

Cone size is related to branching architecture in conifers

Andrew B. Leslie^{1,4}, Jeremy M. Beaulieu², Peter R. Crane¹ and Michael J. Donoghue³

¹School of Forestry and Environmental Studies, Yale University, 195 Prospect Street, New Haven, CT 06511, USA; ²National Institute for Mathematical and Biological Synthesis, University of Tennessee, 1122 Volunteer Blvd, Ste. 106, Knoxville, TN 37996, USA; ³Department of Ecology and Evolutionary Biology, Yale University, PO Box 208106, New Haven, CT 06520, USA;

⁴Present address: Department of Ecology and Evolutionary Biology, Brown University, Box G-W, 80 Waterman Street, Providence, RI 02912, USA

Summary

Author for correspondence:
Andrew B. Leslie
Tel: +1 2034327168
Email: andrew.leslie@yale.edu

Received: 27 January 2014
Accepted: 24 April 2014

New Phytologist (2014) **203**: 1119–1127
doi: 10.1111/nph.12864

Key words: allometry, conifer, Corner's rules, pollen cone, seed cone.

- The relationship between branch diameter and leaf size has been widely used to understand how vegetative resources are allocated in plants. Branching architecture influences reproductive allocation as well, but fewer studies have explored this relationship at broad phylogenetic or ecological scales. In this study, we tested whether pollen-producing and seed-producing cone size scales with branch diameter in conifers, a diverse and globally distributed lineage of nonflowering seed plants.
- Branch diameter and cone size were analyzed using multiple regression models and evolutionary models of trait evolution for a data set of 293 extant conifer species within an explicit phylogenetic framework.
- Branch diameter is a strong predictor of cone size across conifer species, particularly for pollen cones and dry seed cones. However, these relationships are complex in detail because leaf morphology and seed dispersal biology influence the specific ways in which they are expressed.
- The ubiquity and strength of these scaling relationships across conifers suggest that reproductive and vegetative morphologies are coupled in the group, and it is therefore difficult to disentangle the evolution of cone size from the evolution of branching architecture.

Introduction

Biologists have long sought general rules that scale across levels of organization, such as organs, organisms, and ecosystems, and that will have predictive value in anticipating what will happen as such systems undergo change. One such generalization, known as 'Corner's rules' or the 'leaf size–twig size spectrum', describes a set of scaling relationships between plant stems and the structures that they bear (Niklas, 1994; Ackerly & Donoghue, 1998; Westoby & Wright, 2003; Sun *et al.*, 2006). Corner's rules broadly state that plants with large-diameter branches produce large appendages, but have more widely spaced branches. By contrast, plants with small-diameter twigs branch more densely and bear smaller appendages (Corner, 1949; White, 1983; Brouat *et al.*, 1998; Olson *et al.*, 2009).

These relationships have been widely used to describe how resources are allocated within and among plant species (Price *et al.*, 2007; Olson *et al.*, 2009; Bentley *et al.*, 2013), although the exact mechanisms underlying them have been debated. They are often regarded as resulting from a combination of biophysical constraints and selection against self-shading (White, 1983; Ackerly & Donoghue, 1998; Westoby *et al.*, 2002). For example, small-diameter branches cannot support large leaves mechanically or hydraulically, whereas larger branches can. Closely spaced large leaves would self-shade, however, and selection therefore favors architectures with widely separated branches. Other work has suggested that allometric scaling

relationships such as Corner's rules may be a general result of optimizing energy flow and space filling in branched networks (West *et al.*, 1997, 1999; Price *et al.*, 2007; Savage *et al.*, 2010). Building on these studies, Olson *et al.* (2009) suggested that carbon fixation rates per unit volume of canopy were similar among species, but trade-offs in how this carbon is allocated result in the relationships between branching density, tissue density, and appendage size. Regardless of their underlying cause, these scaling relationships have been noted in reproductive appendages as well (Primack, 1987; Niklas, 1993; Ackerly & Donoghue, 1998). For example, individual flower size has been shown to scale with the size of the inflorescence axis and inflorescence size with branch and leaf size (see Bond & Midgley, 1988; Midgley & Bond, 1989; Le Maitre & Midgley, 1991; Diggle, 1995). If these relationships are widespread, total allocation to reproductive structures may scale across plants (see Niklas & Enquist, 2003) and generally follow the same kinds of trade-offs as noted in vegetative architecture; that is, larger branches bear larger flowers, inflorescences, or fruits, but there are fewer of these structures in the plant canopy. However, reproductive structures respond to a wide variety of additional selective pressures that could affect both total reproductive allocation and how reproductive tissues are deployed in the canopy. Seed size, for example, responds to a wide variety of ecological and life history factors (see Westoby *et al.*, 1992) and does not show a clear scaling relationship with leaf size (Cornelissen, 1999; Wright *et al.*, 2007).

In this study, we used conifers to test the strength of Corner's rules relationships in reproductive structures, specifically asking whether large-diameter branches bear large cones. Conifers are a diverse (*c.* 630 living species) nonflowering seed plant group with a well-resolved phylogeny (Rai *et al.*, 2008; Leslie *et al.*, 2012) and a global distribution (Enright & Hill, 1995; Woodward *et al.*, 2004; Farjon & Filer, 2013), and they also have the advantage of a relatively simple reproductive biology that is comparable across all species. Conifers are all wind-pollinated (Owens *et al.*, 1998) and bear separate pollen-producing and seed-producing cones. Previous studies have noted that large seed cones are associated with long needles in pines (McCune, 1988) and suggested that conifers devote a similar amount of tissue to pollen production within a given volume of fertile canopy foliage, but that branch diameter influences the size of individual pollen cones and how they are deployed in the plant canopy (Leslie, 2012). Here, we greatly expanded the taxonomic sampling of these studies to test whether conifer species with large-diameter branches generally bear both large pollen-producing and seed-producing cones. Within this context, we also tested whether two additional features, imbricate scale leaves and seed cones with fleshy tissues, influence cone size scaling relationships. These features are potentially important because scale leaves are associated with a specific branching habit that probably results in smaller cone size (Leslie, 2012), and cones with fleshy tissues for animal-mediated seed dispersal are typically smaller than woody conifer cones (Leslie, 2011).

Materials and Methods

Branch diameter, pollen cone volume, and seed cone volume were measured for 293 extant conifer species, as were seed volume and cone dry mass for subsets of these species (204 species for seed volume; pollen cones from 55 species and seed cones from 87 species for mass). For branch diameter, distal vegetative twigs were measured close to the growing branch tip. For estimating volume, cones and seeds were treated as ellipsoids where their length and maximum width were used as the major and minor axes, respectively. In taxa with irregularly shaped seed cones (e.g. some genera within Podocarpaceae), the volume of each constituent cone structure was calculated as an ellipsoid and then summed. Measurements were primarily based on herbarium specimens from the Harvard University Herbaria, supplemented with data from the Kew herbarium and the herbarium of the New York Botanical Garden. Because the number of cones per specimen was limited (often to a single cone), most data used in this study were measurements from one branch, one pollen cone, and one seed cone per species. This does not account for interspecific variability, but such variation is minor compared with the total size range across all conifer species. For subsequent analyses, cone and branch size data were log-transformed.

All taxa were scored for two discrete characters that may influence cone size–branch diameter scaling relationships: leaf type and seed cone type. For leaf type, we distinguish between species with scale leaves and those that produce other types of foliage. Here, 'scale leaves' refers to scale-shaped or awl-shaped leaves that

lack a well-defined petiole and lamina, and that are usually imbricated. Taxa with scale leaves often have a characteristic branching habit consisting of very small, highly ramified distal branches completely covered by small leaves. Taxa with other types of leaves (e.g. *Pinus* needles or the broad multivein leaves of *Agathis*) were grouped together as 'needle/blade leaves' because these species all exhibit a more typical distinction between leaves and visible branches. For seed cone type, species with 'fleshy cones' produce succulent or pulpy tissues at maturity, while species with 'dry cones' produce woody or sclerified tissues. Additionally, each species was scored for the position in which cones are borne on branches, distinguishing between cones that are borne singly at the distal tip of vegetative branches (here referred to as 'terminal') and those with other arrangements (e.g. terminal clusters of cones or lateral cones from axillary buds; see Supporting Information Notes S1).

We tested the association between branch diameter and cone size for pollen cones and seed cones using both simple and multiple regression models. For multiple regression models, leaf type and seed cone type were included as predictor variables in addition to branch diameter. We treated leaf type and seed cone type as dummy variables (leaf type: 0, needle/broad; 1, scale leaves; cone type: 0, dry; 1, fleshy) that indicate the presence or absence of a categorical effect that may shift the slope and intercept of the relationship between cone volume and branch diameter (see Notes S2 for more details). We evaluated each model using ordinary least squares (OLS) as well as phylogenetic least squares (PGLS), which estimates model parameters while incorporating the statistical effect of phylogenetic relationships among the taxa (Martins & Hansen, 1997; Garland & Ives, 2000). This approach relied upon a previously published time-calibrated molecular phylogeny for extant conifers (Leslie *et al.*, 2012), and assumes that unexplained residual variation is correlated among related species with the correlation following either a Brownian motion (BM) process or an Ornstein–Uhlenbeck (OU) process (see the following paragraph). Evaluation of model fit and estimates of the model parameters were carried out using custom scripts written in R by J. M. Beaulieu. For the specific form of the models tested here, see Supporting Information Notes S2.

We next fit our data to several different models of trait evolution in order to test whether cone size and branch diameter evolve differently in clades with scale leaves and fleshy seed cones. In order to implement these models, the most likely ancestral states for leaf type and seed cone type were reconstructed using the R package *corHMM* (Beaulieu *et al.*, 2013) and then used to delimit character state-based 'regimes' (e.g. whether clades have fleshy versus dry seed cones) for all branches in the phylogeny. Using this framework, we evaluated a set of models that were fit separately to the pollen cone size, seed cone size, and branch diameter data sets (see Beaulieu *et al.*, 2012). The simplest models assumed a BM process, where the values of a given trait (e.g. pollen cone volume) evolve away from an ancestral value at some rate of stochastic evolution (σ^2). This rate can be constant for all taxa ('BM₁') or can vary among regimes ('BM₂'). We also fit more complex models that assume an OU process, where trait values evolve toward an optimum (θ). This can be a single value for all

taxa ('OU₁' model) or can vary among regimes. Multiple optima models differed in the rate of stochastic evolution away from the optima (σ^2) and the strength of directed evolution toward the optima (α); we fit models in which different phenotypic optima (θ) had one global α and one global σ^2 ('OU_M') or where α or σ^2 varied among regimes ('OU_{MA}' and 'OU_{MV}', respectively; see also Notes S3).

Because the three continuous traits (pollen cone size, seed cone size, and branch diameter) are likely to covary, we also implemented a joint optimization procedure to estimate parameter values for the models already described. This maximizes a single joint likelihood function while exploring parameter space for all three traits simultaneously. By linking models across traits, we exploit a natural property of the optimization procedure, which assumes that all parameters are linked through a variance–covariance matrix, derived from inverting the Hessian, or the matrix of second-order derivatives (see Beaulieu *et al.*, 2012). This variance–covariance matrix describes not only the influence each parameter has on the overall likelihood, but also how it covaries with other parameters in the model. These analyses were carried out using a new function implemented in the R package *OUwie* (Beaulieu *et al.*, 2012).

Results

Pollen cone dry mass was strongly associated with pollen cone volume (Fig. 1) in both OLS ($R^2 = 0.94$; $P \ll 0.01$) and PGLS ($R^2 = 0.91$; $P \ll 0.01$) regression models. Pollen cone volume was also positively associated with branch diameter (Fig. 2b), regardless of whether OLS or PGLS approaches were used to evaluate the regression models (Table 1). The best-supported model was a multiple regression OU model that incorporated the effect of both leaf and seed cone type (Table 1), although an OU model incorporating only leaf type received nearly equal support (Table S1). In all these models, the relationship between pollen cone volume and branch diameter had a higher intercept in scale-leaf taxa than in needle/blade-leaf taxa, although their slopes were similar (Table S1). These results were consistent with bivariate plots, which indicated that the pollen cones of scale-leaf taxa were generally larger for a given branch diameter but showed no obvious difference in the slope of the scaling relationship (Fig. 2b).

The pollen cones of scale-leaf taxa also differed in their position, which was nearly always solitary and terminal on branches (97% of species) in contrast to the lateral cones found in most species (88%) with other types of leaves (Fig. 2a; Table S2). In taxa with lateral pollen cones, the volume of all the individual cones produced per branch can be summed to generate a total pollen cone volume, which can then be directly compared with the volume of solitary terminal pollen cones. This calculation is relatively straightforward for members of the Pinaceae because they often exhibit a clustering of cones near branch tips. After this transformation, the relationship between total pollen cone volume and branch diameter more closely resembled that of taxa with scale leaves (Fig. 2c). Total pollen cone volume per branch in Pinaceae species was also more strongly associated with branch diameter than when cones were considered individually

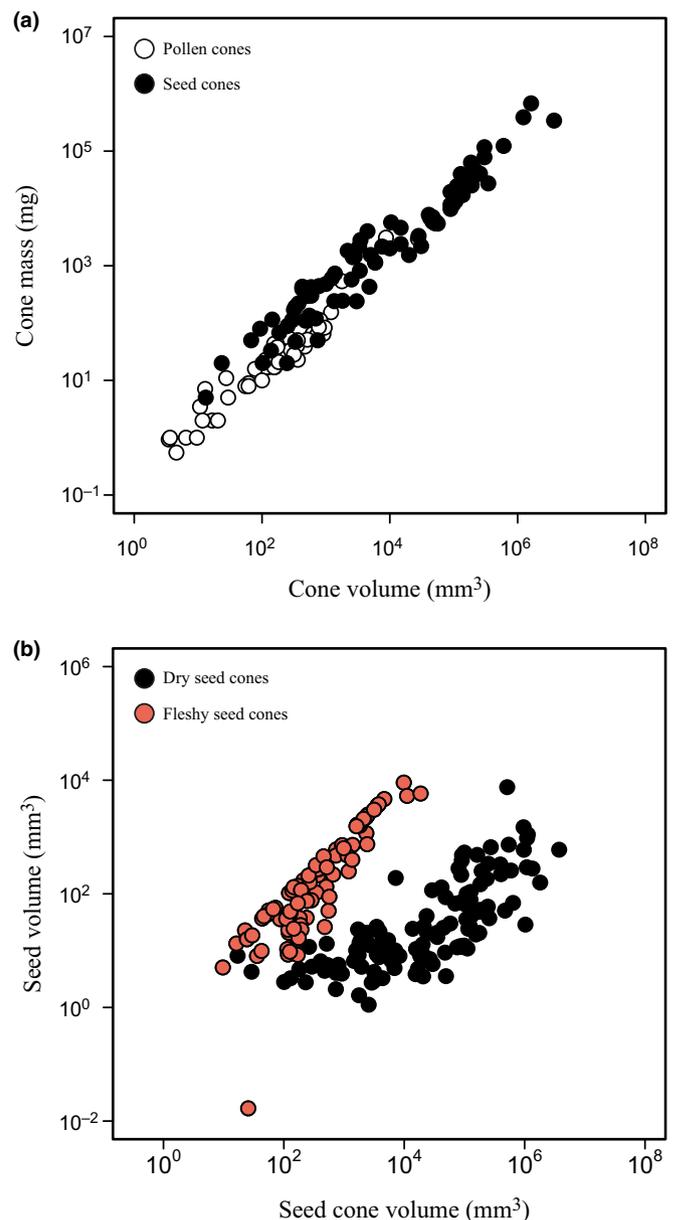


Fig. 1 Relationships between selected cone traits and cone volume in conifer species. (a) Dry mass of pollen and seed cones plotted against their estimated volume; $R^2 = 0.91$ and 0.94 , respectively, using simple regression models. (b) Estimated seed volume plotted against the estimated volume of the cones that bear them; $R^2 = 0.51$ using a multiple regression model with cone type as a predictor variable. Seeds make up nearly the entire cone volume in many species with fleshy cones, which results in the hard upper bound seen in the relationship. See main text for more detailed discussion of the regression models used in (a) and (b).

($R^2 = 0.61$ and 0.47 , respectively; $P \ll 0.05$; based on the best supported regression models).

As in pollen cones, seed cone dry mass was strongly associated with cone volume (Fig. 1a) in both OLS and PGLS regression models ($R^2 = 0.94$; $P \ll 0.01$ in both cases). Seed volume was also positively associated with seed cone volume (Fig. 1b; $R^2 = 0.51$; $P \ll 0.05$), although the form of the relationship was strongly influenced by cone type and the best supported model incorporated cone type as a predictor.

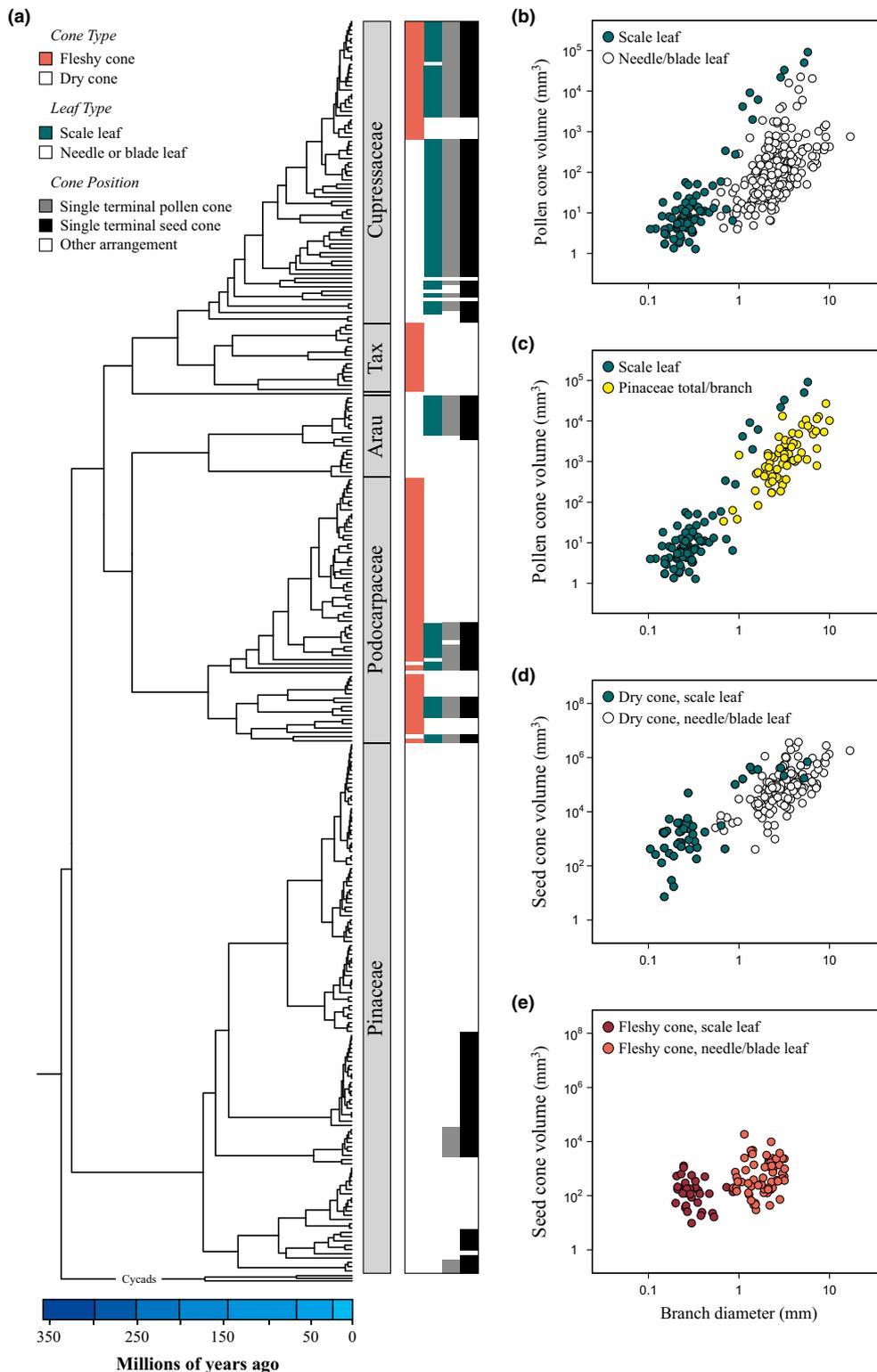


Fig. 2 Conifer phylogeny and the relationships between cone volume and branch diameter. (a) Time-calibrated conifer phylogeny based on two chloroplast genes (*rbcl*, *matK*) and two nuclear genes (*18S*, *PHYB*) with major clades listed: Araucariaceae, Taxaceae. (b) Estimated pollen cone volume plotted against distal branch diameter for scale-leaf species and needle/blade-leaf species; $R^2 = 0.50$ using a multiple regression model with leaf type as a predictor variable. (c) Estimated pollen cone volume plotted against distal branch diameter for scale-leaf species and a subset of needle/blade-leaf species (all members of Pinaceae) with multiple lateral pollen cones per branch. For these taxa, total cone volume per branch was calculated by summing individual cone volumes; $R^2 = 0.61$ using a simple regression model. (d) Estimated seed cone volume plotted against branch diameter in species with dry, woody seed cones. (e) Estimated seed cone volume plotted against branch diameter in species with fleshy seed cones. Taxa are also partitioned by leaf type in (d) and (e). For seed cone volume and branch diameter overall, $R^2 = 0.80$ using a multiple regression model with cone type as a predictor variable. See main text for more detailed discussion of the regression models used in (b–e).

Table 1 Parameter estimates and statistical support for regression models of pollen cone and seed cone volume on branch diameter

Model	Parameter	β	SE	<i>P</i>	R^2	AIC	<i>F</i>
Pollen cone ~ Axis OLS	Intercept	1.61	0.04	<0.01	0.51	576.4	297.3
	Axis diameter	1.32	0.08	<0.01			
Pollen cone ~ Axis OU PGLS	Intercept	1.64	0.06	<0.01	0.43	425.2	223.5
	Axis diameter	1.44	0.10	<0.01			
Pollen cone ~ Axis + Scale + Fleshy OLS	Intercept	1.27	0.07	<0.01	0.60	519.7	143.3
	Axis diameter	2.04	0.13	<0.01			
	Scale leaves	0.99	0.13	<0.01			
	Fleshy	-0.17	0.07	0.02			
Pollen cone ~ Axis + Scale + Fleshy OU PGLS	Intercept	1.41	0.09	<0.01	0.50	399.9	95.9
	Axis diameter	1.87	0.12	<0.01			
	Scale leaves	0.77	0.15	<0.01			
	Fleshy	-0.22	0.12	0.06			
Seed cone ~ Axis OLS	Intercept	3.53	0.04	<0.01	0.46	834.9	244.3
	Axis diameter	1.86	0.08	<0.01			
Seed cone ~ Axis OU PGLS	Intercept	3.45	0.10	<0.01	0.25	697.2	98.8
	Axis diameter	1.53	0.15	<0.01			
Seed cone ~ Axis + Scale + Fleshy OLS	Intercept	4.04	0.10	<0.01	0.80	549.0	230.5
	Axis diameter	1.80	0.18	<0.01			
	Scale leaves	0.26	0.15	0.07			
	Fleshy	-1.58	0.08	<0.01			
	Axis × scale	0.26	0.26	0.31			
	Axis × fleshy	-0.97	0.18	<0.01			
Seed cone ~ Axis + Scale + Fleshy OU PGLS	Intercept	4.04	0.10	<0.01	0.80	551.1	230.4
	Axis diameter	1.80	0.18	<0.01			
	Scale leaves	0.26	0.15	0.07			
	Fleshy	-1.58	0.08	<0.01			
	Axis × scale	0.26	0.26	0.31			
	Axis × fleshy	-0.97	0.18	<0.01			

For pollen cones and seed cones, results are given for both a simple regression model and for the best fit multiple regression model. Bold indicates the best fit model overall for pollen cones and seed cones. We evaluated each model using ordinary least squares (OLS) and phylogenetic least squares (PGLS) regression, which uses an Ornstein–Uhlenbeck (OU) process to incorporate phylogenetic covariance among species data. PGLS models using Brownian motion received poor support. For each model, we show regression coefficients (β) with standard error (SE) and *P*-values, as well as measures of model fit and support (AIC (Akaike Information Criterion), *F*). All *F*-statistic values are significant at the 0.01 level.

Seed cone volume was positively associated with vegetative branch diameter, although the relationship was not strong in the PGLS regression model (Table 1). Branch diameter was a strong predictor of seed cone volume ($R^2 = 0.80$) if leaf type and seed cone type were also included as predictor variables (Table 1). These models also received much higher support than simple regression models, regardless of whether they were evaluated using OLS or PGLS (Table 1). The best supported regression model was an OLS model that included both leaf type and seed type, although an OLS model with only seed cone type received nearly equal support (Table S3). Among regression models with high support, the relationship between fleshy seed cone volume and branch diameter had a lower slope and intercept than the relationship between dry seed cones and branch diameter (Table S3). These results were consistent with bivariate plots of the data, where fleshy seed cones were generally smaller than dry cones and did not obviously increase in size with increasing branch diameter (Fig. 2d,e). Seed cones were generally smaller in taxa with scale leaves relative to taxa with other types of leaves, but showed less of a difference in the intercept of their scaling relationship with branch diameter as compared with pollen cones (Fig. 2d,e; Table S3). As in pollen cones, taxa with scale leaves produce terminal

seed cones while taxa with other types of leaves typically produce lateral cones (78% of species).

Evolutionary models also suggest that cone size evolves differently in clades with scale leaves and fleshy seed cones. We show here results from joint optimization, although the results were similar when parameter values were optimized individually (see Notes S3 and Tables S4, S5, S6). In this case, the best supported model (OU_{MV} model; Table S4) was one in which the optima (θ) and rates of stochastic evolution (σ^2) were free to vary for each character state regime (scale versus needle/blade leaves and fleshy versus dry seed cones). Under this model, the estimated optimum size (θ) for pollen cone volume, seed cone volume, and branch diameter were significantly smaller sizes in clades with scale leaves (Fig. 3a). The rate of stochastic evolution showed less difference between scale-leaf taxa and needle/blade-leaf taxa; it was significantly different only for seed cone volume (0.064 and 0.133, respectively; Table S5). If taxa were analyzed according to dry or fleshy seed cone type rather than leaf morphology, θ was significantly lower for fleshy seed cones than for dry seed cones (Fig. 3b). By contrast, θ values for pollen cone volume and branch diameter were not statistically different between taxa with fleshy or dry seed cones (Fig. 3b). Rates of stochastic evolution

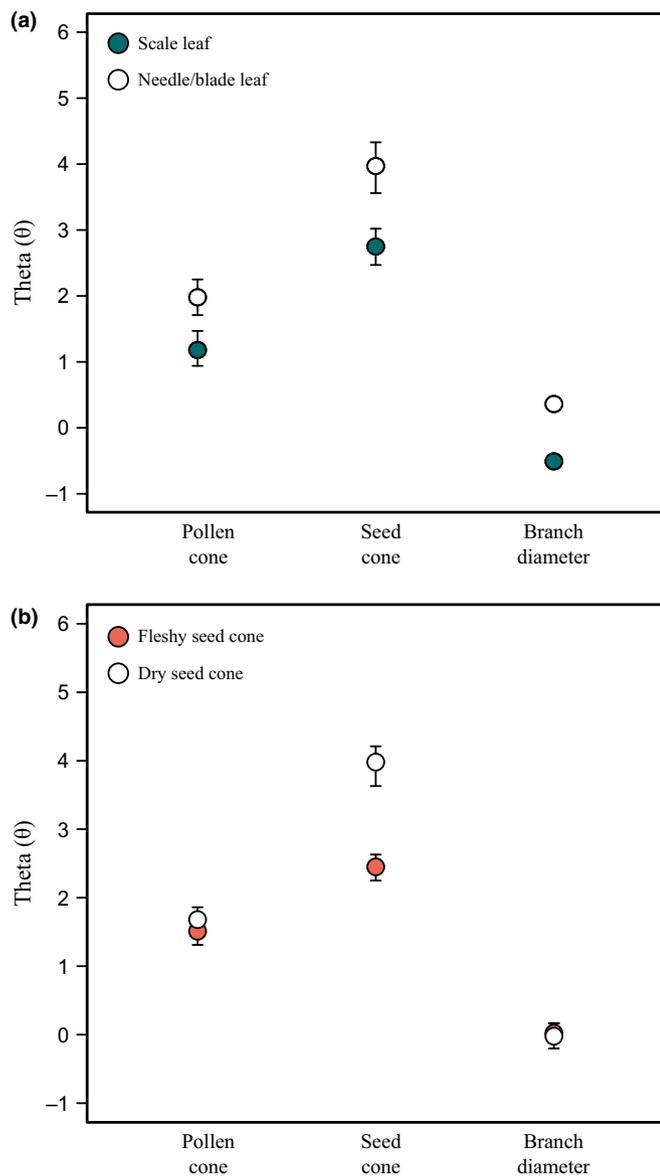


Fig. 3 Results of best fit evolutionary models of cone size and branch diameter in conifers. (a) Estimates of the optimal value (θ) for pollen cone volume, seed cone volume, and branch diameter based on the best fit model when data are partitioned by leaf type (a jointly optimized OU_{MV} model; see main text). This value is consistently smaller for taxa with scale leaves. (b) θ values for pollen cone volume, seed cone volume, and branch diameter based on the best fit evolutionary model when data are partitioned by cone type (a jointly optimized OU_{MV} model). Note that θ is significantly different only for seed cone volume. Error bars represent 95% confidence intervals based on a parametric bootstrap resampling routine.

(σ^2) were significantly different between fleshy and dry taxa for branch diameter (0.004 and 0.010, respectively), pollen cone size (0.038 and 0.086, respectively), and seed cone size (0.049 and 0.163, respectively; see Table S5).

Discussion

The strong association between pollen cone volume and branch diameter is consistent with previous work suggesting that conifers

produce a similar amount of pollen cone tissue within a given volume of fertile canopy foliage, but that branch diameter and branching habit determine how these tissues are deployed (Leslie, 2012). Specifically, large pollen cones tend to be borne on large branches and small cones on small branches, but the higher density with which small branches occur in the plant canopy results in a similar amount of reproductive tissue in total. This is perhaps not surprising because all conifers are wind-pollinated (Owens *et al.*, 1998) and selection may favor devoting a similar proportion of resources to pollen production regardless of the species or clade.

Within this general scaling relationship, however, individual pollen cone size reflects a more complicated set of interactions among leaf morphology, branching habit, and cone position. In particular, taxa with imbricated scale leaves usually have tiny, highly ramified distal branches (although not in *Araucaria* section *Eutacta*), and these consequently produce very small pollen cones borne at the terminal ends of branches. Although these cones are generally small, they are actually larger for a given branch diameter than the lateral cones of taxa with other leaf types (Fig. 2b; Table S1). We might expect this difference based on development, because terminal cones are derived from the entire branch meristem rather than from smaller lateral buds. Furthermore, we might also expect lateral cones to show more variation in size among species (see Le Maitre & Midgley, 1991) because there are many potential ways in which a similar amount of tissue could be deployed by a given branch (i.e. as a few large cones or as many small cones). Cone size in the needle-leaf Pinaceae clade is consistent with these expectations, as adding the volume of all the lateral pollen cones produced per branch results in values that are similar to those of terminal cones and strengthens the scaling relationship between cone volume and branch diameter (Fig. 2c).

The strong scaling relationship between woody seed cones and branch diameter is also consistent with the hypothesis that conifers devote a similar proportion of resources to reproduction within fertile sections of the canopy, and, as in pollen cones, branch diameter determines how this tissue is deployed. This is surprising for seed cones, however, because they experience a wide variety of selective pressures that should alter patterns of resource allocation among species. For example, differences in predation intensity and seed dispersal biology select for differences in the size and robustness of individual cones (Benkman, 1995; Parchman & Benkman, 2002), life-history strategies such as serotiny change cone morphology and how cones are deployed in the canopy (Lamont *et al.*, 1991; Gauthier *et al.*, 1996; Moya *et al.*, 2008), and a variety of environmental factors influence total cone and seed production in the canopy foliage (Caron & Powell, 1989; Woodward *et al.*, 1994; Koenig & Knops, 2000). The long lifespan of conifer seed cones, often up to 2 yr and occasionally 3 yr (Farjon, 2010), may also potentially complicate scaling relationships. For example, seed cones are terminal in scale-leaf taxa, as in pollen cones, but this has less of an obvious effect on scaling relationships, perhaps because mature cones are borne on stems whose growth and development have obscured any direct effect of position. Given all these potential factors influencing reproductive allocation and scaling relationships, it

seems unlikely that conifer species should generally devote a similar proportion of resources to seed cone production, and further studies are necessary to directly test this.

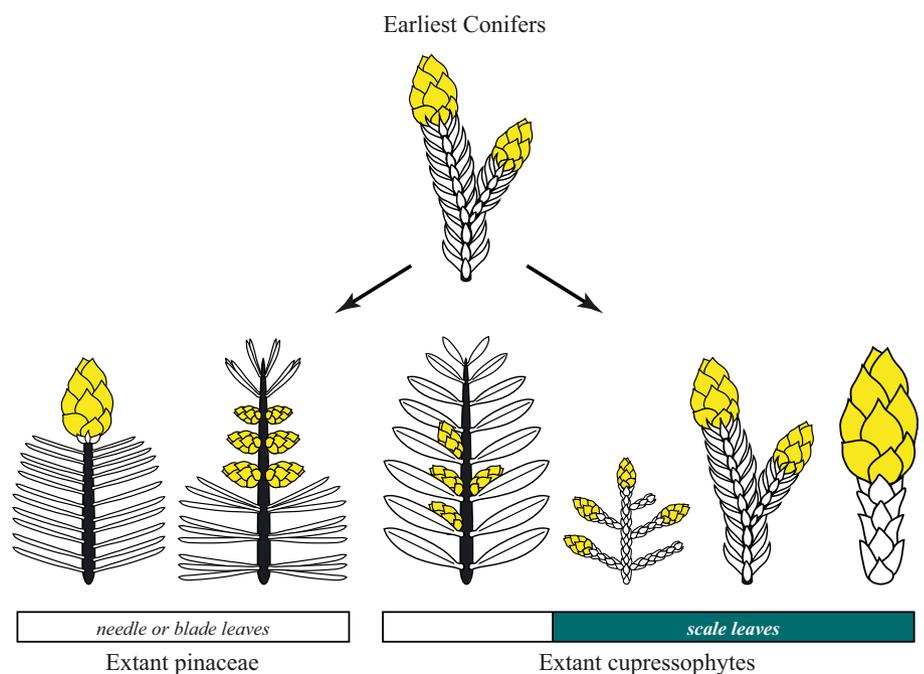
Our data suggest that selective pressures relating to reproductive biology can in fact significantly alter scaling relationships, at least for individual cone size. For example, seed cones with fleshy tissues are small regardless of vegetative branch diameter. These cones almost always consist of just one or two seeds and associated cone scales (Leslie, 2011), and their fleshy tissues attract dispersal agents such as birds and small mammals (Geldenhuys, 1993; Tellería *et al.*, 2014). The evolution of fleshiness, however, appears to have little or no direct relationship with the evolution of pollen cone size or branch diameter; clades with small fleshy seed cones do not also evolve toward small diameter branches or small pollen cones (Fig. 3a). Seed cone size in fleshy clades is therefore effectively decoupled from the scaling relationships exhibited by other types of conifer reproductive cones. This occurs independently in three distantly related lineages (*Juniperus* within the Cupressaceae, Podocarpaceae, and Taxaceae), suggesting that animal seed dispersal consistently shifts the way in which female reproductive tissues are allocated to individual cones.

Despite the decoupling of fleshy seed cone size from branch diameter, our data indicate that there is generally a high degree of integration between conifer reproductive and vegetative structures. It can therefore be difficult to disentangle the evolution of cone size from the evolution of leaves and branching habit. As noted previously, we can divide conifers into two general architectural regimes based on leaf type: those with scale leaves and those with other leaf types (see Fig. 4). The earliest, now extinct, conifer groups had relatively large scale-shaped to awl-shaped leaves similar to those of some modern *Araucaria* species (e.g. *Araucaria heterophylla*) and to *Cryptomeria* within the Cupressaceae (see Florin, 1945; Hernandez-Castillo *et al.*, 2001), suggesting that other leaf types may represent transitions away from an

ancestral scale-leaf condition (see Fig. S1; see also Biffin *et al.*, 2012). In clades that stay within the scale-leaf architectural regime, there has been evolution toward both larger leaves (e.g. extant *Araucaria rulei*) and smaller leaves that closely cover the branch surface (various Cupressaceae and Podocarpaceae), and this is associated with corresponding changes in cone size (Fig. 4). The appearance of other leaf types, however, is associated with a qualitative shift in branching habit that also influences reproductive cones. These taxa have a clearer distinction between leaves and branches, as well as a larger average branch diameter with lower canopy branching density (Figs 2b–d, 4). The evolution of needle or broad leaves from scale-like leaves may therefore be thought of as ‘resetting’ conifer branching architecture and, with it, the likely range of cone size and the specific scaling relationships they exhibit (see Fig. 2b,d). Such resetting would mechanistically occur through developmental correlations (i.e. larger branches produce larger meristems that yield larger cones) and/or selection for optimal allocation of resources (see Olson, 2013).

In a broader sense, these associations illustrate how branching habit can exert a pervasive influence on reproductive tissue allocation in woody seed plants. The extent to which such coordination influences the evolution of vascular plant groups more broadly requires further exploration, but we expect that it will vary widely depending on the morphology and reproductive biology of the group. At one end of this spectrum, reproductive structures might be most strongly coupled with vegetative evolution in plant groups such as *Equisetum* and lycopsids, where strobili have few functional demands other than the abiotic release of spores. At the other end of this spectrum, correlations between vegetative and reproductive morphologies may place relatively few constraints on flowering plant evolution as a consequence of the complexity and modularity of their reproductive structures, where the sizes of their inflorescences, flowers, fruits, and seeds could all potentially vary independently in response to specific

Fig. 4 Schematic line drawings of the various conifer architectural regimes discussed in this study. Distal branches are represented by black bars, which bear typical leaf types in white and reproductive cones in yellow. Representative forms of several major clades are illustrated, including extant Pinaceae, Cupressophytes (which includes all other living conifer species), and early conifer groups. Scale-leaf forms (shown above the green bar) typically have imbricated leaves on ramified branches, which almost always terminate in a solitary pollen or seed cone. Needle or blade leaf forms (shown above the white bar) usually have longer, more widely spaced leaves and most often produce lateral cones, although terminal cones sometimes occur. Drawings are not shown to exact scale, but are meant to indicate the relative sizes of branches, leaves, lateral cones, and terminal cones in the different regimes.



selective pressures. Conifers, as well as other groups of nonflowering seed plants such as cycads and Gnetales, may fall between these extremes.

Acknowledgements

We would like to thank two anonymous reviewers whose comments improved the quality and clarify of the manuscript. We would also like to thank the Harvard University Herbaria, Kew Herbarium, and Steere Herbarium of the New York Botanic Garden Herbarium for access to specimens and collections, and Emily Wood, Aljos Farjon, and Douglas Daly for their assistance. Support for J.M.B. has been provided by the National Institute for Mathematical and Biological Synthesis, an Institute sponsored by the National Science Foundation, the US Department of Homeland Security, and the US Department of Agriculture through NSF Awards #EF-0832858 and #DBI-1300426, with additional support from The University of Tennessee, Knoxville.

References

- Ackerly DD, Donoghue MJ. 1998. Leaf size, sapling allometry, and Corner's rules: phylogeny and correlated evolution in maples (*Acer*). *American Naturalist* 152: 767–791.
- Beaulieu JM, Jhwueng D-C, Boettiger C, O'Meara BC. 2012. Modeling stabilizing selection: expanding the Ornstein–Uhlenbeck model of adaptive evolution. *Evolution* 66: 2369–2383.
- Beaulieu JM, O'Meara BC, Donoghue MJ. 2013. Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid angiosperms. *Systematic Biology* 62: 725–737.
- Benkman CW. 1995. The impact of tree squirrels (*Tamiasciurus*) on Limber Pine seed dispersal adaptations. *Evolution* 49: 585–592.
- Bentley LP, Stegen JC, Savage VM, Smith DD, von Allmen EI, Sperry JS, Reich PB, Enquist BJ. 2013. An empirical assessment of tree branching networks and implications for plant allometric scaling models. *Ecology Letters* 16: 1069–1078.
- Biffin E, Brodribb TJ, Hill RS, Thomas P, Lowe AJ. 2012. Leaf evolution in southern hemisphere conifers tracks the angiosperm ecological radiation. *Proceedings of the Royal Society B: Biological Sciences* 279: 341–348.
- Bond WJ, Midgley J. 1988. Allometry and sexual differences in leaf size. *American Naturalist* 131: 901–910.
- Brouat C, Gibernau M, Amsellem L, McKey D. 1998. Corner's rules revisited: ontogenetic and interspecific patterns in leaf-stem allometry. *New Phytologist* 139: 459–470.
- Caron GE, Powell GR. 1989. Patterns of seed-cone and pollen-cone production in young *Picea mariana* trees. *Canadian Journal of Forest Research* 19: 359–364.
- Cornelissen JHC. 1999. A triangular relationship between leaf size and seed size among woody species: allometry, ontogeny, ecology, and taxonomy. *Oecologia* 118: 248–255.
- Corner EJH. 1949. The durian theory or the origin of the modern tree. *Annals of Botany* 13: 368–414.
- Diggle PK. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. *Annual Review of Ecology, Evolution, and Systematics* 26: 531–552.
- Enright NJ, Hill RS. 1995. *Ecology of the Southern Conifers*. Washington, DC, USA: Smithsonian Institution Press.
- Farjon A. 2010. *A handbook of the world's conifers*. Leiden, the Netherlands: Koninklijke Brill.
- Farjon A, Filer D. 2013. *An atlas of the world's conifers: an analysis of their distribution, biogeography, diversity, and conservation status*. Leiden, the Netherlands: Koninklijke Brill.
- Florin R. 1938–1945. Die koniferen des Oberkarbons und des unteren Perms I–VII. *Palaeontographica. Abteilung B* 85: 1–729.
- Garland T Jr, Ives AR. 2000. Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *American Naturalist* 155: 346–364.
- Gauthier S, Bergeron Y, Simon J-P. 1996. Effects of fire regime on the serotiny level of jack pine. *Journal of Ecology* 84: 539–548.
- Geldenhuys CJ. 1993. Reproductive biology and population structures of *Podocarpus falcatus* and *P. latifolius* in southern Cape forests. *Botanical Journal of the Linnean Society* 112: 59–74.
- Hernandez-Castillo GR, Rothwell GW, Mapes G. 2001. Thucydiaeace fam. nov., with a review and reevaluation of Paleozoic walchian conifers. *International Journal of Plant Sciences* 162: 1155–1185.
- Koenig WD, Knops JMH. 2000. Patterns of annual seed production by Northern Hemisphere trees: a global perspective. *The American Naturalist* 155: 59–69.
- Lamont BB, Le Maitre DC, Cowling RM, Enright NJ. 1991. Canopy seed storage in woody plants. *The Botanical Review* 57: 277–317.
- Le Maitre DC, Midgley JJ. 1991. Allometric relationships between leaf and inflorescence mass in the genus *Protea* (Proteaceae): an analysis of exceptions to the rule. *Functional Ecology* 5: 476–484.
- Leslie AB. 2011. Shifting functional roles and the evolution of conifer pollen-producing and seed-producing cones. *Paleobiology* 37: 587–602.
- Leslie AB. 2012. Branching habit and the allocation of reproductive resources in conifers. *Annals of Botany* 110: 915–921.
- Leslie AB, Beaulieu JM, Rai HS, Crane PR, Donoghue MJ, Mathews S. 2012. Hemisphere-scale differences in conifer evolutionary dynamics. *Proceedings of the National Academy of Sciences, USA* 109: 16217–16221.
- Martins EP, Hansen TF. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into analysis of interspecific data. *American Naturalist* 149: 646–667.
- McCune B. 1988. Ecological diversity in North American pines. *American Journal of Botany* 75: 353–368.
- Midgley J, Bond WJ. 1989. Leaf size and inflorescence size may be allometrically related traits. *Oecologia* 78: 427–429.
- Moya D, Saracino A, Salvatore R, Lovreglio R, de Las Heras J, Leone V. 2008. Anatomic basis and insulation of serotinous cones in *Pinus halepensis* Mill. *Trees* 22: 511–519.
- Niklas KJ. 1993. The allometry of plant reproductive biomass and stem diameter. *American Journal of Botany* 80: 461–467.
- Niklas KJ. 1994. *Plant allometry: the scaling of form and process*. Chicago, IL, USA: The University of Chicago Press.
- Niklas KJ, Enquist BJ. 2003. An allometric model for seed plant reproduction. *Evolutionary Ecology Research* 5: 79–88.
- Olson ME. 2013. The developmental renaissance in adaptationism. *Trends in Ecology and Evolution* 27: 278–287.
- Olson ME, Aguirre-Hernández R, Rosell JA. 2009. Universal foliage-stem scaling across environments and species in dicot trees: plasticity, biomechanics and Corner's rules. *Ecology Letters* 12: 210–219.
- Owens JN, Takaso T, Runions CJ. 1998. Pollination in conifers. *Trends in Plant Science* 3: 479–485.
- Parchman TL, Benkman CW. 2002. Diversifying coevolution between crossbills and black spruce on Newfoundland. *Evolution* 56: 1663–1672.
- Price CA, Enquist BJ, Savage VM. 2007. A general model for allometric covariation in botanical form and function. *Proceedings of the National Academy of Sciences, USA* 104: 13204–13209.
- Primack RB. 1987. Relationships among flowers, fruits, and seeds. *Annual Review of Ecology, Evolution, and Systematics* 18: 409–430.
- Rai HS, Reeves PA, Peakall R, Olmstead GR, Graham SW. 2008. Inference of higher-order conifer relationships from a multi-locus plastid data set. *Botany* 86: 658–669.
- Savage VM, Bentley LP, Enquist BJ, Sperry JS, Smith DD, Reich PB, von Allmen EI. 2010. Hydraulic trade-offs and space filling enable better predictions of vascular structure and function in plants. *Proceedings of the National Academy of Sciences, USA* 107: 22722–22727.

- Sun S, Jin D, Shi P. 2006. The leaf size–twig size spectrum of temperate woody species along an altitudinal gradient: an invariant allometric scaling relationship. *Annals of Botany* 97: 97–107.
- Tellería TL, Carrascal LM, Santos T. 2014. Species abundance and migratory status affects large-scale fruit tracking in thrushes (*Turdus* spp.). *Journal of Ornithology* 155: 157–164.
- West GB, Brown JH, Enquist BJ. 1997. A general model for the origin of allometric scaling laws in biology. *Science* 276: 122–126.
- West GB, Brown JH, Enquist BJ. 1999. A general model for the structure and allometry of plant vascular systems. *Nature* 400: 664–667.
- Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33: 125–159.
- Westoby M, Jurado E, Leishman M. 1992. Comparative evolutionary ecology of seed size. *Trends in Ecology and Evolution* 7: 368–372.
- Westoby M, Wright IJ. 2003. The leaf size – twig size spectrum and its relationship to other important spectra of variation among species. *Oecologia* 135: 621–628.
- White PS. 1983. Evidence that temperate east North American evergreen woody plants follow Corner's rules. *New Phytologist* 95: 139–145.
- Woodward A, Silsbee DG, Schreiner EG, Means JE. 1994. Influence of climate on radial growth and cone production in subalpine fir (*Abies lasiocarpa*) and mountain hemlock (*Tsuga mertensiana*). *Canadian Journal of Forest Research* 24: 1133–1143.
- Woodward FI, Lomas MR, Kelly CK. 2004. Global climate and the distribution of plant biomes. *Philosophical Transactions of the Royal Society of London B* 359: 1465–1476.
- Wright IJ, Ackerly DD, Bongers F, Harms KE, Ibarra-Manriquez G, Martinez-Ramos M, Mazer SJ, Muller-Landau HC, Paz H, Pitman NCA *et al.* 2007. Relationships among ecologically important dimensions of plant trait variation in seven Neotropical forests. *Annals of Botany* 99: 1003–1015.

Supporting Information

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

Fig. S1 Ancestral character state reconstructions for leaf type and seed cone type.

Table S1 Regression model results for the pollen cone data set

Table S2 Results of correlated characters test

Table S3 Regression model results for the seed cone data set

Table S4 Support values for evolutionary models

Table S5 Results from best fit evolutionary model with parameter values estimated jointly for all traits in each character regime

Table S6 Results from best fit evolutionary models with parameter values estimated separately for each trait in each character regime

Notes S1 Explanation of the character scoring scheme used in this study.

Notes S2 Description of the phylogenetic regression models and correlated character tests used in this study.

Notes S3 Description of the models of character evolution used in this study.

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.



About New Phytologist

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <25 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@ornl.gov)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**