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Leaf Form Evolution in *Viburnum* Parallels Variation within Individual Plants

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ABSTRACT: Few studies have critically evaluated how morphological variation within individual organisms corresponds to variation within and among species. Subindividual variation in plants facilitates such studies because their indeterminate modular growth generates multiple serially homologous structures along growing axes. Focusing on leaf form, we evaluate how subindividual trait variation relates to leaf evolution across *Viburnum*, a clade of woody angiosperms. In *Viburnum* we infer multiple independent origins of wide/lobed leaves with toothed margins from ancestors with elliptical, smooth-margined leaves. We document leaf variation along the branches of individual plants of 28 species and among populations across the wide range of *Viburnum dentatum*. We conclude that when novel leaf forms evolved in *Viburnum*, they were intercalated at the beginning of the seasonal leaf sequence, which then generated a repeated spectrum of leaf forms along each branch (seasonal heteroblasty). We hypothesize that the existence of such a spectrum then facilitated additional evolutionary shifts, including reversions to more ancestral forms. We argue that the recurrent production of alternative phenotypes provides opportunities to canalize the production of particular forms and that this phenomenon has played an important role in generating macroscale patterns.

Keywords: Viburnum, heteroblasty, leaf shape, phylogeny, phenotypic plasticity.

Introduction

Much attention has been focused on whether and how phenotypic plasticity might influence adaptive evolution, speciation, and macroevolutionary patterns (e.g., Schlichting and Pigliucci 1998; Price et al. 2003; West-Eberhard 2003; Pfennig et al. 2010; Moczek et al. 2011; Wund 2012). One popular idea is that the recurrent plastic expression of a novel phenotype may precede the genetic changes that regularize or internalize the developmental control of the production of that phenotype (Waddington 1957; Schlichting and Wund 2014). Under this phenotype-first model, traits that are plastic in an ancestral lineage can be fixed or elaborated in descendant species through genetic accommodation (West-Eberhard 2003). The plausibility of this model of evolution is a key point of dissension in an ongoing debate over the adequacy of traditional evolutionary theory (e.g., see Laland et al. 2014; Wray et al. 2014). At issue is also the question of how developmental regulation can make some evolutionary transitions more likely than others, that is, how development influences evolvability. The evolution of a plastic developmental pathway can bias the production of phenotypic variation, making certain outcomes more likely than others (Riedl 1978). Ultimately, understanding when and how phenotypic plasticity can influence macroevolution will help us determine how novel traits evolve, how and why particular phenotypes evolve recurrently, and the extent to which we can accurately predict phenotypic evolution.

Although the conceptual theory surrounding the phenotype-first model is well developed, clear empirical examples are rare and often not fully explored. Here we focus particularly on a phylogenetic perspective, which we suggest has been underutilized. The phenotype-first model of evolution should leave a distinct phylogenetic signature: trait divergences among species within a clade should parallel plastic variation within the species of that clade. Is this a pattern that we observe over deep timescales? To date, phylogenetic comparisons of this sort have been made in only a few clades and then with relatively few species (Losos et al. 2000; Gomez-Mestre and Buchholz 2006; Wund et al. 2008; Allf et al. 2016). The paucity of phylogenetic studies likely reflects the difficulty of collecting the appropriate intraindividual data for an adequate number of species. In vertebrates, for example, the relevant traits are often behavioral or developmental and nearly impossible to measure outside of controlled laboratory settings.

Plants, by contrast, are generally far more amenable to studies of the relationship between phenotypic plasticity and trait evolution across multiple scales. This is because

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morphological variation that arises during the course of development of an individual plant is preserved in a series of iterated structures. The modular indeterminate growth of plants generates spatially and temporally structured variation that is repeatedly and predictably exposed to selection. Leaves, for instance, vary from node to node along the axes of a plant because of factors such as the position of the leaf on the branch (e.g., Critchfield 1960), the developmental status of the plant (e.g., Kaplan 1980), and environmental cues such as sunlight or temperature (Taiz and Zeiger 2002). Herrera (2009, p. 339) argued convincingly that this type of "sub-individual variability is part of the essence of being a plant" and that it is an underestimated force in both ecology and evolution.

Our study of a diverse group of flowering plants was designed specifically to compare broadscale phylogenetic trends of trait evolution with the phenotypic variation observed within and among populations but especially within individual organisms. We focus here on leaf form because this varies considerably and predictably within individual organisms, among populations within species, and among species within clades. Leaf variation within individual plants is typically classified on the basis of whether it is driven by internal or external cues (Nicotra et al. 2011; Zotz et al. 2011). "Heterophylly" is the term applied to plastic leaf form variation that is induced by the local environment of a leaf, such as differences between sun and shade leaves or between leaves of aquatic plants produced above and below the water. In contrast, the term "heteroblasty" is used for shifts in leaf form that are due to regular changes that take place through the course of development, such as the sometimes dramatic differences associated with the transition from juvenile to mature life stages in some species of *Acacia* (Kaplan 1980), *Hedera* (Goodin 1965), and *Eucalyptus* (Zotz et al. 2011). Subtler forms of heteroblasty are evident within many plants in the form of constitutive variation associated with the position of a leaf along a shoot (Ashby 1948; Critchfield 1960; Chitwood et al. 2016). In many cases, however, leaf form is affected by both external and internal factors simultaneously, and these can be differentiated only with careful experiments (Lee and Richards 1991; Jones 1995; Winn 1996). We do not attempt to separate these effects here and simply refer to consistent variation from node to node over the course of a growing season as seasonal heteroblasty (Jones 1999).

Seasonal heteroblasty is a widespread phenomenon in plants and can be remarkably consistent both within individuals and within species. In some cases, individual plants constitutively produce a repeated spectrum of leaves along each branch, a variety of leaf forms that can potentially be targeted by selection. It is easy to imagine that leaf evolution might occur by reducing the range of forms expressed along an axis by canalizing the production of a particular leaf type, perhaps shifting toward the production of leaves at one end of the spectrum or the other. Although seasonal heteroblasty has been characterized in a number of species, it has been directly compared with leaf form diversity among species to a limited extent only in grape vines (*Vitis*; Chitwood et al. 2016).

Here we document leaf form variation at multiple levels in the plant clade *Viburnum* to try to understand the origins of macroevolutionary trait diversity. We first analyzed leaf form across 105 species, identifying broad phylogenetic trends in *Viburnum* leaf evolution. We then evaluated leaf variation within 28 different *Viburnum* species, quantifying seasonal heteroblasty along individual branches. We focused specifically on three species native to eastern North America—*Viburnum acerifolium*, *Viburnum rafinesquianum*, and *Viburnum dentatum*—that were collected from multiple populations across their geographic ranges. Finally, we compared these results with leaf form variation within and among populations in the widespread *V. dentatum* species complex. Overall, we discovered a striking parallelism across these levels, fully consistent with the idea that variation first expressed within individual plants may have facilitated leaf evolution across the clade.

Methods

Study Organisms

Viburnum (Adoxaceae) is a flowering plant clade of approximately 165 species of shrubs and small trees. Most *Viburnum* species today occur in Northern Hemisphere temperate forests, although there are around 20 species (in multiple clades) distributed in tropical forests in Southeast Asia, and the *Orienotinus* clade (with around 36 species) occupies cloud forests in Central and South America (Clement and Donoghue 2011; Clement et al. 2014; Spriggs et al. 2015). Recent phylogenetic studies have proposed a tropical or subtropical origin for *Viburnum*, with as many as 10 subsequent transitions into cool-temperate environments (Clement and Donoghue 2011; Clement et al. 2014; Spriggs et al. 2015; Edwards et al. 2017*a*). A previous study of leaf form in *Viburnum* demonstrated that shifts into the temperate zone were associated with the evolution of round and/or lobed leaves, leaf teeth, and the deciduous leaf habit (Schmerler et al. 2012). The phylogenetic correlation between leaf form and leaf habit (evergreen vs. deciduous) is exceptionally strong, and we have suggested that there may even be a mechanistic connection between the appearance of complex leaf shapes and the packing of many partially developed preformed leaves inside of overwintering buds (Edwards et al. 2016).

Extensive field observations indicate that heteroblasty in *Viburnum* is closely linked with growth architecture and based more on leaf position along the branch than on the precise timing of leaf emergence (for a more detailed discussion of *Viburnum* architecture, see Edwards et al. 2014). Throughout this study, we therefore classify leaves on the basis of position (fig. 1). We refer to the leaf positions as early and late, following Critchfield (1960), who was among the first to systematically document seasonal heteroblasty. In *Viburnum*, we refer to the pair of (opposite) leaves at the first node as the early leaves; these are preformed in the resting bud, and they emerge rapidly in a spring leaf flush. We refer to the leaves at the second node as intermediate. These intermediate leaves are less well developed inside the bud, and they expand as part of the initial leaf flush. What we refer to as the late leaves appear from the third node onward on branches that continue to grow after the initial leaf flush. They are not (or only barely) developed within the bud and are therefore neoformed during the growing season. These late leaves are most common on fast-growing shoots and young plants. They are rare on most mature plants and even rarer on branches that bear inflorescences, and hence they are rarely present on herbarium specimens. A random sample of leaves from an adult *Viburnum* plant will contain almost exclusively early and intermediate leaves. In this study, we focus on documenting the degree of seasonal heteroblasty exhibited by species from eight distinct temperate deciduous lineages and sampled specifically to collect as many leaf positions as possible. While we have not yet formally demonstrated the absence of seasonal heteroblasty in the more tropical evergreen viburnums, our extensive field observations indicate that heteroblasty is absent or very limited in these species. Hence, seasonal heteroblasty appears to have originated along with the evolution of the deciduous habit in multiple lineages.

We chose the *Viburnum dentatum* species complex to quantify leaf form variation within and among populations across its geographic range. This clade is variable in many leaf traits and occurs more or less continuously along the east coast of North America (from northern Florida to southeastern Canada) and west into Texas, Arkansas, and Illinois (Kartez 2015). We refer to this as a species complex because the taxonomy of this group has been controversial, with some authors describing it as one or two species (e.g., Gleason and Cronquist (1991) and others splitting it into as many as seven (McAtee 1956; Weakley et al. 2011). All phylogenetic analyses to date have strongly supported the monophyly of this complex (Clement et al. 2014; Spriggs et al. 2015; Eaton et al. 2017).

Evolution of Leaf Form in Viburnum

Much of the data for the *Viburnum*-wide analysis were derived from Schmerler et al. (2012), incorporating 24 leaves from each of 81 species. For the two species (*V. dentatum* and *Viburnum lantana*) that were represented multiple times in Schmerler et al.'s (2012) study, we randomly sampled 24 leaves from a single accession. For the current study, we increased the taxon sampling by 24 species, including six to 24 leaves from herbarium specimens for each new spe-

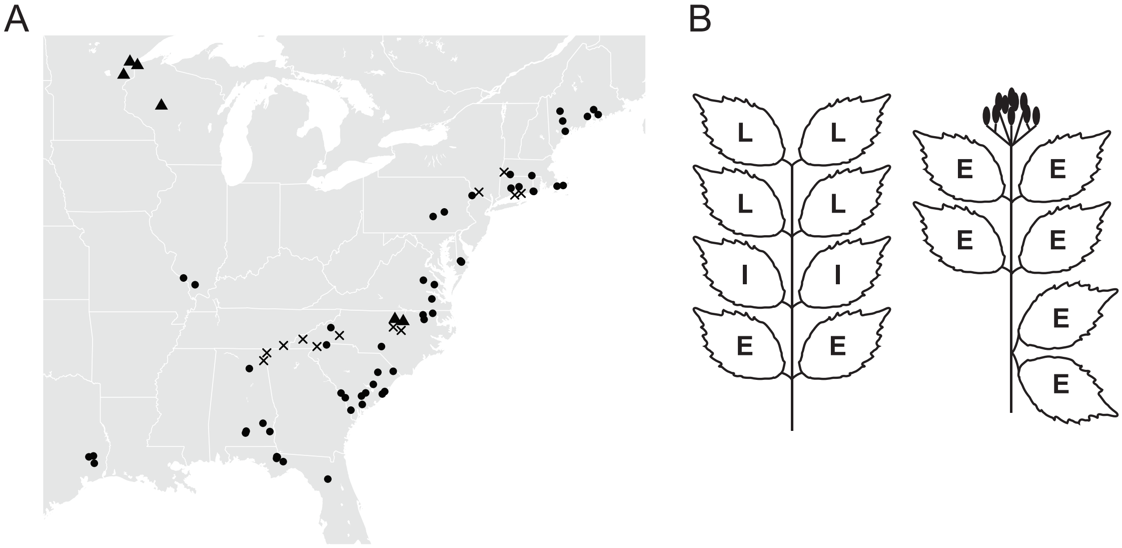

Figure 1: Sampling design to survey leaf form variation among populations and within individuals. *A*, Locations of collected populations of *Viburnum dentatum* (circles), *Viburnum rafinesquianum* (triangles), and *Viburnum acerifolium* (X's). *B*, Leaf position classification scheme used for all heteroblasty analyses shown on a vegetative branch (*left*) and an inflorescence branch (*right*). The first-position leaves are early (E), the second-position leaves are intermediate (I), and third-position leaves and beyond are late (L). We classified the pair of leaves directly subtending the inflorescence as early because these are preformed in bud and emerge early in the season.

cies. Our final data set contains 2,235 leaves from 105 *Viburnum* species. We note that leaf position is not scored in this data set; however, because all leaves were collected from mature plants or herbarium specimens, we are confident that these are early or intermediate leaves.

Leaf area was measured from full leaf outlines in ImageJ (http://rsbweb.nih.gov/ij), and the number of leaf teeth per centimeter of leaf margin (in the middle of the leaf) was counted using the ObjectJ (http://simon.bio.uva.nl/objectj) plugin for ImageJ. In both cases, we log-transformed these measurements for our *Viburnum*-wide analysis. To characterize the major axes of *Viburnum* leaf shape variation, we first performed elliptical Fourier analysis (Kuhl and Giardina 1982) both on all 2,235 leaves and on a data set subsampled to standardize the number of leaves per species (630 leaves). The contours of leaf images were converted to chain code in SHAPE (Iwata and Ukai 2002) and imported into the R package Momocs (Bonhomme et al. 2014) for all subsequent processing. To ensure proper alignment of leaf outlines, two homologous landmarks were defined on each leaf, one at the point of attachment of the petiole and a second at the leaf tip. Shape analyses were then conducted independent of leaf size using 30 harmonic descriptors, and principal component analysis (PCA) was used to generate a morphospace. For a more detailed description of this methodology, see Bonhomme et al. (2014).

To directly compare leaf shape across different data sets (within species, across *Viburnum*), we constructed an external reference data set made up of a series of a priori morphospaces against which we scored all leaves. This is essentially a form of rapid phenotyping that can be used to quantify leaves along predetermined axes of shape variation that remain constant. We constructed three a priori axes of variation (fig. 2): the first describes the length : width ratio or sphericity (henceforth, width), ranging from a narrow to a wide ellipse. The second axis describes the center of balance of a shape, from ovate to obovate; and the third is an axis of lobing. The choice of these axes was informed by morphospaces constructed with more typical PC analyses of *Viburnum* data sets that included leaves from single species or from across *Viburnum*. Our aim was to create roughly orthogonal axes that each describes a distinct aspect of leaf shape (fig. A1; figs. A1–A11 are available online). The major difference between our a priori axes and those constructed with traditional PCA is that lobed leaves, which have a relatively extreme shape, here do not directly impact the quantification of the more subtle leaf variation captured in a priori axes 1 and 2. Instead, leaf lobing is separated as a distinct axis that is correlated with the others in that lobed leaves also tend to be wide and ovate.

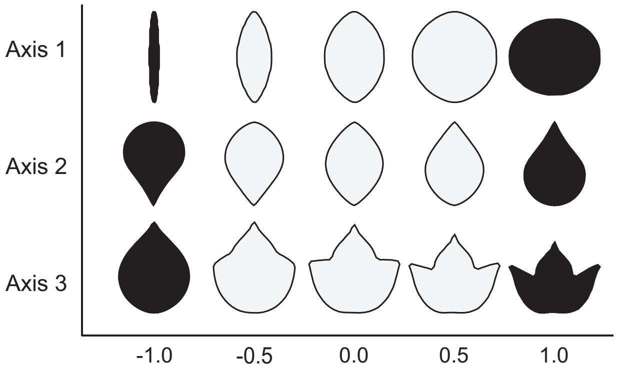

Figure 2: A priori axes used to quantify leaf shape. In each row, the two reference shapes used to generate each axis are black, while intermediate eigenleaves generated in Momocs are gray. *X*-axis shows standard deviations along the principal component axis.

To construct the a priori axes, two reference shapes—for example, a narrow ellipse and a wide ellipse (fig. 2)—were each copied 500 times. Elliptical Fourier analysis of only these reference shapes necessarily finds that 100% of the shape variation is described by PC1, an axis of variation from a narrow to a wide ellipse. We sequentially added each *Viburnum* leaf to this 1,000 ellipse data set, performed elliptical Fourier and PCA, and saved the PC1 score of the leaf. Because there are 1,000 ellipses and only one *Viburnum* leaf in each analysis, PC1 remains essentially unchanged, and each leaf is scored on the basis of where its shape lies along the axis between the two reference shapes. We conducted similar analyses for an axis of ovate to obovate shapes and for lobed to unlobed shapes (fig. 2).

This a priori axis method deviates from the standard practices used to analyze morphological data sets, but we argue that it is highly useful for comparing complex traits across data sets. Like many morphological traits, leaf shape is most often measured with either elliptical Fourier analysis or Procrustes aligned landmarks (Schmerler et al. 2012; Chitwood et al. 2016), both of which require PCA to construct the major axes of variation. These analyses can be sensitive to uneven sampling, and, more importantly, they generate data-dependent axes of variation. The three a priori shape axes that we use here capture major shape variation within *Viburnum* and allow us to quantify the variation within individuals with the same metric used across species.

We note that some leaves were found to have PC1 scores more extreme than the reference shapes. On the unlobed to lobed axis, for example, a narrow unlobed leaf could have a lower PC1 score than the unlobed reference leaf (a priori axis 3 score <-1.0 in fig. 2). When this happened, the upper or lower extreme of PC1 reflected specific characteristics of the leaf in that particular analysis; for instance, a leaf that is narrower than the reference leaf on a priori axis 3 will have an extreme score. To avoid comparing extremes that may or may not otherwise be similar, we imposed the PC values of the reference leaves as strict upper and lower limits and assigned leaves with scores that were <-1.0 to the -1.0 category and scores >1.0 to the 1.0 category. Thus, in the previous example, the narrow unlobed leaf would be assigned -1.0, the same PC1 value as the unlobed reference leaf. Extreme scores were most common along a priori axis 3 (scores for 1,837 leaves were constrained), relatively rare for a priori axis 2 (141 leaves constrained), and entirely absent for a priori axis 1 (0 leaves constrained). This practice also helped to minimize the overlap across our a priori axes. Leaf data and code for this a priori morphospace analysis is deposited in the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.15249 (Spriggs et al. 2018).

Phylogenetic Analyses

Our phylogenetic analyses were conducted using the maximum clade credibility tree from Spriggs et al. (2015) with the crown age set to 1. Using the R package APE (Paradis et al. 2004), this tree was pruned from 138 tips to 105 tips to match our leaf form data set. We used the a priori morphospace technique to score all *Viburnum* leaves on our three predetermined axes of shape variation. We then performed maximum likelihood ancestral state reconstructions in the R package Phytools (Revell 2012) using simple species means for the a priori shape axes, leaf teeth, and leaf area. Most species in this *Viburnum*-wide data set are represented by a single individual, and we did not account for leaf position or individual in the calculation of species mean traits; however, the full range of variation within each species is plotted in all figures. Because this *Viburnum*-wide data set is primarily composed of early and intermediate leaves, this analysis specifically infers ancestral forms for leaves that were preformed to some degree within resting buds.

Heteroblasty in Three Focal Viburnum *Species*

Between June and October in 2013, 2014, and 2015, leaves were collected for *V. dentatum*, *Viburnum rafinesquianum*, and *Viburnum acerifolium* from locations spanning the Eastern United States (fig. 1*A*; table A1; tables A1, A2 are available online). At least 10 leaves per plant and up to six individuals per population were collected in the field and scanned using a Cannon LiDE110 color image scanner. Leaves with minor insect damage were repaired in Photoshop (http://www.adobe.com) and used for shape analyses, but these were excluded for leaf area and leaf tooth measurements. Drawings made in the field documented the position of each leaf, which we classified as early, intermediate, or late (fig. 1*B*). In total, 1,532 leaves were used for *V. dentatum*, 362 for *V. rafinesquianum*, and 285 for *V. acerifolium*. Voucher specimens from all populations are deposited in the Yale University Herbarium. To quantify the teeth in these species more precisely, all leaf teeth were counted along one side of each leaf in ObjectJ. These tooth counts were standardized by an approximate perimeter of the same side of the leaf, modeling an entire margin by connecting the tooth sinuses from the petiole to the leaf tip. For each of the three focal species, we generated a species-specific morphospace in Momocs and also scored all leaves along our three a priori shape axes.

Heteroblasty across Viburnum

In July 2016, 30–60 leaves were sampled from individuals of 25 different *Viburnum* species growing at the Arnold Arboretum of Harvard University, Jamaica Plain, Massachusetts (tables A1, A2). Following the same protocol as above, all leaves were scanned and classified as early, intermediate, or late and were scored using the a priori shape axes. Most of the species sampled from the Arnold Arboretum are not native to Massachusetts but are from temperate regions in Asia with relatively similar climates. On the basis of our extensive field and herbarium studies of *Viburnum* in China and Japan, we are confident that the normal leaf shapes of these nonnative species have been maintained in the Arboretum.

Statistical Analyses

Testing for Seasonal Heteroblasty. To determine whether seasonal heteroblasty was significant for our three focal species, we treated each one separately. We generated morphospaces and PC axes of shape variation with the leaves collected for each species to visually assess leaf shape differences. We then performed linear discriminant analysis (LDA) using all leaf form variables for each species (leaf area, leaf tooth count, a priori axis 1, a priori axis 2, and (for *V. acerifolium*) a priori axis 3). For these analyses, we used a one-third prior probability for membership to each class (early, intermediate, or late) and leave-one-out cross-validation to calculate the percentage of leaves that could be correctly classified as early, intermediate, or late. We confirmed these estimates by creating training data sets for each species that contained a randomly sampled leaf for each position for each individual. We then classified the remaining leaves on the basis of the training data set model. This random sampling procedure was conducted 100 times for each species, and in each case, the range of correctly classified leaves is reported.

Within-Individual Variation in Multiple Viburnum *Species.* To test whether there were shifts within individuals in the same leaf traits that evolved across *Viburnum*, we focused on a priori axes 1 and 2. For each species, we used a one-way ANOVA to evaluate whether the means of the a priori axis scores were significantly different among the different leaf positions (early, intermediate, or late). For our three focal species, we tested for an effect of leaf position on tooth number and tooth number per leaf perimeter, traits that also vary among *Viburnum* species.

Relationship between Species Leaf Form and the Degree of Seasonal Heteroblasty. We used linear regression to test for a correlation between early leaf shape and the degree of seasonal heteroblasty displayed within individuals. For this analysis, we compared the average early leaf width of each species (i.e., the mean a priori axis 1 score for early leaves) to the range of a priori axis 1 scores for all leaves of the same species. We also fit a phylogenetic linear model for these data in the R package Phylolm (Ho and Ané 2014) using a phylogeny pruned down to match the 25 species in the Arnold Arboretum heteroblasty survey.

Variation across the Range of the Viburnum dentatum *Species Complex.* To assess leaf variation among populations, we focused on the *V. dentatum* complex, the only species in our data set that was sampled across more than 10 populations. Leaf variation within the complex was assessed for 22 populations, each containing three to six individuals. For this analysis, we excluded leaves with extensive insect damage, and individuals with fewer than 8 leaves were removed. The contribution of different levels to the total variance in leaf traits was estimated with intercept-only random effects models in the R package lme4 with REML estimation. We included leaf position nested within individual nested within population as random effects. We performed a second analysis with only early leaves, requiring five leaves per individual and three to six individuals per population. To meet these requirements, six individuals and one population were removed.

Results

Viburnum-*Wide Analysis*

The *Viburnum* morphospace constructed from all 2,235 leaves (fig. 3) was indistinguishable from the morphospace constructed with 630 leaves (six per species; fig. A2), and we subsequently consider only the larger data set. Both here and in the study by Schmerler et al. (2012), the first principal component (PC1) describes an axis that ranges from narrow to wide leaves and explains more than 75% of the total variation. Our second principal component (PC2) primarily represents a left-right axis that describes leaf asymmetry. There may be a good biological interpretation of PC2, possibly related to which side of the branch each leaf is on. However, because we did not have the data to test this, we excluded this axis from further consideration. The third axis (PC3), however, is more relevant and ranges from ovate to obovate shapes (fig. 3). Ancestral state reconstructions based on our a priori shape axes indicate that early *Viburnum* species likely had unlobed, relatively narrow, slightly ovate leaves (figs. 4, A3, A4). From this ancestral leaf type, lobed leaves appear to have evolved at least twice, and wide leaves with mean width (a priori axis 1) scores ≥.04 evolved up to 10 times. Distinctly wide leaves likely evolved along the branch subtending the *Pseudotinus* clade (*Viburnum nervosum* to *Viburnum furcatum* in fig. 4; clade names follow Clement et al. 2014; see fig. A5), the *Opulus* clade (*Viburnum edule* to *Viburnum sargentii* in fig. 4), and the *Porphyrotinus* clade (*Viburnum ellipticum* to *Viburnum seemenii* in fig. 4). In most other cases, however, notably wide leaves appear to have evolved relatively recently in singleton species (e.g., *Viburnum dilatatum*, *Viburnum molle*) or in small clades near the tips of the tree (e.g., *Viburnum carlesii* plus *Viburnum bitchiuense* within the *Euviburnum* clade; fig. 4). We also see cases of reversal to more narrowly elliptical leaves (e.g., *Viburnum integrifolium* from Taiwan within the *Succotinus* clade; *Viburnum hartwegii* and *Viburnum costaricanum* from Mexico and Central America within the *Oreinotinus* clade; fig. 4).

Our ancestral state reconstructions also show that early *Viburnum* species likely had medium-sized leaves with few leaf teeth (figs. A6, A7). Although leaf size varies by two orders of magnitude across *Viburnum*, with the largest leaves in the *Pseudotinus* clade and the smallest (e.g., *Viburnum utile*, *Viburnum parvifolium*, and *Viburnum loeseneri*) nested within several clades, we do not observe clear phylogenetic patterns in leaf size. Most early-branching *Viburnum* lineages are reconstructed as intermediate in size, with subsequent shifts occurring in both directions. In contrast, leaf teeth seem to have evolved multiple times across *Viburnum* from an entire or irregularly-toothed ancestor (fig. A7). Classifying species as either entire or toothed can be difficult in *Viburnum* because some species are mostly entire but produce teeth occasionally and irregularly. This mostly entire condition (evident in species such as *Viburnum clemensiae*, *Viburnum amplificatum*, *Viburnum taiwanianum*, and *Viburnum propinquum*) is inferred to be ancestral for *Viburnum*. From this starting point, we reconstruct ~10 origins of conspicuously and regularly toothed leaf margins.

Heteroblasty in Three Focal Viburnum *Species*

We generated separate morphospaces for each of the three focal species, using all of the leaves collected (figs. 5, A8). For *Viburnum dentatum* and *Viburnum rafinesquianum*, the intraspecific morphospace was nearly identical to the all-*Viburnum* morphospace. PC1 was again an axis of leaf width (containing 72.4% and 71.6% of the variation, respectively), PC2 captured right-left balance (15.1% and 16.0%), and PC3 described ovate-obovate variation (2.88% and 2.77%). There was a slightly different pattern in the lobed leaves of *Viburnum acerifolium*: PC1 (48.8%) ranged from narrow, unlobed

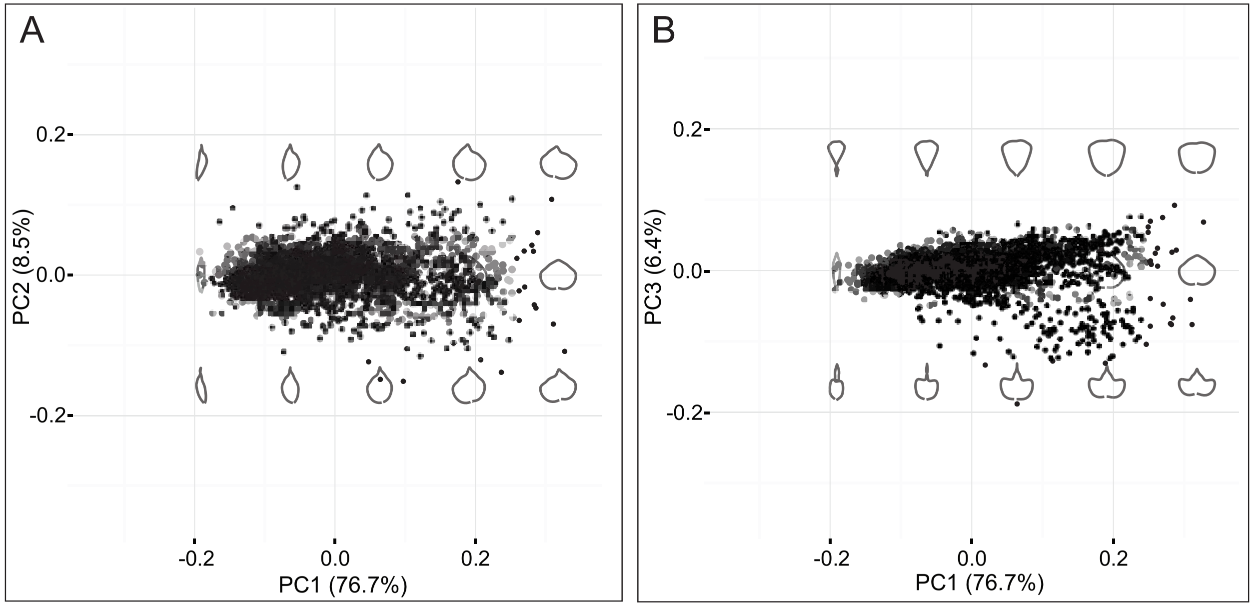

Figure 3: *Viburnum*-wide principal component analysis (PCA) and morphospace displays the major axes of leaf shape variation in *Viburnum*. *A*, Morphospace for principal component (PC) 1 and PC2. *B*, Morphospace for PC1 and PC3. Each point represents a single leaf, and eigenleaves are plotted in the background.

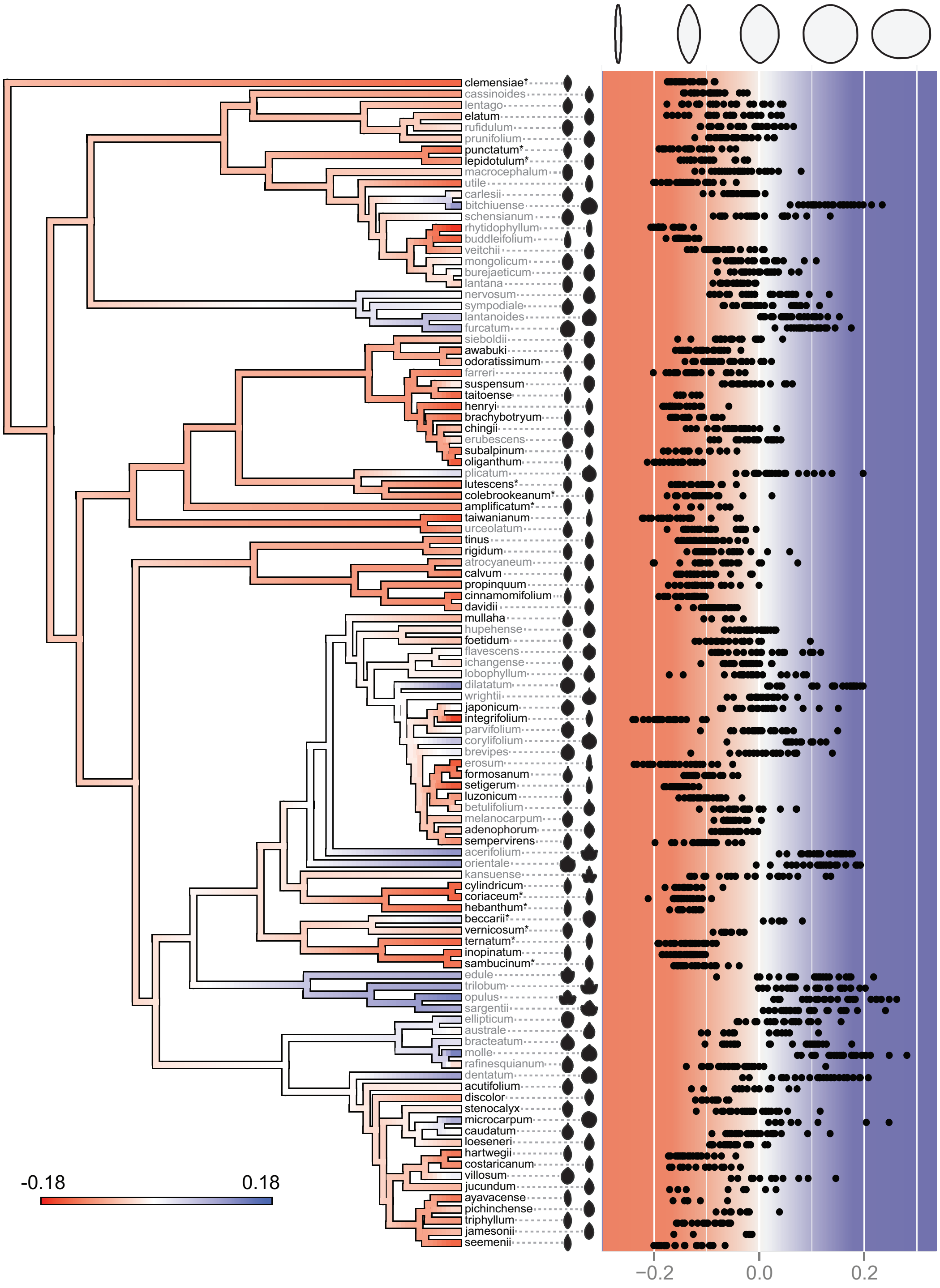

Figure 4: Wide leaf shapes evolved multiple times in *Viburnum* from a relatively narrow ancestor. *Viburnum* phylogeny showing inferred ancestral states for a priori axis 1. On the right, the a priori axis 1 score of each leaf is indicated with a circle; the mean leaf shape for each species (not to scale) is connected by dashed lines to the corresponding species name. Branches are colored on the basis of inferred a priori axis 1 value (color scale on bottom left). Species occupying tropical forests in Southeast Asia are marked with an asterisk, and temperate *Viburnum* species are gray. These scorings follow those of Edwards et al. (2017*a*), except for species not present in Edwards et al. (2017*a*), which were scored on the basis of Schmerler et al. (2012) and Spriggs et al. (2015).

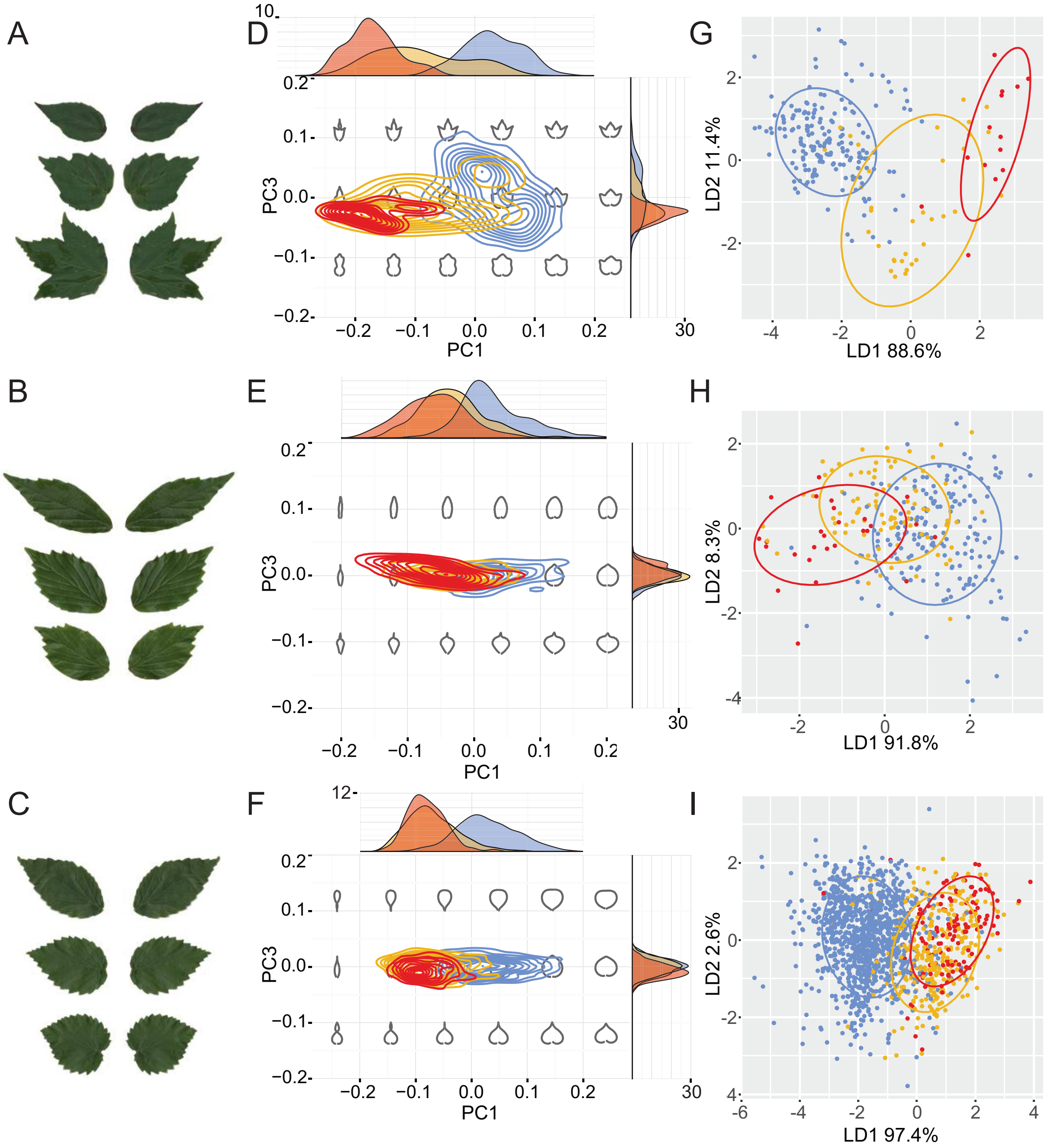

Figure 5: Leaf shape is significantly different across leaf positions in three *Viburnum* species. *A–C*, Representative leaf images for *Viburnum acerifolium* (*A*), *Viburnum rafinesquianum* (*B*), and *Viburnum dentatum* (*C*). In each case, leaf pairs are ordered early, intermediate, and late from bottom to top (see fig. 2*B*). *D–F*, Contour lines for early (red), intermediate (yellow), and late (blue) leaves are drawn into the principal component (PC) 1 versus PC3 morphospace for *V. acerifolium* (*D*), *V. rafinesquianum* (*E*), and *V. dentatum* (*F*). Density plots indicate the relative distributions of the leaf positions along the PC axes. *G–I*, Linear discriminant analysis plots with 80% confidence ellipses drawn around early (red), intermediate (yellow), and late (leaves) for *V. acerifolium* (*G*), *V. rafinesquianum* (*H*), and *V. dentatum* (*I*).

leaves to wide, lobed leaves; PC2 (21.0%) described right-left balance; and PC3 (12.3%) described the degree of lobing (fig. A8).

LDA performed within each species determined that leaf positions (early, intermediate, and late) were distinguishable on the basis of shape alone (fig. 5). When all three leaf positions were considered, LDA correctly predicted the leaf position of 86.2%–91.4% of *V. acerifolium* leaves, 80.0%–83.6% of *V. dentatum* leaves, and 56.0%–74.5% of *V. rafinesquianum* leaves. We also removed intermediate leaves and repeated the LDA. Using only early and late leaf categories, LDA correctly predicted 97.9% of *V. acerifolium* leaves, 94.5% of *V. dentatum* leaves, and 87.1% of *V. rafinesquianum* leaves.

Comparison of Leaf Form Variation within Individuals and across Species

In all species, leaf position had a significant effect on leaf width, as measured by one-way ANOVA of a priori axis 1 scores ($P < .005$), and early leaves were consistently wider than late leaves (fig. 6). Some species also showed heteroblastic patterns in a priori axis 2, but these were less common and varied in direction (fig. 6). In our three focal species, the number of leaf teeth and leaf teeth as a function of leaf perimeter were also significantly related to leaf position ($P < .001$; fig. A9).

Species Leaf Form and the Degree of Heteroblasty

Using both phylogenetic and nonphylogenetic linear models, we found a significant positive correlation between the leaf width (a priori axis 1) of the early leaves within species and the degree of seasonal heteroblasty measured as the entire range of widths occupied by each species ($P < .001$ for both; fig. A10).

Leaf Form Variation across the Viburnum dentatum *Species Complex*

We assessed five traits within the *V. dentatum* species complex: leaf teeth, leaf teeth per leaf perimeter, a priori axis 1 (our leaf width), a priori axis 2 (ovate to obovate), and leaf area (table A2). For leaf area and a priori axis 2, the trait variation was distributed relatively evenly within individuals, among individuals within populations, and among populations. For a priori axis 1 and leaf teeth, however, the patterns were more complex. When all leaf positions were considered, most of the variation in leaf teeth per leaf length and a priori axis 1 was within individuals (41.7% and 66.7%, respectively). The variation among individuals within populations was 0% in both cases, but both traits varied substantially among populations (37.6% and 12.3%). When only early leaf positions were considered, much less variation was found within individuals (11.2% for leaf teeth/leaf length, 20.8% of a priori axis 1), and more variation was attributed to differences among populations (67.1%–19.9% among populations and 3.6%–22.4% among individuals within populations).

Discussion

Across *Viburnum* we identified recurrent transitions in leaf form that parallel patterns of subindividual variation. We found remarkably consistent shifts in leaf form along branches in all 28 investigated species, with wider, toothier early leaves shifting toward narrower leaves with fewer teeth later in the season. Populations across the range of the *Viburnum dentatum* complex diverge along this same axis, as do *Viburnum* species at a deeper timescale. Taken together, these results document a phylogenetic pattern of trait evolution that is fully consistent with the phenotype-first model of evolution, where variation expressed routinely within individual organisms may predispose specific evolutionary transitions at a macroscale.

Using the a priori morphospace approach, our *Viburnum*-wide findings are entirely congruent with those of Schmerler et al. (2012) that *Viburnum* leaf form is evolutionarily labile and that wide and/or lobed shapes evolved in concert with transitions into temperate or boreal environments. However, now with increased sampling and phylogenetic resolution, we have been able to infer the polarity of leaf form evolution with greater certainty, showing that ancestral *Viburnum* species likely produced tropical-looking leaves that were relatively narrow, elliptical, and mostly entire margined. This leaf form appears to have persisted throughout much of the initial diversification of *Viburnum*, with subsequent evolutionary transitions coinciding with adaptation to colder, more seasonal environments (fig. 4) as well as shifts to a deciduous leaf habit (Edwards et al. 2017*a*). These results support the previous suggestion that *Viburnum* originated in more tropical climates than most of its species occupy today (Clement and Donoghue 2011), and the conclusion that transitions from tropical to temperate forests occurred as many as 10 times within the group (Spriggs et al. 2015).

Evidence of seasonal heteroblasty in all 28 investigated *Viburnum* species suggests that it is common throughout the clade. In all species, we found that early leaves were significantly wider (along a priori axis 1) than late leaves (fig. 6). In contrast, heteroblastic variation in leaf size and along a priori axis 2 tended to be species specific (figs. 6, A8). For instance, while early leaves were larger than late leaves in *Viburnum acerifolium*, leaf size was constant across leaf positions in *V. dentatum* (fig. A9). These differences demonstrate that the covariance among leaf traits varies across species and that seasonal heteroblasty can be associated with different

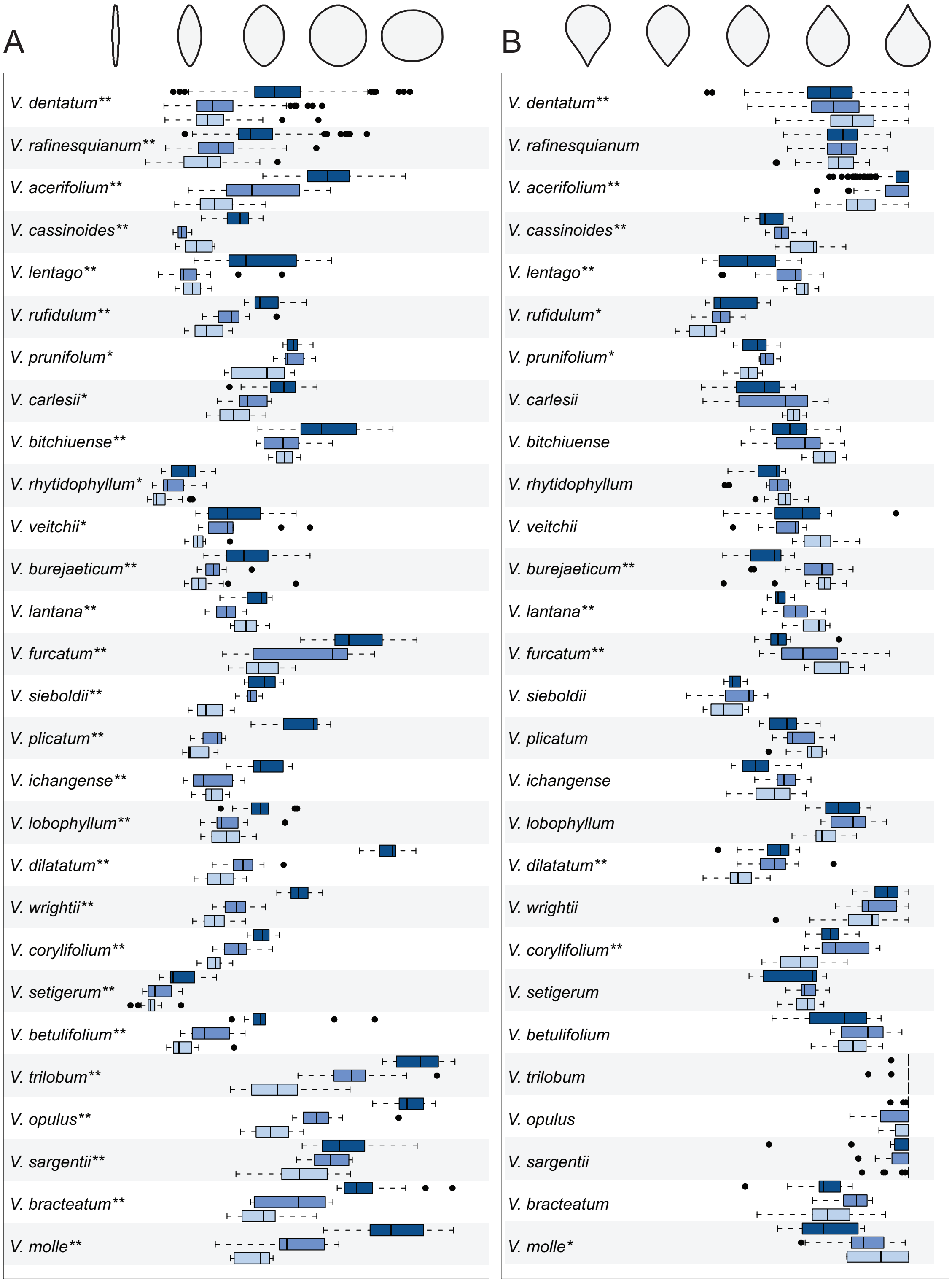

Figure 6: Seasonal heteroblasty is evident in all evaluated *Viburnum* species. *A*, Box plot shows the distribution of a priori axis 1 scores for early (dark blue), intermediate (medium blue), and late (light blue) leaves of each species. Shapes at the top represent a priori axis 1 (left) and a priori axis 2 (right). One asterisk, $P < .005$; two asterisks, $P < .001$.

traits in different species. However, it is significant that leaf width and leaf teeth were the most consistently variable traits among different leaf positions, because these are two of the main traits that also vary among species. In other words, the primary axes of variation within individual plants correspond to the primary axes of variation across all of *Viburnum*.

The pattern of population divergence that we document in the *V. dentatum* complex is consistent with recent evolution along the axes of heteroblastic leaf variation. In agreement with our heteroblasty data, we found that a high proportion of the variation in leaf width and in leaf teeth exists within individual plants. We also found evidence for divergence among *V. dentatum* populations in all leaf traits, including leaf width and leaf teeth. It is clear that the ancestral lineage of the *V. dentatum* complex displayed seasonal heteroblasty, and it would appear that some populations have accentuated the production of either the early leaf form (wider and with more teeth in the north) or the late leaf form (narrower and with fewer teeth in the south). Our ongoing phylogeographic analyses of the *V. dentatum* complex show considerable genetic structure across its geographic range (E. L. Spriggs, D. Eaton, E. Edwards, and M. Donoghue, unpublished manuscript), but it is not yet clear whether the leaf form variation observed among populations reflects genetic differentiation or plastic response to differing environments.

Considering all of our data sets simultaneously, we conclude that when novel leaf forms (wider and more ovate or lobed) evolved within *Viburnum*, they were intercalated at the beginning of the seasonal leaf sequence. Intriguingly, we found that the species with the most derived leaf forms also display the most seasonal heteroblasty (fig. A10). The late leaf forms of all species are similar and ancestral in appearance, while the early leaves reflect evolutionary divergences among species. We suggest that the recurrent nature of the variation produced by seasonal heteroblasty makes it particularly important for evolution. Specifically, seasonal heteroblasty differs in its regularity and persistence from other forms of developmental or plastic variation that produce rare anomalies or alternative phenotypes under extreme conditions. On each individual, seasonal heteroblasty produces a regularized spectrum of leaf forms that are exposed to selection each year. The specificity and predictability of this variation suggest that there are developmental controls guiding leaf form at each node and that these could be easily altered to initiate evolutionary transitions among leaf types.

What underlying mechanisms could drive such consistent and repeated transitions in leaf form along branches? It now appears that a common molecular mechanism involving a gradient in the production of micro-RNAs may underlie conspicuous juvenile-to-adult phase changes in a number of species (Poethig 2013; Hudson et al. 2014; Chitwood and Sinha 2016). However, it is currently not known whether such a mechanism underlies seasonal heteroblasty of the type described here. At another causal level, variation in meristem size or shape along the growing axis could also be a factor. It is well established that leaf size is positively correlated with the size of the meristem that produced it, and this might also account for variation in other leaf traits (Sinnott 1921; Whaley 1939; Abbe et al. 1941; Corner 1949). Herrera (2009) proposed a model in which the decoupling of growth and development could lead to consistent longitudinal variation in the size or shape of primordia and therefore to variation among organs. In *V. acerifolium* we observed a clear trend in size across leaf positions, with late leaves being smaller than early leaves (fig. A9). However, there are not clear size trends in either *Viburnum rafinesquianum* or *V. dentatum*, and, if anything, the leaves of *V. rafinesquianum* increase in size over the course of the season (fig. A9). These conflicting patterns in leaf size cast doubt on a simple meristem-level effect as the main factor driving the positional transition to narrower, less toothy leaves in multiple *Viburnum* species.

Much has been written about the evolution of leaf traits and particularly about potential factors that underlie the global correlation between leaf teeth and temperature (reviewed in Givnish and Kriebel 2017). Recently, we suggested a possible alternative mechanism based on the packing of leaf primordia into resting buds (Edwards et al. 2016, 2017*b*). The analyses presented here are entirely consistent with that bud-packing argument if the apparent reversion at the end of the season to leaves that are more ancestral in appearance is due to different developmental circumstances experienced by early leaves versus late leaves. In particular, early leaves are preformed inside of a resting bud and undergo a phase of arrested growth, while later leaves are neoformed during the growing season and develop more or less continuously (without resting) from primordia into mature leaves. If derived leaf traits are a function of the amount of leaf development that occurs within resting buds, as suggested by Edwards et al. (2016), then late leaves and ancestral *Viburnum* leaves may be similar in form simply because they both develop free of the constraints imposed by a resting bud environment, without a significant pause in their developmental trajectory. In this sense they represent a default *Viburnum* leaf. If a change in the rhythm of growth—specifically, the initiation of a prolonged dormancy—is linked to the evolution of derived *Viburnum* leaf traits, then the leaf form diversity that we observe across *Viburnum* can largely be credited to multiple transitions from warmer, less seasonal environments into colder, more seasonal ones. This raises important questions that we cannot yet answer with certainty; for example, what exactly is the rhythm of growth in more tropical *Viburnum* species? They produce resting buds (i.e., they do not develop continuously; Edwards et al. 2017*a*), but how much leaf development occurs within resting buds in these possibly ancestral environments? How exactly does the content of resting buds differ between tropical and temperate species, and how is this reflected in the degree of seasonal heteroblasty? Our preliminary studies indicate that development within resting buds is limited in tropical species as compared with temperate ones and that tropical species show correspondingly diminished seasonal heteroblasty (E. J. Edwards and M. J. Donoghue, personal observations). However, definitive answers to these questions will require years of careful monitoring in the field (cf. Edwards et al. 2017*a*).

Finally, we emphasize that although seasonal heteroblasty has been studied in detail in only a few plant species, it appears to be a very widespread phenomenon in angiosperms (Zotz et al. 2011), with remarkable similarities in distantly related lineages (the sequence of leaf forms in *V. acerifolium* closely matches that of other species with lobed leaves; e.g., *Liriodendron* Jackson 1899, *Acer* Critchfield 1971). Therefore, we believe that it may have had important evolutionary consequences in many angiosperm lineages. Given the existence of a consistently repeated spectrum of leaf shapes along a branch, selection might favor the production of one leaf form over others. Small changes in timing could potentially canalize the production of a derived leaf form throughout branch development (cf. Chitwood et al. 2016). Alternatively, such changes could promote reversions to an ancestral leaf form by favoring leaves produced later in development. Indeed, in *Viburnum* our ancestral state reconstructions reveal several evolutionary shifts from round, toothy leaves to the regular production of more ancestral leaf types (e.g., the derivation of *Viburnum sempervirens* and related species within the *Succotinus* clade, possibly related to a shift into a warmer climate). In any case, the overall result is precisely the one that we have shown here for *Viburnum*, namely, the alignment of the differences seen among related species with the primary axes of heteroblastic trait variation seen within individual organisms.

We have demonstrated a clear parallelism in leaf variation at three different levels in *Viburnum*. Although we find a strong positive correspondence among levels in this case, numerous other outcomes are possible, and additional studies that directly compare phenotypic variation across scales will be necessary to establish a general understanding of the role of subspecific variation in shaping evolutionary trajectories. We have focused here on seasonal heteroblasty and variation in leaf form, but analogous forms of structured variation occur in other plant organs (e.g., flowers, fruits, tendrils) and even in some major animal clades (e.g., corals, gorgonians; Hughes 2005). In all such cases, the repeated production within an organism of fully integrated alternative organ or segment phenotypes provides an evolutionary opportunity to canalize the production of a single phenotype and therefore potentially to rapidly alter a population or species (West-Eberhard 2003). In plants, we believe that this process is very likely to link patterns of variation across different scales. Most generally, we strongly concur with Herrera (2009) that the integration of subindividual variation into evolutionary analyses will generate important new insights into the origin and evolution of novel morphological traits.

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Literature Cited

Abbe, E. C., L. F. Randolph, and J. Einset. 1941. The developmental relationship between shoot apex and growth pattern of leaf blade in diploid maize. American Journal of Botany 28:778–784.

Allf, B. C., P. A. P. Durst, and D. W. Pfennig. 2016. Behavioral plasticity and the origins of novelty: the evolution of the rattlesnake rattle. American Naturalist 188:475–483.

Ashby, E. 1948. Studies in the morphogenesis of leaves. I. An essay on leaf shape. New Phytologist 47:153–176.

Bonhomme, V., S. Picq, C. Gaucherel, and J. Claude. 2014. Momocs: outline analysis using R. Journal of Statistical Software 56:1–24.

Chitwood, D. H., L. L. Klein, R. O'Hanlon, S. Chacko, M. Greg, C. Kitchen, A. J. Miller, et al. 2016. Latent developmental and evolutionary shapes embedded within the grapevine leaf. New Phytologist 210:343–355.

Chitwood, D. H., and N. R. Sinha. 2016. Evolutionary and environmental forces sculpting leaf development. Current Biology 26:R297–R306.

Clement, W. L., M. Arakaki, P. W. Sweeney, E. J. Edwards, and M. J. Donoghue. 2014. A chloroplast tree for *Viburnum* (Adoxaceae) and its implications for phylogenetic classification and character evolution. American Journal of Botany 101:1029–1049.

Clement, W. L., and M. J. Donoghue. 2011. Dissolution of *Viburnum* section Megalotinus (Adoxaceae) of Southeast Asia and its implications for morphological evolution and biogeography. International Journal of Plant Sciences 172:559–573.

Corner, E. J. H. 1949. The durian theory or the origin of the modern tree. Annals of Botany 13:367–414.

Critchfield, W. B. 1960. Leaf dimorphism in *Populus trichocarpa*. American Journal of Botany 47:699–711.

———. 1971. Shoot growth and heterophylly in *Acer*. Journal of the Arnold Arboretum 52:240–266.

Eaton, D. A., E. L. Spriggs, B. Park, and M. J. Donoghue. 2017. Misconceptions on missing data in RAD-seq phylogenetics with a deep-scale example from flowering plants. Systematic Biology 66: 339–412.

Edwards, E. J., D. S. Chatelet, B.-C. Chen, J. Ong, S. Tagane, H. Kanemitsu, K. Tagawa, et al. 2017*a*. Convergence, consilience, and the evolution of temperate deciduous forests. American Naturalist 190 (suppl.):S87–S104.

Edwards, E. J., D. S. Chatelet, L. Sack, and M. J. Donoghue. 2014. Leaf life span and the leaf economic spectrum in the context of whole plant architecture. Journal of Ecology 102:328–336.

Edwards, E. J., D. S. Chatelet, E. L. Spriggs, E. S. Johnson, C. V. Schlutius, and M. J. Donoghue. 2017*b*. Correlation, causation, and the evolution of leaf teeth: a reply to Givnish and Kriebel. American Journal of Botany 104:509–515.

Edwards, E. J., E. L. Spriggs, D. S. Chatelet, and M. J. Donoghue. 2016. Unpacking a century-old mystery: winter buds and the latitudinal gradient in leaf form. American Journal of Botany 103:1–4.

Givnish, T. J., and R. Kriebel. 2017. Causes of ecological gradients in leaf margin entirety: evaluating the roles of biomechanics, hydraulics, vein geometry, and bud packing. American Journal of Botany 104:354–366.

Gleason, H. A., and A. Cronquist. 1991. Manual of vascular plants of Northeastern United States and adjacent Canada. New York Botanical Garden Press, Bronx.

Gomez-Mestre, I., and D. R. Buchholz. 2006. Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. Proceedings of the National Academy of Sciences of the USA 103:19021–19026.

Goodin, J. R. 1965. Anatomical changes associated with juvenile-to-mature growth phase transition in *Hedra*. Nature 208:504–505.

Herrera, C. M. 2009. Multiplicity in unity. University of Chicago Press, Chicago.

Ho, L. S. T., and C. Ané. 2014. A linear-time algorithm for Gaussian and non-Gaussian trait evolution models. Systematic Biology 63: 297–408.

Hudson, C. J., J. S. Freeman, R. C. Jones, B. M. Potts, M. M. L. Wong, J. L. Weller, V. F. G. Hecht, et al. 2014. Genetic control of heterochrony in *Eucalyuptus globulus*. G3 Genes Genomes Genetics 4: 1235–1245.

Hughes, R. N. 2005. Lessons in modularity: the evolutionary ecology of colonial invertebrates. Scientia Marina 69:169–179.

Iwata, H., and Y. Ukai. 2002. SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptical Fourier descriptors. Journal of Heredity 93:384–385.

Jackson, R. T. 1899. Localized stages in development in plants and animals. Memoirs of the Boston Society of Natural History 5:89–153.

Jones, C. S. 1995. Does shade prolong juvenile development? a morphological analysis of leaf shape changes in *Cucurbita argyrosperma* subsp. *sororia* (Cucurbitaceae). American Journal of Botany 82: 346–359.

———. 1999. An essay on juvenility, phase change, and heteroblasty in seed plants. International Journal of Plant Sciences 160(suppl.): S105–S111.

Kaplan, K. R. 1980. Heteroblastic leaf development in *Acacia*: morphological and morphogenetic implication. La Cellule 73:135–203.

Kartez, J. T. 2015. The Biota of North America Program (BONAP), Taxonomic Data Center.

Kuhl, F. P., and C. R. Giardina. 1982. Elliptical Fourier features of a closed contour. Computer Graphics and Image Processing 18:236–258.

Laland, K. N., T. Uller, M. W. Feldman, K. Sterelny, G. B. Muller, A. Moczek, E. Jablonka, et al. 2014. Does evolutionary theory need a rethink? Nature 514:161–164.

Lee, D. W., and J. H. Richards. 1991. Heteroblastic development in vines. Pages 205–244 *in* F. E. Putz and H. A. Mooney, eds. The biology of vines. Cambridge University Press, Cambridge.

Losos, J. B., D. A. Creer, D. Glossip, R. Goellner, A. Hampton, G. Roberts, N. Haskell, et al. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. Evolution 54:301–305.

McAtee, W. L. 1956. A review of the nearctic *Viburnum*. Chapel Hill, NC.

Moczek, A. P., S. Sultan, S. Foster, C. Ledon-Rettig, I. Dworkin, H. F. Nijhout, E. Abouheif, and D. W. Pfennig. 2011. The role of developmental plasticity in evolutionary innovation. Proceedings of the Royal Society B 278:2705–2713.

Nicotra, A. B., A. Leigh, C. K. Boyce, C. S. Jones, K. J. Niklas, D. L. Royer, and H. Tsukaya. 2011. The evolution and functional significance of leaf shape in the angiosperms. Functional Plant Biology 38:535–552.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetic and evolution in R language. Bioinformatics 20:298–290.

Pfennig, D. W., M. A. Wund, E. C. Snell-Rood, T. Cruickshank, C. D. Schlichting, and A. P. Moczek. 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends in Ecology and Evolution 25:459–467.

Poethig, S. R. 2013. Vegetative phase change and shoot maturation in plants. ~~Current Topics in Developmental Biology~~ 105:125–152.

Price, T. D., A. Qvarnstrom, and D. E. Irwin. 2003. The role of phenotypic plasticity in driving genetic evolution. Proceedings of the Royal Society B 270:1433–1440.

Revell, L. J. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3:217–223.

Riedl, R. 1978. Order in living organisms. Wiley, New York.

Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, MA.

Schlichting, C. D., and M. A. Wund. 2014. Phenotypic plasticity and epigenetic marking: an assessment of evidence for genetic accommodation. Evolution 68:656–672.

Schmerler, S. B., W. L. Clement, J. M. Beaulieu, D. S. Chatelet, L. Sack, M. J. Donoghue, and E. J. Edwards. 2012. Evolution of leaf form correlates with tropical-temperate transitions in *Viburnum* (Adoxaceae). Proceedings of the Royal Society B 279:3905–3913.

Sinnott, E. W. 1921. The relation between body size and organ size in plants. American Naturalist 55:385–403.

Spriggs, E. L., W. L. Clement, P. W. Sweeney, S. Madriñán, E. J. Edwards, and M. J. Donoghue. 2015. Temperate radiations and dying embers of a tropical past: the diversification of *Viburnum*. New Phytologist 207:340–354.

Spriggs, E. L., S. B. Schmerler, E. J. Edwards, and M. J. Donoghue. 2018. Data from: Leaf form evolution in *Viburnum* parallels variation within individual plants. American Naturalist, Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.15249.

Taiz, L., and E. Zeiger. 2002. Plant physiology. Sinauer, Sunderland, MA.

Waddington, C. H. 1957. The strategy of the genes. Allen & Unwin, London.

Weakley, A. S., R. J. LeBlond, B. A. Sorrie, C. T. Witsell, D. Estes, K. Gandhi, K. G. Mathews, et al. 2011. New combinations, rank changes, and nomenclatural and taxonomic comments in the vascular flora of the southeastern United States. Journal of the Botanical Research Institute of Texas 5:437–455.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.

Whaley, W. G. 1939. Developmental changes in apical meristems. Proceedings of the National Academy of Sciences of the USA 25: 445–448.

Winn, A. A. 1996. The contributions of programed developmental change and phenotypic plasticity to within-individual variation in leaf traits in *Dicerandra linearifolia*. Journal of Evolutionary Biology 9:737–752.

Wray, G. A., H. E. Hoekstra, D. J. Futuyma, R. E. Lenski, T. F. C. Mackay, D. Schluter, and J. E. Strassmann. 2014. Does evolutionary theory need a rethink?—COUNTERPOINT No, all is well. Nature 514:161–164.

Wund, M. A. 2012. Assessing the impacts of phenotypic plasticity on evolution. Integrative and Comparative Biology 52:5–15.

Wund, M. A., J. A. Baker, B. Clancy, J. L. Golub, and S. A. Foster. 2008. A test of the "flexible stem" model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. American Naturalist 172:449–462.

Zotz, G., K. Wilhelm, and A. Becker. 2011. Heteroblasty—a review. Botanical Review 77:109–151.

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