance and we demonstrated a significant negative relationship between the two strategies, which suggests an avoidance–tolerance trade-off.
- Negative relationships may result from costs that each strategy incurs or from constraints imposed by physical laws on plant tissues. The existence of such a trade-off has important ecological and evolutionary consequences. It would lead to constraints on the evolution and variation of both strategies, possibly limiting their evolution and may constrain many morphological, anatomical and architectural traits that underlie avoidance and tolerance.

Introduction

External mechanical forces resulting from the pressure exerted by wind or water movement are a major stress factor for sessile organisms such as plants and may cause regular disturbances in many ecosystems (Ennos, 1997; Read & Stokes, 2006). Mechanical failure occurs when the forces encountered by plants exceed their capacity to resist breakage, buckling and/or uprooting (Koehl, 1982; Vogel, 2003; Schutten *et al.*, 2005). Different strategies have been defined to describe plant adaptations to adverse environmental conditions (e.g. herbivory, toxicity, drought, flooding; Rosenthal & Kotanen, 1994; Fineblum & Rausher, 1995; Mauricio *et al.*, 1997). The terminologies may vary according to the environmental factor considered and whether plant responses are studied at the individual-, population- or community-level. Our definitions derive from Levitt (1972) and are used for many abiotic stresses (e.g. drought, heat, salt, light stresses; Fitter & Hay, 2002; Schulze *et al.*, 2005; Verslues *et al.*, 2006). Plant resistance, which is the plant's ability to minimize the negative impact of environmental adverse conditions, is based either on avoidance or tolerance. Avoidance entails traits that enable plants to resist adverse conditions by preventing the deleterious effects of these conditions whereas tolerance consists in traits that enable plants to endure adverse conditions (Fitter & Hay, 2002; Schulze *et al.*, 2005). In the case of exposure to mechanical forces, plant resistance relies either on minimizing the forces encountered, which by analogy can be regarded as an avoidance strategy, or on maximizing their resistance to breakage, which can be regarded as a tolerance strategy (Puijalon *et al.*, 2008).

The magnitude of the mechanical forces encountered by plants exposed to moving fluids (air or water) depends on several parameters, some of them being linked to plant morphology, particularly size and shape (Fig. 1). The main morphological traits that enable plants to reduce the mechanical forces that they experience (e.g. given wind speed or water flow distribution) are a small area exposed to the fluid, and a shape that reduces the force encountered for a given area (Fig. 1). A small area can be achieved either through the production of a small growth form or through reconfiguration, at high fluid velocity, of the canopy in a compact form (Speck, 2003; Rudnicki *et al.*, 2004; Puijalon *et al.*, 2005). The main traits that reduce the force encountered for a given area are linked to the shape of the stems and leaves and, more generally, of the canopy (for example, its streamlining; Telewski & Jaffe, 1986b; Sand-Jensen, 2003; Puijalon *et al.*, 2008). Concerning tolerance, the force required to break a plant structure (e.g. a stem or petiole) is the product of its cross-sectional area and its material strength (Denny, 1988; Niklas, 1992; Vogel, 2003; Fig. 1). Consequently, plant traits leading to high resistance to breakage are a large cross-section of plant organs or a high proportion of strengthening tissues (Telewski & Jaffe, 1986a; Ennos, 1997; Read & Stokes, 2006).

When a plant is exposed to external mechanical forces, both avoidance and tolerance may bring benefit to the plant, through a reduced risk of mechanical failure and hence an increased probability of survival. Investigations of plant strategies involved in defence against herbivores and in response to herbicides, have demonstrated that strategies that are partly redundant can be negatively correlated (Fineblum & Rausher, 1995; Fornoni *et al.*, 2003; Baucom & Mauricio, 2008), while other studies failed to detect such negative correlations (Mauricio *et al.*, 1997; Weinig *et al.*, 2003). Despite their ubiquitous nature and ecological relevance, the strategies of resistance to mechanical stress have been little investigated to date. The few studies that did consider both avoidance through low forces encountered and at the same time tolerance of these forces did not test the relationships between them (Telewski & Jaffe, 1986b; Schutten *et al.*, 2005). Consequently, it is unclear to what extent avoidance and tolerance in relation to resistance to mechanical forces are negatively correlated or, conversely, vary independently. The question of the existence of a trade-off is of primary ecological interest, as it may reveal constraints that limit the evolution of the traits involved in that trade-off. As nearly all plants are exposed to external mechanical forces, an avoidance/tolerance trade-off could thus indicate constraints on the evolution and variation in a broad range of architectural traits of canopies, leaves and stems, all involved in these strategies.

Submerged aquatic vegetation is an excellent model to investigate strategies of resistance to mechanical constraints. Indeed, as aquatic plants experience reduced gravitational force as a result of the buoyant nature of water, their morphologies and mechanical architecture are not constrained by fundamental mechanical adaptations required for self-supporting growth forms (Niklas, 1992; Rowe *et al.*, 2004) and are thus characterized by a great variety of morphologies and growth forms (Cook, 1990). Moreover, aquatic plants occur in all places of Angiosperm phylogeny, including both deeply branching groups such as Nymphaeaceae, and shallow branching groups such as Apiaceae (APGIII, 2009). All these properties minimize the possible bias resulting from the study of a particular morphology, growth form or phylogenetic group. Finally, many aquatic plants may have been exposed to higher selection for their resistance to external mechanical forces (e.g. induced by floods, waves) as the hydrodynamic forces encountered by aquatic plants greatly exceed forces imposed on terrestrial species because of the high density of water.

The aim of our study was to investigate the resistance strategies of plants to mechanical forces and, more particularly, to test whether avoidance and tolerance are negatively corre-

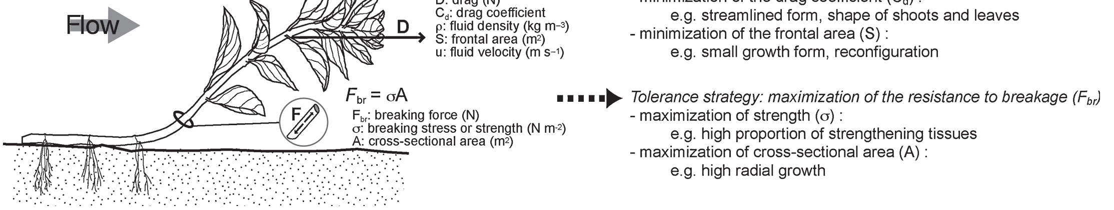

Fig. 1 Resistance strategies (avoidance and tolerance) in the case of exposure to a unidirectional moving fluid. (a) Key mechanical parameters of a plant exposed to a unidirectional moving fluid and the main forces involved in the risk of breakage (drag, D, i.e. hydrodynamic force in flow direction and breaking force F_{br}). (b) The strategies resulting in minimization of the risk of breakage, that is, minimization of the mechanical force encountered or maximization of the resistance to breakage.

lated across species. In order to test this hypothesis, we studied the ability of submerged aquatic vegetation to resist mechanical forces induced by unidirectional steady flow. Avoidance and tolerance were evaluated by measuring two integrative traits (Fig. 1). In order to have a positive relationship between avoidance ability and the integrative trait that describes it, avoidance was evaluated by drag^{-1}, drag being the main force encountered by plants in unidirectional steady flow (Vogel, 2003). Tolerance was described by the breaking force of plant organs exposed to flow (stems or petioles): breaking force and tolerance are consequently positively related. We used a phylogenetically corrected analysis (Felsenstein, 1985) to test the relationship between the two traits, after construction of a molecular phylogeny for the species considered.

Materials and Methods

Plant sampling

Twenty-eight species of aquatic plants characterized by contrasting morphologies and phylogenetic groups were selected (see the Supporting Information Table S1). The species sampled represented 14 families and 22 genera. Four growth forms were identified (Fig. 2): unbranched caulescent (leaves inserted on a developed, unbranched or poorly branched stem), branched caulescent (leaves inserted on a developed, branched stem) and linear and nonlinear leaved rosette (leaves inserted on a reduced, partly buried stem). For two heterophyllous species, whose submerged and floating leaves differed morphologically (*Nuphar lutea*, *Potamogeton nodosus*), individuals only composed of submerged leaves as well as individuals only composed of floating leaves were sampled and treated separately. For each species, fully developed individuals were collected under stress-free conditions (standing, totally submerged or floating conditions). One individual consisted of a ramet (rosette species and unbranched caulescent species) or a fully developed branch (branched caulescent species, Fig. 2).

All species were collected in their natural habitats (wetlands of the Rhône and Ain floodplains, France, Table S1), except individuals of *Vallisneria spiralis*, which were obtained from glasshouse cultivation of plants from the same origin. For each species, individuals were sampled in widely spaced patches to minimize the risk of sampling the same clone. Plants were held in cold (16°C), aerated freshwater and exposed to artificial light after collection and before measurement. All measurements were carried out in July–August.

Integrative traits describing plant strategies

As aquatic plants are flexible, they bend in the flow direction and they are thus primarily exposed to tensile forces (Schutten *et al.*, 2005). Consequently, we used tensile breaking forces to evaluate tolerance. The way a given organ encounters mechanical forces depends on its growth form, so the drag and breaking forces used to evaluate avoidance and tolerance, respectively, were defined for organs playing the same functional role, without having necessarily the same developmental origin. In a caulescent species, the highest stress levels induced by drag forces occur at the basal part of its stem, which supports the whole plant. Hence, the drag of the whole plant and the tensile breaking force of the basal part of the stem were used to indicate avoidance and tolerance, respectively (Fig. 2). For rosette species, the basal part of the stem is buried, and therefore not vulnerable to rupture under drag forces. Consequently, the average drag of each leaf (total drag divided by leaf number) and the

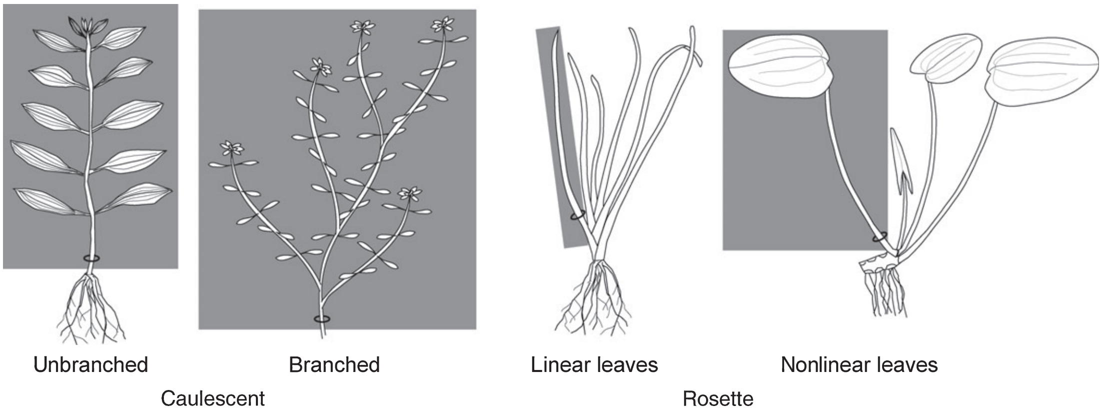

Fig. 2 Drag and breaking force used to describe avoidance and tolerance strategies for different growth forms. The plant represented for each growth form corresponds to the definition of an individual used in the present study. The drag used to describe avoidance is represented by a grey square: whole-plant drag for caulescent species and average drag of leaf for rosette species. The breaking force used to describe tolerance is represented by a black circle and was measured on the basal part of stem for caulescent species and on the basal part of leaves or petioles for rosette species.

breaking force of the basal part of the petioles was used, or the base of the leaves in the case of monocots (Fig. 2). The traits describing plants strategies were not corrected for size in order to take into account the two components of each strategy (Fig. 1): a size-dependent component (frontal area and cross-sectional area) and a size-independent one (drag coefficient and strength).

Drag measurements

Hydrodynamic traits were measured in a unidirectional flow flume (full description in Bouma *et al.*, 2005). The width of the straight working section of the flume was 30 cm and the height of the water column was 25 cm. Drag was measured using a force transducer (WL|Delft Hydraulics, Delft, the Netherlands, described in Bouma *et al.*, 2005). A hole fitting the size of the transducer was made in the bottom of the flume so that the transducer was placed underneath with its top part perfectly level with the bottom of the flume. Plants were attached to the transducer by their stem, after elimination of below-ground parts and positioned in a natural growth position. This device enabled both positive and negative horizontal forces exerted on plants to be measured. Drag was recorded over 20 s at a frequency of 20 Hz and these values were used to calculate the average force. Eight individuals were measured for each species. As measurements made without plants attached to the force transducer revealed that the force induced by the transducer itself was negligible, no correction for transducer drag was made.

Drag measurements were performed at the maximum velocity attainable in the flume. The three-dimensional (3D) velocity was measured with an acoustic Doppler velocimeter (ADV; Nortek AS, Oslo, Norway). Vertical velocity profiles consisting of 10 points (at 1, 2, 3, 4, 5, 7, 9, 11, 15 and 19 cm above the bottom of the flume) were measured using a grid. The grid consisted of three downstream (i.e. 0, 5 and 10 cm) × three cross-stream (i.e. −5, 0 and 5 cm) locations, together with location 0, with 0 being the position of the force transducer. For each point, the velocity was measured at 25 Hz over 15 s. Free stream velocity was calculated by averaging the velocity out of the boundary layer. The average free stream velocity was 82.3 ± 2.29 cm s^{-1}.

Measurements of biomechanical traits

We measured tensile breaking force on 19–25 individual samples per species. An individual sample consisted of a basal fragment of stem for caulescent species and a basal fragment of petiole rosette species. All fragments were collected on different plants to avoid pseudo-replication. The tensile tests were performed on a universal testing machine (Instron 5542, Canton, MA, USA). Each sample was attached at both ends to pneumatic clamps. A constant extension (5 mm min^{-1}) was applied to the upper clamp until the sample broke.

Genetic data acquisition and phylogenetic reconstruction

A molecular phylogeny was constructed for the species sampled based on the rbcL sequence data. RbcL sequences were fetched for 18 species from NCBI database using a BLAST search and *Berula erecta* (AM234813) as seed. When multiple hits were found the longest and least degenerated sequence was chosen (see Table S1). *Gnetum parvifolium* (NC011942) and *Cycas taitungensis* (NC009618) were used to root the tree. To complete this data set, rbcL sequences were determined for 10 species (see Table S1). Total plant genomic DNA was extracted following a sorbitol DNA extraction method (Storchova *et al.*, 2000) for *Myosotis scorpioïdes* species and following the CTAB (cetyltrimethyl ammonium bromide) method (Doyle & Doyle, 1990) for the other species. The rbcL gene was amplified using a PCR with the primers rbcL1F (5′-ATGTCACCACAAACAGAGACT-3′) and rbcL1369R (5′-TTCCATACTTCACAAGCAGC-3′). Amplifications were performed in 25-μl reactions containing: 30 ng DNA solution, 1× PCR buffer (New England BioLabs, Ozyme, Saint-Quentin-en-Yvelines, France), 0.8 mM dNTPs, 0.5 μM of each primer, 2 U of *Taq* polymerase (New England BioLabs). The PCR was carried out in a PTC-200 thermocycler (MJ Research Inc., Watertown, MA, USA) with the following settings: an initial denaturation at 94°C for 2 min, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 90 s at 72°C, followed by one step of 10 min at 72°C. The PCR products were purified and bidirectionally sequenced using the rbcL1F and rbcL1369R primers by MWG Biotech (Ebersberg, Germany). Sequences were edited and assembled using SEQUENCHER (Gene Codes Corp., Ann Arbor, MI, USA). All sequences have been deposited in GenBank (for accession numbers see Table S1).

Alignment was unambiguous and thus performed by eye using SEAVIEW (Galtier *et al.*, 1996). The most probable topology was inferred using PHYLOBAYES 3.2e (Lartillot & Philippe, 2004) under a CAT-GTR model. Two independent runs were performed in parallel and were stopped when the largest discrepancy observed across all bipartitions between these two independent runs reached 0.05 (considering a burn-in of 100 trees and sampling every 2 topologies; i.e. maxdiff < 0.05).

Phylogenetically independent contrast

To be statistically valid, conventional analyses assume that traits values are independent from the phylogeny of the taxa that harbour them or that species are phylogenetically equidistant (e.g. no taxa subsets share an exclusive common ancestor). To test whether our traits contained phylogenetic signal we calculated Blomberg's *K* indices (Blomberg *et al.*, 2003). In a second step, we implemented Felsenstein's phylogenetically independent contrast (Felsenstein, 1985) to evaluate the relationship between drag^{-1} and breaking force. Ultrametrization of the trees used for contrasts was done by using a semi-parametric method based on penalized likelihood (Sanderson, 2002) assuming two levels of smoothing determined by parameter λ: either $\lambda = 0$ (very low autocorrelation, i.e. different rates for branches) or $\lambda = 100$ (i.e. nearly the same rate for all branches). Blomberg's *K*, tree ultrametrization and contrasts were computed using PICANTE-1.2-0, APE-2.6-3 and PHYLOGR 1.0.7 packages in R-2.9.0. Following Garland *et al.* (1992), the absence of relationships between the absolute value of standardized contrasts and their standard deviation were

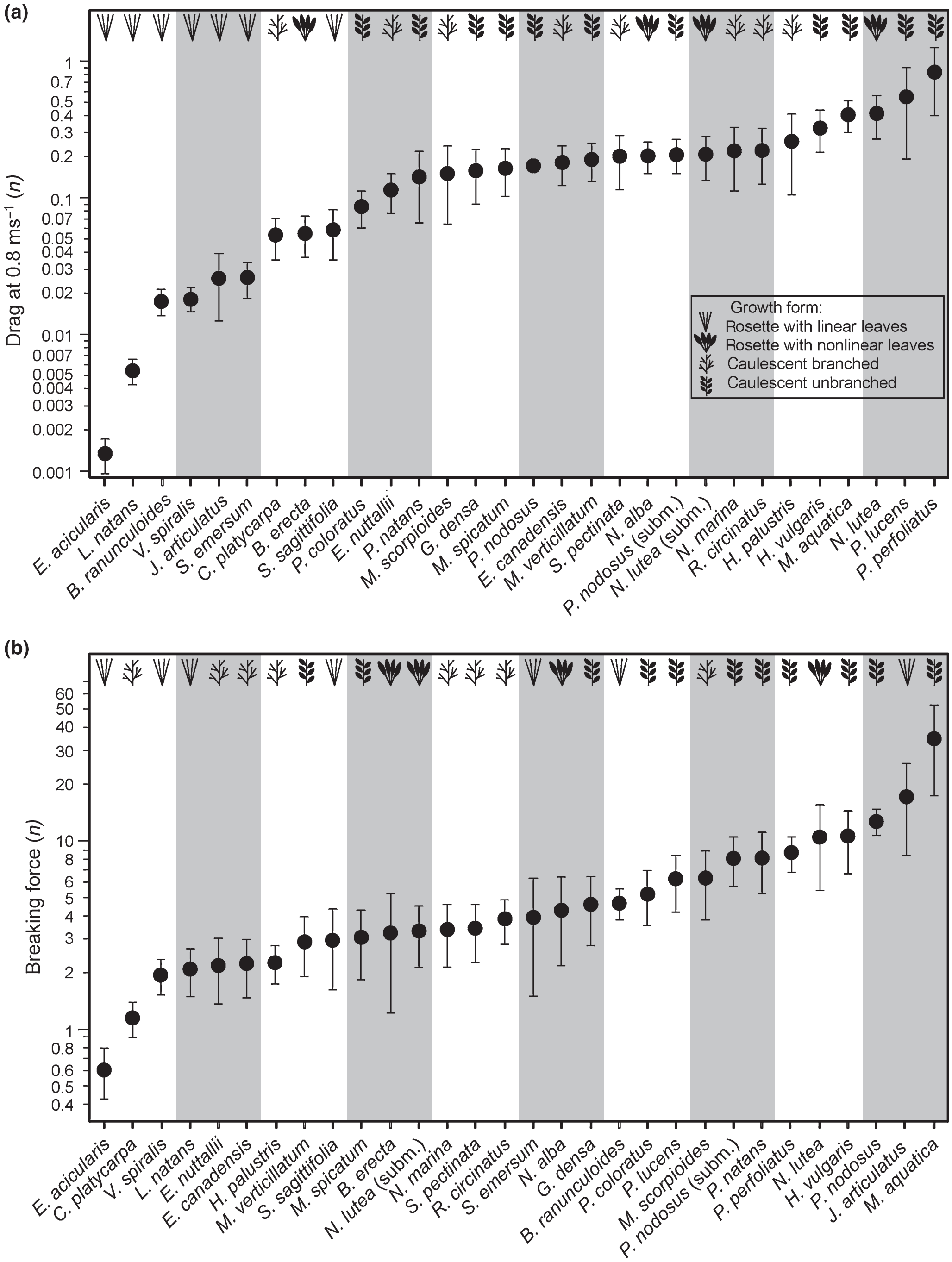

Fig. 3 (a) Drag (n = 8 per species) and (b) breaking force (n = 19–25 per species) (mean ± SD) represented on a log-scale. Growth form is represented for each species on the top part of each panel of the plot.

verified (none of the four relationships were significant, $P \geq 0.36$). The relationship between standardized contrast of drag^{-1} and breakage was tested using a regression through the origin (the intercept is set to zero).

R-2.9.0 (R Development Core Team, 2009) software was used for all statistical analyses.

Results

Drag differed significantly between species ($F_{29,214} = 104.4$, $P < 10^{-4}$; linear model using log-transformed drag as dependent variable and species as main effect) and varied by a factor of > 600 (Fig. 3a). The breaking force also differed significantly between species and varied by a factor of 56 ($F_{29,620} = 147.8$, $P < 10^{-4}$; generalized least-squares model with unequal variance, using log-transformed breaking force as the dependent variable and species as the main effect, Fig. 3b).

Phylogenetic space explorations were stopped after visiting 8004 (run 1) and 7905 (run 2) topologies (maxdiff < 0.04). The most probable tree inferred using PHYLOBAYES (Fig. 4) was in overall agreement with current knowledge about angiosperm phylogenic relationships (APGIII, 2009). Blomberg's K indicates that drag^{-1} and breaking force contain significant phylogenetic signals except breaking force for $\lambda = 0$ (Table 1). Without correction for phylogenetic distance, avoidance (drag^{-1}) and tolerance (breaking force) were significantly negatively correlated. This relationship was tested including either the floating ($R^2 = 0.30$, $P = 0.0028$), or the submerged ($R^2 = 0.27$, $P = 0.0044$) growth forms of the two species, *P. nodosus* and *N. lutea*, occurring either with submerged or floating growth forms (Fig. 5a). After correction for the phylogenetic distance between species, avoidance and tolerance remained significantly negatively correlated whatever the growth forms and the values of λ considered (Table 1, Fig. 5b,c).

Discussion

Evidence of an avoidance–tolerance trade-off

The present study demonstrates the contrasting avoidance and tolerance abilities of different species to mechanical force, with differences of more than 1 and 2 orders of magnitude for both strategies, respectively. Our results also demonstrate a negative correlation between avoidance and tolerance, which strongly suggests a trade-off between these two strategies. The correction performed for phylogeny shows that this correlation was not an artefact caused by assumption violations (i.e. conventional correlation assumes that species are phylogenetically equidistant or that traits evolve independently from the phylogeny; Felsenstein, 1985; Blomberg *et al.*, 2003; Garland *et al.*, 2005). Many trade-offs between plant strategies, life-history or morphological

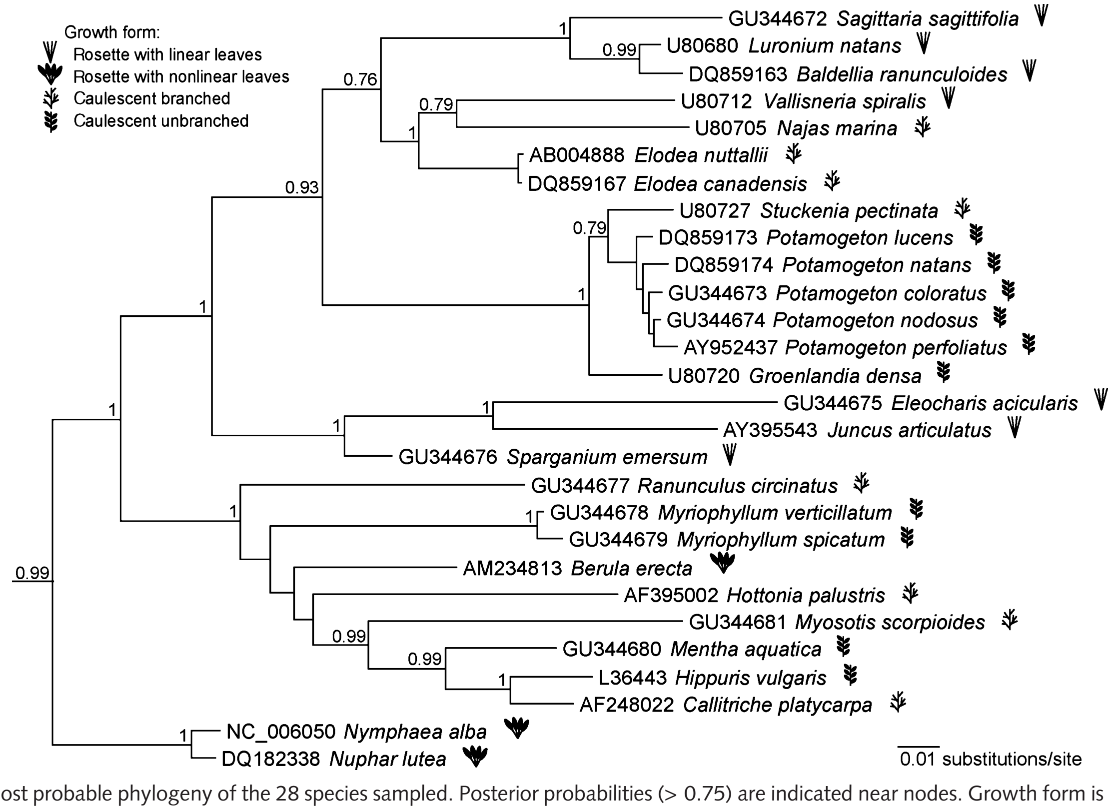

Fig. 4 Most probable phylogeny of the 28 species sampled. Posterior probabilities (> 0.75) are indicated near nodes. Growth form is represented for each species. Ultrametric trees (not shown) for contrast calculations were obtained assuming either λ = 0 or λ = 100.

Table 1 Blomberg's K for breaking force and drag^{-1}, adjusted R^2 of the relationship between drag^{-1} and breaking forces and associated P-values

		Floating growth forms	Submerged growth forms
$\lambda = 0$	Blomberg's K (breaking force)	$K = 0.27; P = 0.17$	$K = 0.28; P = 0.18$
	Blomberg's K (drag^{-1})	$K = 0.47; P = 0.005$	$K = 0.48; P = 0.003$
	Breaking force–drag^{-1} relationship	$R^2 = 0.31; P = 0.001$	$R^2 = 0.36; P = 0.0004$
$\lambda = 100$	Blomberg's K (breaking force)	$K = 0.32; P = 0.013$	$K = 0.36; P = 0.006$
	Blomberg's K (drag^{-1})	$K = 0.36; P = 0.015$	$K = 0.37; P = 0.015$
	Breaking force–drag^{-1} relationship	$R^2 = 0.16; P = 0.02$	$R^2 = 0.26; P = 0.003$

The values are calculated in relation to two growth forms of heterophyllous species (floating vs submerged) and for two values of the parameter λ (0 and 100).

traits have been investigated, but these studies mostly focused on traits related to reproduction, leaf functions and defence against herbivory (Agrawal *et al.*, 2002; Agrawal & Fishbein, 2008; He *et al.*, 2009; Ballhorn *et al.*, 2010) and rarely on plant mechanical functions. To our knowledge, this study provides the first evidence of a trade-off between avoidance and tolerance of mechanical forces.

Origin of the trade-off

Avoidance and tolerance are partly redundant as they both lead to reduced risk of breakage and hence enhanced plant survival. Our results are consistent with studies carried out on resistance to herbicide and herbivory, which demonstrated that traits that serve the same function are negatively correlated (Fineblum & Rausher, 1995; Fornoni *et al.*, 2003; Baucom & Mauricio, 2008). Such negative relationships may result from costs that each strategy incurs, which would favour allocation to one of the strategies rather than to both simultaneously (Fineblum & Rausher, 1995; Mauricio *et al.*, 1997; Pilson, 2000; Fornoni *et al.*, 2003; Baucom & Mauricio, 2008). In the present study, both avoidance and tolerance may incur significant costs. The principal traits underlying avoidance are a reduced area exposed to flow, a streamlined canopy and a high capacity for reconfiguration (Sand-Jensen, 2003; Puijalon *et al.*, 2005, 2008). Small-sized or stunted morphologies leading to reduced area may incur direct costs because various components of plant fitness are positively related to plant size (Shipley & Dion, 1992). The costs associated with a streamlined shape could result from higher self-shading, owing to stacking of leaves on top of each other, which reduces light interception and thus photosynthesis (Koehl & Alberte, 1988; Vogel, 2003). Finally, a high reconfiguration capacity could create a less stable leaf form and hence a less efficient positioning of the photosynthetic area in the absence of strong forces applied to the leaves (Vogel, 1989). Traits underlying tolerance are mainly a high cross-sectional area, which requires an increased allocation to radial growth and a high proportion of compounds with structural functions, which are costly to produce (Penning de Vries *et al.*, 1974; Cipollini, 1999). Resources thus expended cannot simultaneously be allocated to fitness-enhancing functions such as flowering and seed production.

The negative relationship between tolerance and avoidance could also be partly caused by constraints imposed by physical laws on plant tissues. Lignin is a major constituent of cell walls and provides mechanical support and increases the tensile breaking force of plant tissue, but also reduces tissue flexibility and thus the capacity for reconfiguration (Niklas, 1992, 1996). Physical constraints because of size could also be a trait partly underlying the negative relationship between avoidance and tolerance because overall size tends to be negatively correlated with avoidance (plant drag is proportional to frontal area) and positively to tolerance (breaking force is proportional to cross-sectional area).

Conclusion

The results presented demonstrate that strategies to resist mechanical forces are negatively correlated. This correlation could lead to constraints on the evolution and variation of both strategies; selection for enhanced avoidance leading to a reduced tolerance, and vice versa, possibly limiting their evolution (Stearns & Hoekstra, 2005; Baucom & Mauricio, 2008). Moreover, both strategies themselves depend on several morphological, anatomical and architectural traits (e.g. size and shape of the canopy or stem anatomical traits), the evolution and expression of which could also be constrained by the trade-off.

Our results also emphasize the possible conflicts between traits involved in mechanical and other plant functions such as photosynthesis or water conduction (Vogel, 1989, 2003; Niklas, 1992), which may lead to other trade-offs. Whether this correlation is observed across species of other plants, and particularly terrestrial ones, remains to be seen. On the individual scale, plastic responses to mechanical stress (thigmomorphogenesis) can result in improved avoidance or tolerance (Puijalon *et al.*, 2008) and it is possible that these responses are constrained by the same negative correlation identified in the present study for aquatic plants (i.e. enhanced avoidance ability is balanced by reduced tolerance and vice versa).

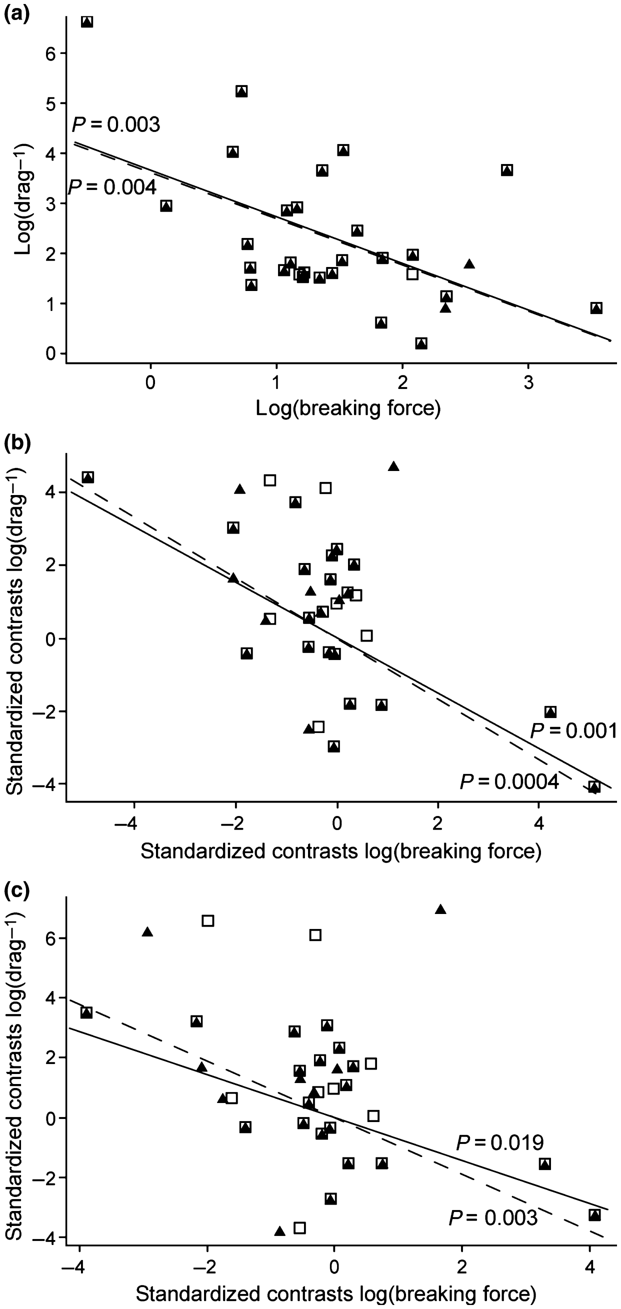

Fig. 5 Correlation between drag^{-1} and breaking force. (a) Correlation between raw data and phylogenetically independent correlation between standardized contrasts calculated for two values of the parameter λ (b) λ = 0 and (c) λ = 100. Points and regression lines are represented by: triangles and full line, for the data set including floating growth forms of *Potamogeton nodosus* and *Nuphar lutea*; and squares and dotted line, for the data set including their submerged growth forms.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Species sampled (phylogeny, growth forms and GenBank accession numbers) and sampling sites