NEWS & VIEWS

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ON THE NATURE OF THINGS: ESSAYS

New Ideas and Directions in Botany

Unpacking a century-old mystery: Winter buds and the latitudinal gradient in leaf form[1]

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This year marks the 100th anniversary of a seminal paper on plant form. In 1916, in the pages of the *American Journal of Botany*, Irving W. Bailey and Edmund W. Sinnott documented a remarkable observation: in wet tropical forests, the percentage of woody plant species with toothed or lobed leaves is close to zero, but it increases toward 100% moving north into cold-temperate regions (Bailey and Sinnott, 1916). This latitudinal gradient has repeatedly been confirmed (e.g., Little et al., 2010; Peppe et al., 2011) and is so robust that paleobotanists use the percentage of leaves with entire margins in paleofloras as a proxy for mean annual temperature (Wolfe, 1971). In the meantime, it has come to light that other aspects of leaf form may be correlated with climate, as temperate leaves also tend to be rounder, while tropical leaves are more elliptical (Schmerler et al., 2012). But, *why* does leaf form vary so predictably? The short answer is that we still don't know. Here we explore a new angle, focusing attention on changes in the rhythm of growth and leaf development that accompanied evolutionary shifts into strongly seasonal climates.

First we must ask: Is this pattern due to many evolutionary shifts in leaf form as lineages moved from tropical into temperate forests (and vice versa)? Or, is it largely driven by just a few successful lineages in northern latitudes that happened to have teeth and lobes (e.g., maples, birches, oaks)? We still don't have a clear idea of the number of tropical–temperate transitions in plants (Donoghue and Edwards, 2014). Yet, the wide taxonomic distribution of lineages with both tropical and temperate ranges supports the assumption that there were multiple biome shifts accompanied by repeated evolutionary changes in leaf form (e.g., temperate *Acer* within Sapindaceae, *Tilia* within Malvaceae, *Hamamelis* within Hamamelidaceae, *Fagus* within Fagaceae). And, judging by our experience with *Viburnum* (Schmerler et al., 2012; Spriggs et al., 2015), additional transitions are likely hidden within many of the clades that span these biomes (Edwards and Donoghue, 2013; Donoghue and Edwards, 2014).

Until now, adaptive explanations for the leaf-form gradient have focused on leaf function either later in development or in mature leaves. For instance, we know that leaf size and shape influence boundary layer dynamics; smaller and more dissected leaves facilitate gas exchange and transpirational cooling (Gates, 1968). But, why then should leaves not instead be more dissected in tropical forests, where the air is often hot and still? A second explanation points to leaf teeth as sites of early-season gas exchange, arguing that rapid maturation of toothy margins provides a boost in photosynthate production when light and water are more available, before the formation of a full forest canopy (Baker-Brosh and Peet, 1997; Royer and Wilf, 2006). Data vary in support of this hypothesis, and there has been no attempt to quantify the total contribution of photosynthesis in teeth of emerging leaves to a plant's carbon budget, which we imagine is exceedingly small. Another hypothesis is that teeth serve as hydathodes that expel water that might otherwise flood developing leaf tissues early in the spring. This may be relevant for temperate species that use positive root pressure to remove freeze–thaw embolisms (Lechowicz, 1984; Feild et al., 2005), but many species with leaf teeth do not generate positive xylem pressure. A fourth explanation is biomechanical: temperate leaves, it is said, are thinner and rely more heavily on structural support from their vein systems. In such leaves, the optimal tissue configuration surrounding each major vein is wedge shaped, which in a pinnately veined leaf would result in a toothy margin (Givnish, 1979). It has even been argued that teeth protect leaves against herbivores (Brown and Lawton, 1991). Each of these hypotheses has some merit and might apply in particular cases. But, in our estimation, none of them is terribly well supported, and little attention has been paid to the alternative possibility that selection on other aspects of the organism might indirectly generate certain leaf characteristics, possibly affecting both teeth and shape simultaneously. Here we consider the idea that the repeated emergence of

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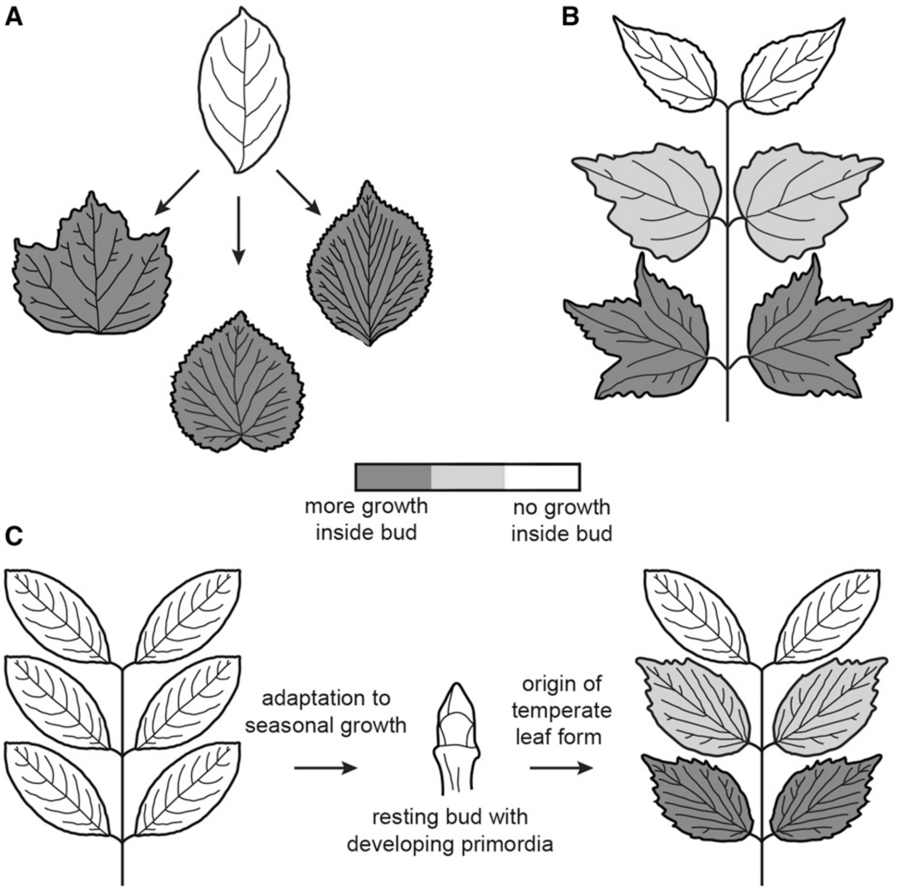

FIGURE 1 A new hypothesis for the origin of temperate leaf form. (A) In *Viburnum*, multiple shifts into cold temperate forests resulted in the repeated evolution of toothed and lobed leaves. Top leaf, tropical *Viburnum amplificatum*; bottom left, *Viburnum trilobum*; bottom center, *Viburnum molle*; bottom right, *Viburnum wrightii*. (B) Seasonal heteroblasty in temperate *Viburnum* species leads to the production of tropical-like leaves. The bottom two pairs of leaves developed inside overwintering buds (preformed), with the basal pair being the most developed at spring bud break. The top leaf pair was neoformed; i.e., produced during the growing season without any time in the bud. (C) The bud-packing hypothesis. On the left is a hypothetical tropical ancestor, with all leaves formed largely outside resting buds (neoformed). During adaptation to a seasonal climate, plants evolved a prolonged resting phase and began to fill their resting buds with leaf and inflorescence primordia to facilitate rapid spring growth. This early leaf development inside a tightly packed bud resulted in the evolution of more complex leaf shapes. Leaf color in each image corresponds to the relative amount of development inside buds.

temperate leaf forms resulted from selection on a different stage of leaf development, namely, leaf primordia inside overwintering buds.

Most broad-leaved trees in the temperate zone are also deciduous. How might the shift to deciduousness—and the corresponding development of leaf primordia inside tightly packed overwintering buds—influence mature leaf shape? A connection between bud packing and leaf shape can be traced back to the 19th century, when Sir John Lubbock described and contrasted the arrangement of leaf primordia inside buds of oaks, beeches, and tulip trees and suggested that the various folds and vernation patterns in bud were directly responsible for their differences in mature leaf form (Lubbock, 1899). More than a century later, Couturier et al. (2011; see also Kobayashi et al., 1998) provided a more general theoretical framework for Lubbock's original ideas. These authors first demonstrated that the shape of maple leaves can be precisely predicted from the principles of kirigami (fold-and-cut origami), with the angles and depths of the sinuses relating to the folds and boundaries of the leaf primordia in bud (Couturier et al., 2011). Then they carried out an experiment in which one leaf primordium in a pair was ablated (Couturier et al., 2012). This removal resulted in dramatic differences in the shape of the remaining leaf, suggesting that the bounded space within which a leaf primordium develops exerts physical pressures that influence adult leaf form.

We find additional support for a bud-packing hypothesis in the seasonal heteroblasty exhibited by temperate woody plants. In a series of studies, William Critchfield documented systematic differences in leaf form associated with position along a branch (e.g., Critchfield, 1971). Specifically, he showed that "preformed" leaves, which undergo a phase of arrested development within a bud, differ in shape from "neoformed" leaves, which develop continuously from primordia not contained within a bud and produced later in the season. Recently, we have documented such regular, seasonal heteroblasty in several *Viburnum* species, representing clades that underwent two independent shifts into temperate forests (Fig. 1) (E. L. Spriggs et al., unpublished manuscript). In these cases, the preformed leaves are consistently rounder (or more lobed) and toothier than the neoformed leaves, which are more elliptical, with greatly reduced teeth. In other words, the leaves of these temperate species that develop fully outside of the overwintering bud look decidedly more tropical. We suspect that seasonal shifts in leaf form may arise from two co-occurring forces: primarily, the different scenarios of early development, presented by the physical confines of the bud itself as well as the significant pause in development caused by winter dormancy; and secondarily, potentially plastic responses to different light and temperature environments experienced in early vs. late season. The relative importance of bud-packing and external environment in establishing seasonal heteroblasty could be easily assessed with the right experiment.

If a connection between leaf form and bud packing has been acknowledged for over a century, why has it never been considered as an explanation for the latitudinal gradient in leaf form? Its relevance rests on a critical assumption: the leaves of temperate species must undergo significantly more development inside buds than do their tropical counterparts. How likely is this? In general, growth rhythms are less obvious in the tropics than they are in seasonal temperate climates, and tropical phenology remains poorly documented. We know that tropical plants don't develop continuously, and leaf flushing is common and often spectacular in tropical forests (Wu et al., 2016). But how much early leaf development occurs inside buds in tropical species? There are very few surveys of bud anatomy and resting times in tropical woody plants, although authors sometimes mention in passing the existence of "resting buds" and bud scales (Richards, 1952; Hallé et al., 1978). In our ongoing work, we have found that tropical *Viburnum* species do in fact produce buds with differentiated scales and that meristems do rest for considerable periods, flushing leaves just once or twice per year. However, it appears that very little development of the leaf primordia takes place inside these buds. Consequently, virtually all their leaves are effectively neoformed.

If a bud-packing hypothesis is supported, it will be necessary to parse which aspects of the syndrome are specifically adaptive. It is hard to argue with the idea that producing a flush of new leaves quickly in the spring is an adaptation to seasonally cold climates. Maybe some basic and repeated changes in leaf form simply reflect differential growth responses to the physical contact of primordia with one another and with the surrounding bud scales (cf. Couturier et al., 2012). Alternatively, perhaps certain shapes and arrangements of leaf primordia inside the buds allow more efficient filling of a small and tightly constrained space. In *Viburnum* again, we are struck by what appear to be repeated shifts to a particular arrangement of leaf primordia in temperate buds (Fig. 2). It would be productive to approach this from a modeling standpoint, to compare whether and how particular vernation and ptyxis types facilitate efficient bud packing.

We are not suggesting that the bud-packing hypothesis is the only explanation for the latitudinal gradient in leaf form, and we certainly don't think that it can explain all the various instances of complex leaf shapes found in nature. Similar phenotypes can clearly arise for many different reasons. But, with respect to the Bailey–Sinnott trend, we think it is as compelling as any other hypothesis, and we are certain that it will be productive to shift the focus away from the function of mature leaves and, instead, to develop a more integrated, whole-plant perspective. We find the bud-packing hypothesis attractive because it has the potential to simultaneously connect evolutionary biome shifts to phenology, branching architecture, bud formation, bud packing, leaf shape, and leaf margins. And, it has the virtue of promoting a much closer look at what's happening inside resting buds. Botanists have long been aware of differences in the arrangement and folding of leaf primordia, but have neglected to "unpack" and explain this hidden diversity. We have ever more powerful tools at our disposal to address this problem, and we look forward to more integrative studies of a fundamental and lingering botanical question.

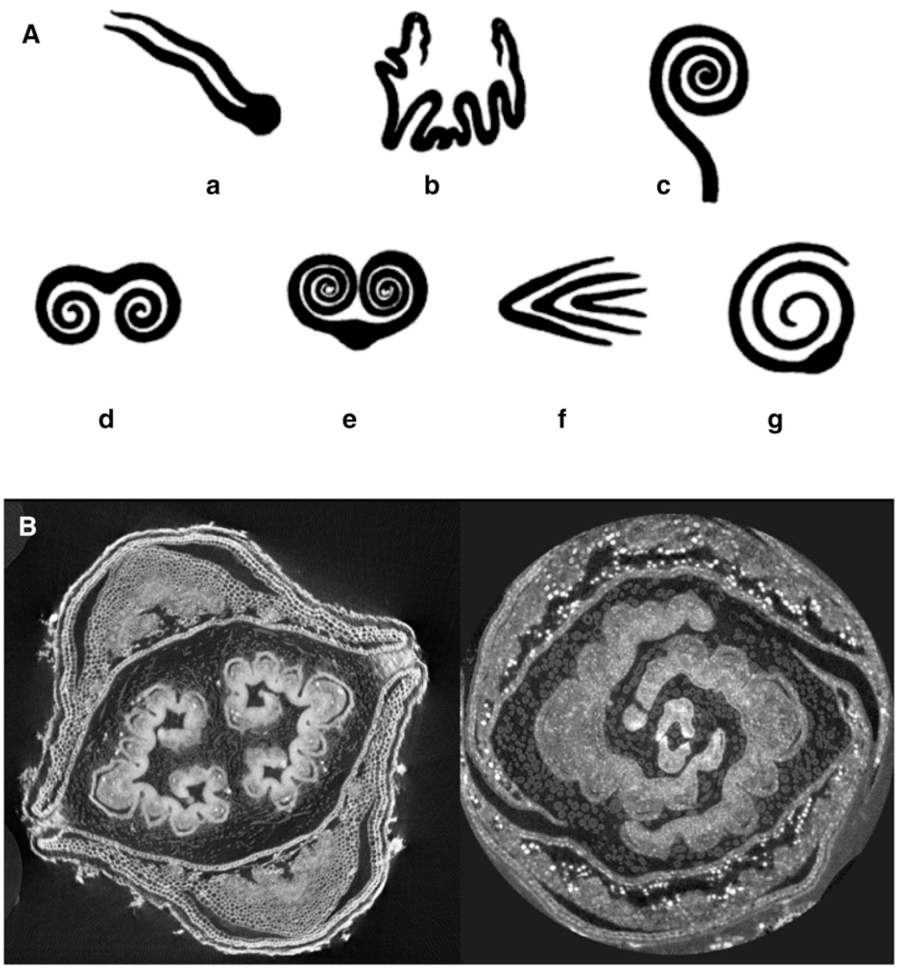

FIGURE 2 Variation in vernation and ptyxis in overwintering buds. A, from Lubbock (1899), who diagrammed common types of leaf folding and arrangement inside of buds. B, micro-CT scans of the buds of *Viburnum plicatum* (left) and *Viburnum dentatum* (right). In each image, the outermost tissue comprises bud scales. In *V. plicatum*, there is one pair of leaves; in *V. dentatum*, there is one well-developed outer pair and a very small inner pair. In *Viburnum*, we have discovered multiple transitions from the arrangement in *V. plicatum*, corresponding to Lubbock's (e) involute rolling, to a modified version of his (g) convolute rolling, with the two leaves of a pair overlapping, represented by *V. dentatum*.

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