Correlation, causation, and the evolution of leaf teeth: A reply to Givnish and Kriebel[1]

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We are grateful to Givnish and Kriebel (2017) for providing a thorough review of the arguments that have been advanced to explain the latitudinal gradient in leaf form. Their paper was motivated by our recent "On The Nature of Things" essay presenting the "bud packing hypothesis" (BP) as an alternative explanation for why leaves in the temperate zone are so often toothed or lobed (Edwards et al., 2016). Although Givnish and Kriebel (2017) added the BP hypothesis to their list of possible explanations and included it as one of many causal arrows in their synthetic model (see their fig. 6), they were generally unconvinced by our arguments and instead strongly favored the "support and supply hypothesis" (SS) advanced by Givnish almost 40 years ago (Givnish, 1979). Here we present new analyses that question the assumptions of the SS model and elaborate further on the possible connections between teeth and bud packing. Most importantly, we reiterate our plea for studies of bud development.

But first, let us note where we seem to agree. We all view leaf boundary layer dynamics, early season photosynthesis, and guttation through hydathodes as unlikely explanations for the latitudinal gradient in leaf margins. Although Givnish and Kriebel portray us as dismissing the idea that spinose teeth might sometimes defend against herbivores, we do not disagree at all. In fact, as we stressed (Edwards et al., 2016, p. 975), "Each of these hypotheses has some merit and might apply in particular cases." As they rightly argued, large herbivores are especially likely to select for spinose teeth in short-statured plants in arid and semiarid environments. Our discussion focused instead on woody plants of mesic forests, where spinose leaves are rare (e.g., *Ilex*). Finally, we strongly agree with Givnish and Kriebel that venation architecture deserves far more attention in relation to this problem, and we return to this topic below.

However, we disagree with their other main points. Most of the Givnish and Kriebel commentary focused on demonstrating a correlation between leaf thickness and toothy margins, which they present as evidence in favor of the SS model. But, we have never doubted a relationship between leaf thickness and leaf teeth. In fact, Givnish and Kriebel used our data on *Viburnum* (Schmerler et al., 2012; Chatelet et al., 2013) to show this relationship, which we were already well aware of. Because we disagree with their scoring of several species, we re-examined this relationship with a revised data set and recovered an even stronger relationship between thickness and margin type than they originally reported (Fig. 1). However, it is very difficult to disentangle leaf thickness and leaf margins from leaf habit and longevity. In *Viburnum*—and we presume in many other clades—teeth and thickness are also strongly correlated with evolutionary shifts in leaf habit (evergreen vs. deciduous; Fig. 1), and, more importantly for our arguments, with changes in the rhythm of leaf production, leaf lifespan, and the extent of leaf development inside of resting buds. Our phylogenetic regression analyses recover a strong association between leaf thickness and margin type ($\beta = -3.361 \pm 0.891$ SE, $p = 0.0002$), but an equally strong relationship between leaf thickness and leaf habit ($\beta = 3.824 \pm 0.944$ SE, $p = 0.0001$), and, as we have shown previously (Schmerler et al., 2012), another very strong relationship between leaf margin type and leaf habit ($p = 2e^{-12}$).

Givnish and Kriebel performed two other analyses along these lines. First, they reduced a ~3500 species data set on leaf form, thickness, and habitat compiled by Royer et al. (2012) to ~600 species to repeat the original analyses in a phylogenetic context. As expected, they found a tight relationship between leaf thickness and margin type. Though we do not question this correlation, in general
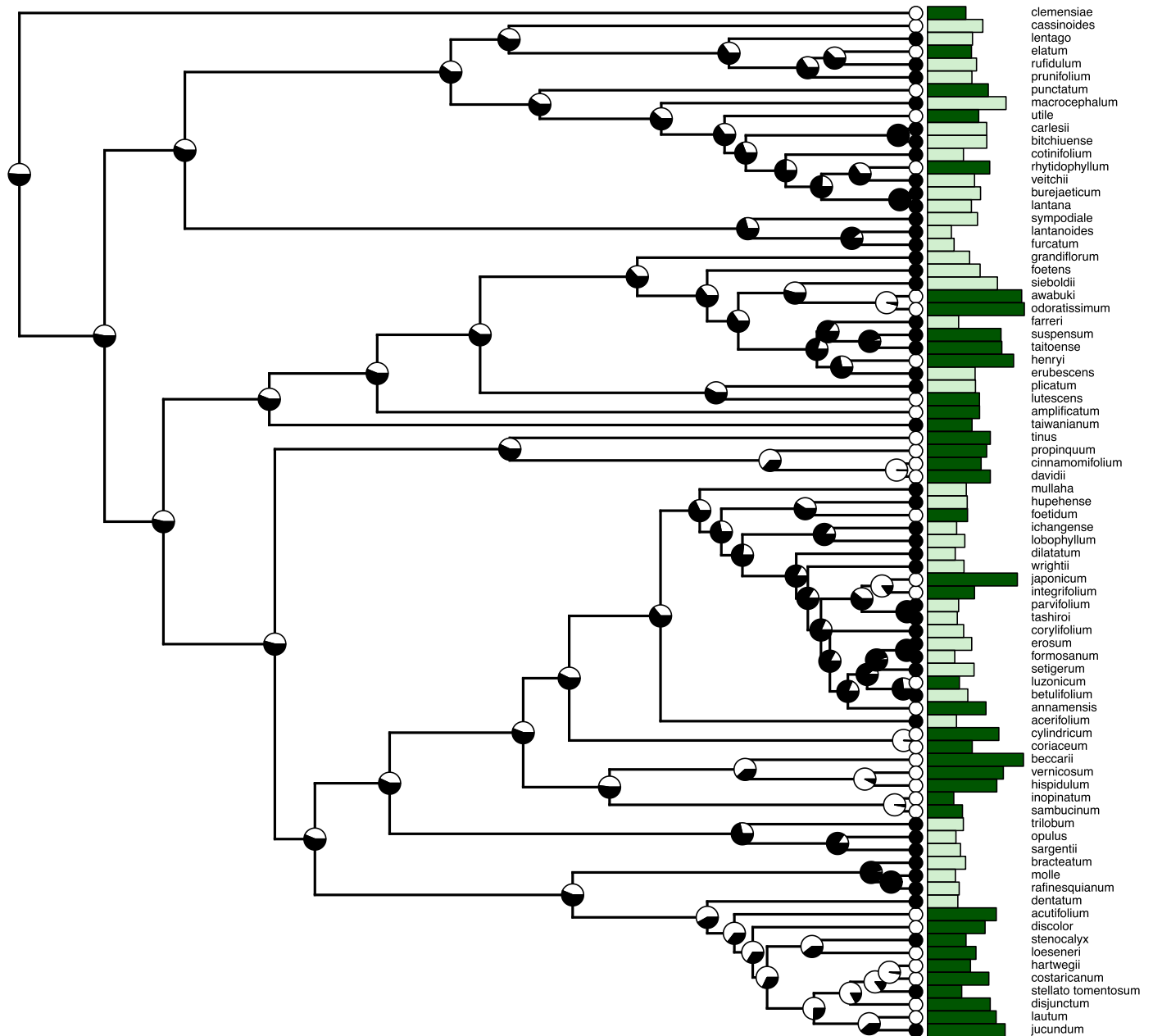
FIGURE 1 The correlation of margin type (black = regular teeth; white = entire or with few, irregularly spaced teeth) with leaf thickness (bar plots at the tips) in a phylogeny of *Viburnum* based on Spriggs et al. (2015). The color of the leaf thickness bars reflects leafing habit: dark green = evergreen; light green = deciduous. The sampling is similar to that of Givnish and Kriebel (2017) (GK) except that our analysis includes *V. jucundum* and excludes several segregate taxa (*V. lantana* var. *discolor* and *V. rafinesquianum* var. *affine*). The scoring of margin type also differs in several taxa. Following the coding of Schmerler et al. (2012), we correct codings for several species: *V. odoratissimum* and *V. henryi* were coded as "toothy" by GK, but "entire" here; *V. awabuki* and *V. amplificatum* were coded as "minutely toothed" by GK, but "entire" here; and *V. foetidum*, *V. japonicum*, and *V. luzonicum* were coded as "toothy" by GK, but "irregular" here. Based on field and herbarium studies conducted since Schmerler et al. (2012), we now code *V. suspensum* as "toothy", and both *V. cinnamomifolium* and *V. propinquum* as "entire"; all three were coded "irregular" by Schmerler et al. (2012) and "minutely toothed" by GK. The remaining differences in comparison to fig. 5 of Givnish and Kriebel (2017) reflect a difference in the delimitation of the states of the binary character; they combined their "minutely toothed" (which we interpret, based on their scorings, to be equivalent to what we term "irregularly toothed", with very few teeth scattered along the margin) with regularly toothed categories. We prefer to combine them with entire-margined leaves, as Givnish and Kriebel (2017) did in their fig. S3. The basic results are the same under both scorings; see text for discussion.

we find it difficult to interpret such "global" analyses that rely on poorly sampled phylogenies (see Edwards et al., 2015). They also repeated an analysis originally carried out by Givnish (1979) to demonstrate a correlation between thickness and margins in evergreen species of the El Yunque rainforest. This dataset provides an interesting wrinkle, as it removes leaf habit (evergreen vs. deciduous)

as a factor. In so doing, it provides a great opportunity to directly test what we see as the real underlying causes (which in the temperate zone are strongly correlated with leaf habit); we predict that the species with thinner, toothier leaves will specifically differ in their patterns of leaf production, longevity, and bud packing from their thicker, entire-margined neighbors.

Our main point is that simply observing the correlation between thickness and margin type predicted by the SS model does little to demonstrate that this model is correct when there are other completely plausible explanations for why those traits would be connected. A relationship between margins and thickness, no matter how strong, cannot differentiate between the SS and BP models, because it is consistent with both hypotheses (Fig. 2). Specifically, the bud-packing hypothesis predicts that thin leaves and leaf teeth are both linked to the period of dormancy and shorter growing seasons that accompany the transition to a deciduous leaf habit. Under this model, one fully expects a strong correlation between leaf thickness and leaf margins—not because thin leaves directly promote the evolution of teeth, as Givnish and Kriebel argued, but because both traits are causally connected to an underlying factor (Fig. 2). Consequently, gathering more data to support this correlation (which they consider a priority for future studies) will not help. More data of this type would be useful for other reasons, but not to distinguish between these two hypotheses.
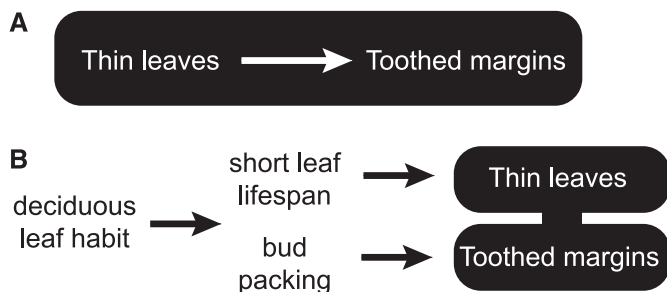
To choose among the competing explanations for the latitudinal gradient in leaf form, it is necessary to carefully evaluate their underlying assumptions. Givnish and Kriebel (2017, p. 354) asserted that "Theoretical and empirical problems undermine all hypotheses except the support–supply hypothesis…" Yet, as we argue below, there are also problems with the SS model, many of which stem from the basically untested claim by Givnish (1979) that the tissue between secondary veins in thinner leaves has lower hydraulic conductance and is biomechanically more "independent" and less well supported than in thicker leaves. The authors present no evidence

FIGURE 2 Alternative explanations for a correlation between leaf thickness and leaf margin type. In both models, thickness and margin type are strongly correlated (indicated by the black background). This trait correlation may arise via a direct causal mechanism, such as envisioned by the SS model (A), where toothed margins emerge as a functional repercussion of a thin lamina. Alternatively, two traits may be correlated because they are each connected mechanistically to a third variable (B). The BP model is an example of this second scenario: in this case, the evolution of a deciduous leaf habit in the temperate zone simultaneously selected for thinner leaves in response to a short growing season and leaf lifespan, and leaves with toothed margins as a result of early leaf development inside of overwintering buds. Both the direct and the indirect model result in a strong trait correlation, meaning that a strong trait correlation, by itself, cannot be used to support one model over the other.

to support this assumption; in fact, they highlight the testing of it as a key research priority moving forward.
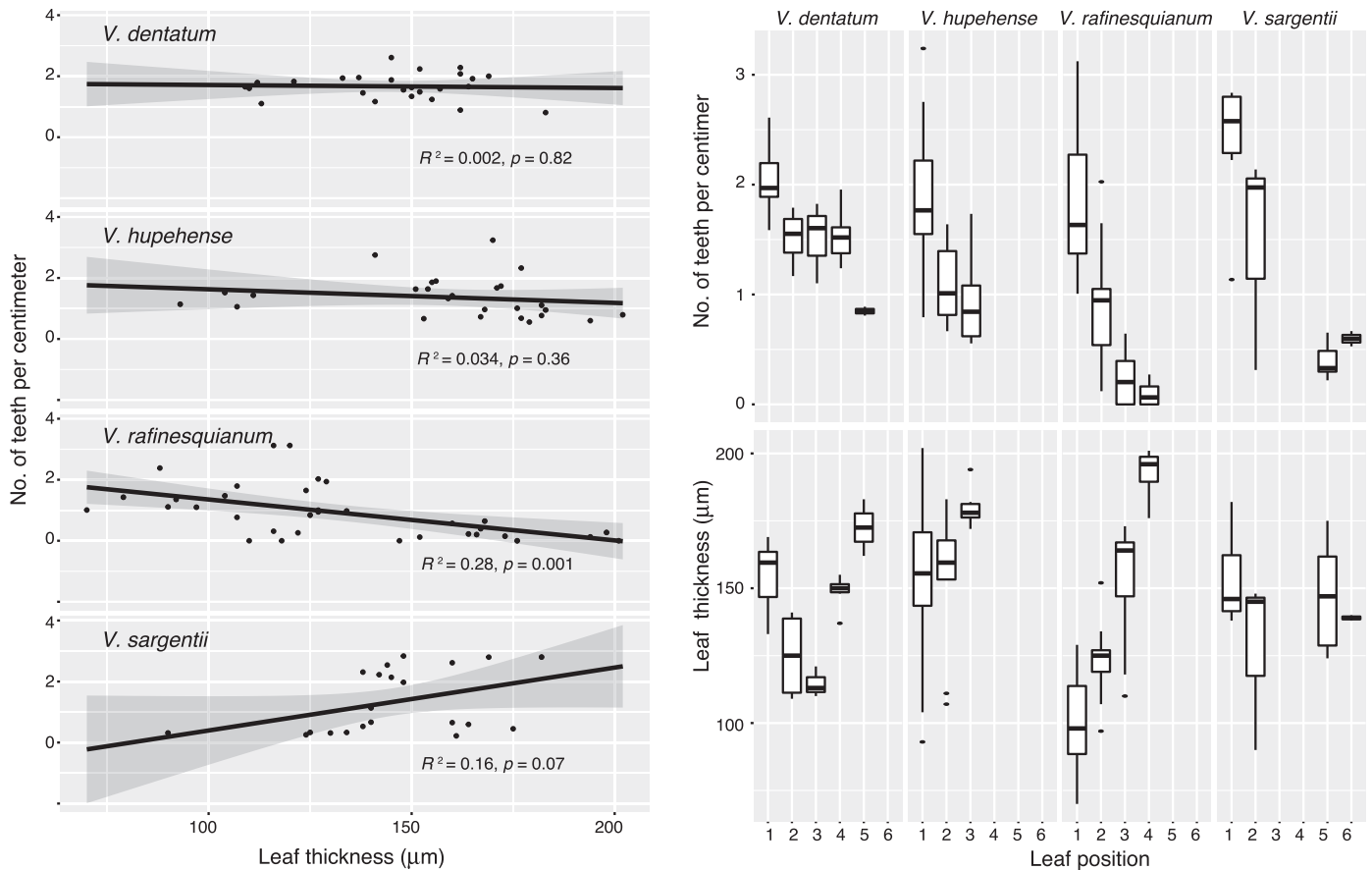
While we agree that this problem needs more attention, some relevant theory and evidence already exists. In the past 15 years, we have witnessed enormous progress in our understanding of leaf hydraulic conductance (K_{leaf}) and the major bottlenecks to water flow from petiole to stomata. In fact, the vein-cutting experiments that Givnish and Kriebel proposed have already been used to great effect, revealing the partitioning of hydraulic resistances to water flow into different segments of the leaf hydraulic pathway (Sack and Holbrook, 2006). Most studies agree that, though variable, the highest hydraulic resistance in leaves generally lies outside of the venation system, as water moves through the mesophyll to stomata (Sack and Frole, 2006; Brodribb et al., 2007). Extraxylary hydraulic conductance (K_{ox}) is influenced by many aspects of leaf mesophyll anatomy, including leaf porosity and cell wall thickness (Buckley et al., 2015), and the relationship between K_{ox} and leaf thickness is surely complex. On the one hand, thicker leaves might allow for more parallel pathways through the apoplast of cell walls, which we interpret as the main argument of the SS model; yet thinner leaves should have a *shorter* hydraulic path length from vein ending to substomatal cavity, which would actually lead to *higher* K_{ox} than in thicker leaves. In a recent modeling study, Buckley et al. (2015) discovered that venation density was the single most important driver of K_{ox}, but that shortening the hydraulic path length was the second most effective way to increase K_{ox}. In other words, all else being equal, thinner leaves should have a higher K_{leaf} than thicker ones. If these results prove robust, they seem to directly contradict a central assumption of the SS model (see fig. 6 of Givnish and Kriebel [2017]).

Here we present three additional preliminary lines of evidence that further question the validity of the SS model. First, according to Givnish and Kriebel (2017, p. 365): "Given that repeated shifts from non-entire to entire leaf margins across species in *Viburnum* are associated with increases in leaf thickness, it would be surprising if developmental shifts within species involving the same leaf forms were not also associated with increases in leaf thickness." This is eminently testable in *Viburnum* since many species exhibit seasonal heteroblasty in which the "preformed" leaves (that initiate development inside of overwintering buds) are toothier or more lobed than the "neoformed" leaves (that are formed later in the season, outside of a resting bud). Givnish and Kriebel (2017) predicted that the preformed, toothed leaves should be thinner than the neoformed, entire-margined leaves. We can clearly reject this prediction in a preliminary analysis of four heteroblastic *Viburnum* species (Fig. 3). In three of these species, we found no significant shifts in leaf thickness in spite of a marked reduction in leaf teeth along a branch. In a fourth species, *V. rafinesquianum*, we found the pattern predicted by the SS model, but in this case we also noted a shift in light environment along the branches that we examined, with the late-season neoformed leaves in full sun, and the preformed leaves more shaded within the canopy. Taken together, these results indicate that the realized thickness in these relatively thin-leaved, deciduous species is variable, but does not have much to do with leaf margins. Perhaps, instead, it is largely determined by other (long recognized) environmental factors, such as light intensity.

Our second line of evidence relevant to the SS hypothesis concerns the assumption, discussed above, that extraxylary hydraulic resistance is higher in thin leaves (Givnish and Kriebel, 2017: fig. 6),

FIGURE 3 The relationship between leaf thickness and toothiness in leaves produced along individual branches in four seasonally heteroblastic species of *Viburnum* growing in the Arnold Arboretum of Harvard University (Jamaica Plain, Massachusetts, USA). Left panel: plotting all measured leaves for each species, only *V. rafinesquianum* shows the relationship predicted by Givnish and Kriebel (2017; see text). Right panel: changes in leaf thickness and toothiness as a function of the location of each leaf (1 = first pair of leaves produced on the branch, 2 = second pair, etc.).

which would create hydraulic stress in the lamina near the leaf margin such that the formation of a sinus (which results in marginal teeth) would be optimal. In our recent paper on *Viburnum* leaf hydraulics (Scoffoni et al., 2016), we showed that the proportion of hydraulic resistance outside of the xylem network is *highly* variable, even across a small number of species. Indeed, K_{ox} varied nearly 40-fold among species and was a primary determinant of whole leaf hydraulic conductance; yet K_{ox} appears to vary quite independently of leaf thickness in *Viburnum* (Fig. 4). We note that Buckley et al. (2015) also failed to recover a significant relationship between K_{ox} and leaf thickness in their empirical dataset. Again, it appears that a key assumption of the SS model is not supported.

Third, we present preliminary data that pertain directly to the perceived biomechanical limitations of thin leaves and the independence of the regions of leaf tissue bounded by secondary veins. Sack et al. (2012) demonstrated a remarkable relationship between leaf size and major vein density across flowering plants: overall, larger leaves have more sparsely spaced secondary veins. Our preliminary analysis of this relationship in *Viburnum* strongly supports this relationship, but with an important caveat: species with secondary veins ending in marginal teeth have significantly higher major vein densities (i.e., more closely spaced secondary veins) for a given leaf size than do entire-margined species (Fig. 5). This result highlights an important detail not considered in the SS model, namely, that plant

species can and do vary in their vein density, so that the areas of mesophyll between major veins can vary tremendously in size. The relative size of "independent" mesophyll zones is not addressed in the original Givnish (1979) model, nor in the recent commentary, and it seems to us to be an open and potentially important area of inquiry. There are already some relevant studies, such as the vein-cutting experiments of Sack et al. (2008), which demonstrate how major venation patterns influence the extent of functional redundancy (and consequently, the non-independence of mesophyll regions), seemingly quite independently of leaf thickness.

We turn now to the BP hypothesis, which focuses attention on the possibility that teeth and/or lobes are more directly connected to what is happening inside of resting buds than to adult leaf function. Givnish and Kriebel (2017) argued against this on several grounds: (1) the papers we cited on packing might explain leaf lobes, but not teeth; (2) some plants (e.g., late-summer herbs) produce teeth without producing resting buds; (3) we provided no mechanistic explanation of why teeth would promote better packing or more rapid unfolding in the spring; (4) even if we were to find a developmental link between teeth and packing, this link would not explain the *adaptive value* of teeth; and (5) our explanation might not be necessary because there is a strong correlation between leaf thickness and margins.
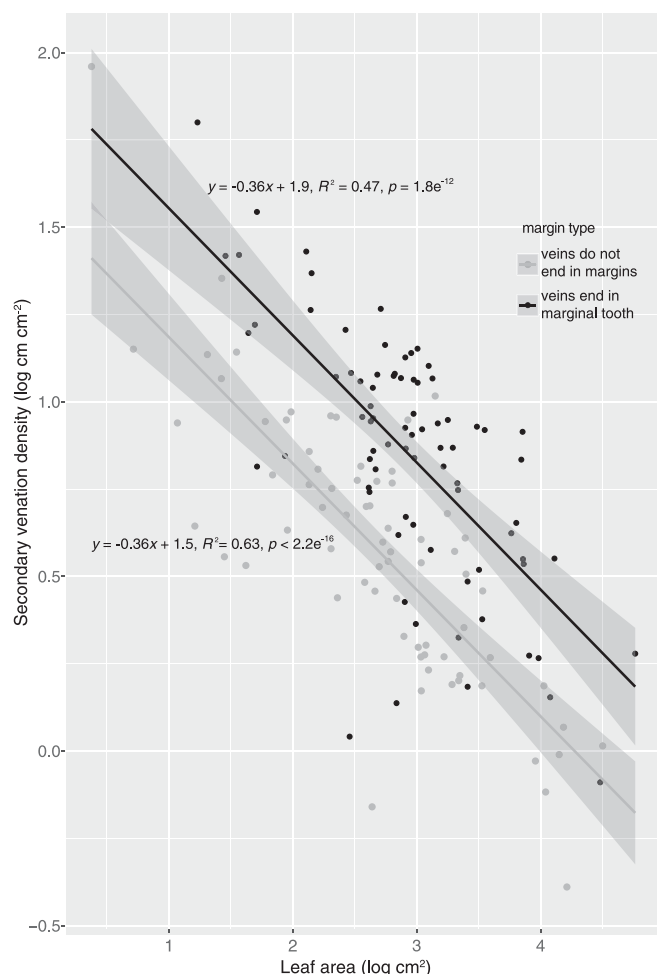
Three of their points are quickly dismissed. They are incorrect on the first point (1): Couturier et al. (2011) and Kobayashi et al. (1998)

$R^2 = 0.011$, $p = 0.69$
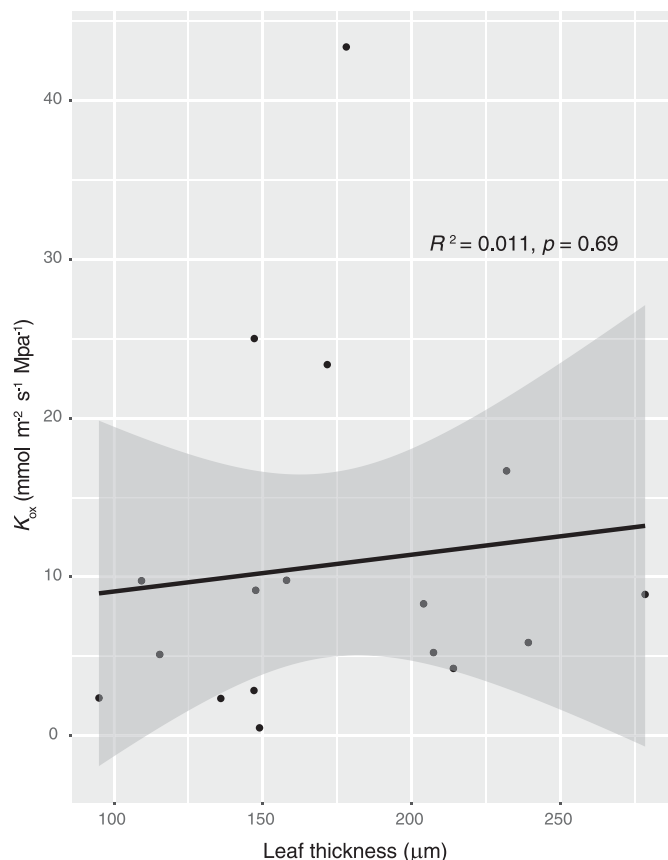
FIGURE 4 Givnish and Kriebel (2017) predicted that extraxylary leaf hydraulic conductance would be lower in thin leaves and higher in thick leaves. For the 16 species of *Viburnum* studied by Scoffoni et al. (2016), there is no significant relationship between K_{ox} and leaf thickness.

$y = -0.36x + 1.9$, $R^2 = 0.47$, $p = 1.8e^{-12}$

margin type
veins do not end in margins
veins end in marginal tooth

$y = -0.36x + 1.5$, $R^2 = 0.63$, $p < 2.2e^{-16}$

FIGURE 5 In 66 *Viburnum* species with toothed leaf margins, the density of secondary veins is higher than in 63 *Viburnum* species with entire margins across a wide range of leaf areas.

did include toothed margins in their models; e.g., they both considered *Fagus* in detail. Regarding point (2), we have already emphasized our view that teeth have arisen for multiple reasons. We focused on woody plants in moist tropical and temperate forests, and not on herbs, arid environments, etc., where very different explanations are potentially called for. We addressed point (5) above: if empirical data are consistent with more than one model, then these data do not help us to choose among them.

Their points (3) and (4) are more interesting. How or why might toothy margins relate to the development of leaf primordia inside of overwintering buds and/or rapid expansion during the spring flush? Here we limit our discussion to craspedodromous leaves in which each secondary vein ends directly in a marginal tooth. Such leaves are especially common in temperate deciduous plants, and are also the focus of the SS model. In our admittedly limited experience, in many craspedodromous leaves the leaf primordia in a bud largely appear to consist of major veins, often with long finger-like projections that will eventually become the teeth, and with very little lamina developed in between (Fig. 6). Depending on phyllotaxis and the way leaves are folded and arranged inside of buds, the vein endings from separate leaves at this early stage sometimes interdigitate with one another and facilitate closer packing around the dome-shaped apical meristem and any additional leaves or flower/inflorescence primordia that are present.

After a long period of arrested development, leaf growth gears up again quickly with budburst. Few detailed comparisons have been made of the rate of leaf expansion in deciduous plants, and we know of none that specifically compare emergence in relation to leaf form. In the study by Lopez et al. (2008), there may be a hint that species with compound, lobed, or toothed leaves show faster leaf expansion (mean of ~28 days) than those with entire leaves (mean of ~42 days), but clearly much more study is needed. As Givnish and Kriebel (2017) noted, *Fagus*, with its craspedodromous venation and plicate folding, was the fastest to emerge in the Lopez et al. (2008) study, and this motivated Givnish and Kriebel to include a connection between plicate venation and rapid expansion in their synthetic model (see their fig. 6). If plication is adaptive (as Givnish and Kriebel themselves propose), and if toothy margins are simply a byproduct of plicate folding (as suggested by the Couturier et al. [2011] model), then, so long as teeth are not positively maladaptive, further explanation of the existence of teeth may be unnecessary.

Another open question is the relative contribution of cell division and cell expansion to leaf emergence. If most of the rapid spring growth is due to cell expansion, then it stands to reason that
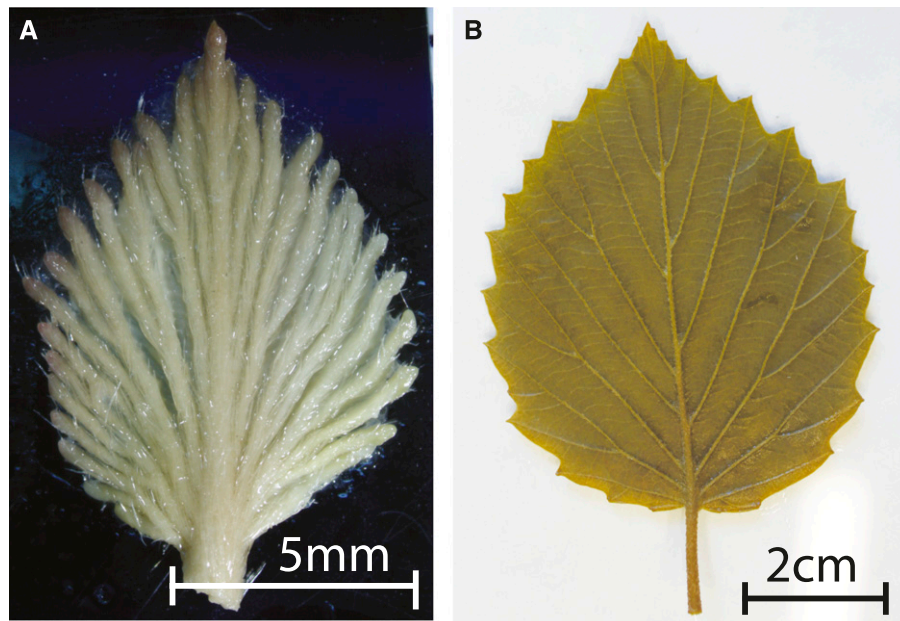
FIGURE 6 *Viburnum dentatum* leaves at two stages of development. (A) Leaf primordium obtained from a resting bud, showing prominent secondary veins ending directly in elongate marginal teeth. (B) Mature leaf showing expanded leaf lamina between the secondary veins and the shape of the mature teeth.

the final leaf form will to some extent reflect the form of the leaf primordium in the resting bud. One possibility is that the "filling in" of tissue never catches up with the elongate vein endings, which then results in a tooth of some size and shape in the mature leaf. Of course, the persistence of meristematic regions in the developing lamina after budbreak could alter this dynamic and effectively override any influence of primordium form. Several other factors that influence the development of leaf primordia could have residual effects on mature leaf form. For example, the tight packing of multiple organs inside a small bud generates physical contacts and stresses between separate leaf primordia and between leaf primordia and bud scales. We imagine that these forces can influence primordium shape and, in turn, mature leaf form (Couturier et al., 2012). An additional factor is the role of auxin and its transport through the venation system. If teeth are long-term auxin accumulators, their rates of growth may simply exceed the rest of the tissues.

These speculations highlight two important points. First, we know far too little, for most species, about the morphology or development of leaf primordia within buds, about leaf expansion rates, or about the mechanisms underlying many aspects of development that are directly relevant to this discussion. Second, the traditional "how" (proximate mechanism) vs. "why" (ultimate evolutionary cause) distinction emphasized by Givnish and Kriebel (2017) is blurred in some of what we have said—not because we misunderstand the basic distinction, but rather because the "how" questions actually do bear on the "why" questions in pointing us toward the attributes that actually require adaptive explanation. It is possible, for example, that teeth in mature leaves do not really require an adaptive explanation per se; they may simply be developmental manifestations or epiphenomena of selection acting on different attributes (e.g., craspedodromous secondary venation) or at another level of organization (efficient bud packing). Maybe, as Gould and Lewontin (1979) stressed long ago, we have yet to properly "atomize" leaf features,

and, consequently, may be focusing our explanations at the wrong level altogether.

A directly related problem is that there may be multiple adaptive explanations that focus on different levels of organization or different life stages. Givnish and Kriebel (2017) themselves provided a fine example of this issue. At one point, they asserted that the adaptive significance of lobed leaves is to minimize the cost of supportive tertiary veins, but elsewhere they appear to accept that the deep sinuses in lobed leaves allow for more efficient folding of the lobes within bud. So, which is it? Or, maybe selection has acted at both levels? To sort out this question, we will need to push beyond simple correlations. The "how" vs. "why" distinction has great heuristic value, of course, and is wonderful for teaching, but in practice the most satisfying evolutionary explanations will integrate across this conceptual divide, ultimately finding consilience in optimality, genetic, developmental, population-level, and phylogenetic evidence (Olson and Arroyo-Santos, 2015).

In summary, we have explained why we doubt the SS model and have addressed the arguments presented by Givnish and Kriebel (2017) against the BP hypothesis. Amassing further evidence of the correlation between leaf thickness and leaf margins will not be helpful in choosing between these two hypotheses, as this relationship is fully consistent with both. Instead, progress requires that we critically test the underlying assumptions of these alternative models. We have presented preliminary data that bear on key assumptions of the SS model, but much more work is needed along these lines. With respect to the BP hypothesis, we stand by the arguments of Edwards et al. (2016), namely, that we need much more detailed analyses of what is going on inside of resting buds—exactly how leaf primordia are folded (ptyxis) and folded around one another (vernation), the efficiency of the packing of different forms of leaf primordia, developmental trajectories of leaves inside and outside of buds, and the consequences that all of these factors have on the form of mature leaves. We are certain that such analyses will be productive, regardless of whether the bud packing explanation turns out to be correct. In any case, we emphasize that we proposed the bud packing hypothesis because we think it bears on the latitudinal gradient first highlighted by Bailey and Sinnott (1916), not because we think it can explain every leaf with non-entire margins. Quite the contrary, the very idea of a single explanation is misguided. Leaf teeth have undoubtedly arisen for many different reasons in different lineages and environments and likely also through different developmental mechanisms. And as Darwin (1859, p. 490) famously noted, "There is grandeur in this view of life."

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