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Testing the hypothesis that biological modularity is shaped by adaptation: Xylem in the *Bursera simaruba* clade of tropical trees

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The study of modularity allows recognition of suites of character covariation that potentially diagnose units of evolutionary change. One prominent perspective predicts that natural selection should forge developmental units that maximize mutual functional independence. We examined the module-function relation using secondary xylem (wood) in a clade of tropical trees as a study system. Traditionally, the three main cell types in wood (vessels, fibers, and parenchyma) have respectively been associated with three functions (conduction, mechanical support, and storage). We collected samples from nine species of the *simaruba* clade of *Bursera* at fifteen sites and measured thirteen anatomical variables that have traditionally been regarded as reflecting the distinct functions of these cell types. If there are indeed (semi) independently evolving modules associated with functions, and cell types really are associated with these functions, then we should observe greater association between traits within cell types than between traits from different cell types. To map these associations, we calculated correlation coefficients among anatomical variables and identified modules using cluster and factor analysis. Our results were only partially congruent with expectations, with associations between characters of different cell types common. These results suggest causes of covariation, some involving selected function as predicted, but also highlighting the tradeoffs and shared developmental pathways limiting the evolutionary independence of some cell types in the secondary xylem. The evolution of diversity across the *simaruba* clade appears to have required only limited independence between parts.

1 | INTRODUCTION

One of the most debated traditions in evolutionary biology is to regard isolated parts of organisms as adaptations (Lewontin, 1978). To the extent that these "parts" are arbitrarily delimited, conclusions regarding their adaptive status seem suspect. Part-speak in biology is often criticized because rather than being a collection of parts, each individual organism functions as a coordinated whole. Yet even though organisms must function as integrated wholes, there is clearly enough developmental independence between parts so as to lead to the evolution of vast morphological diversity even between closely related species (Eble, 2005). The middle finger of the aye–aye is an example. As serially repeated structures, the fingers of the primate hand could plausibly be expected to share developmental machinery, limiting their

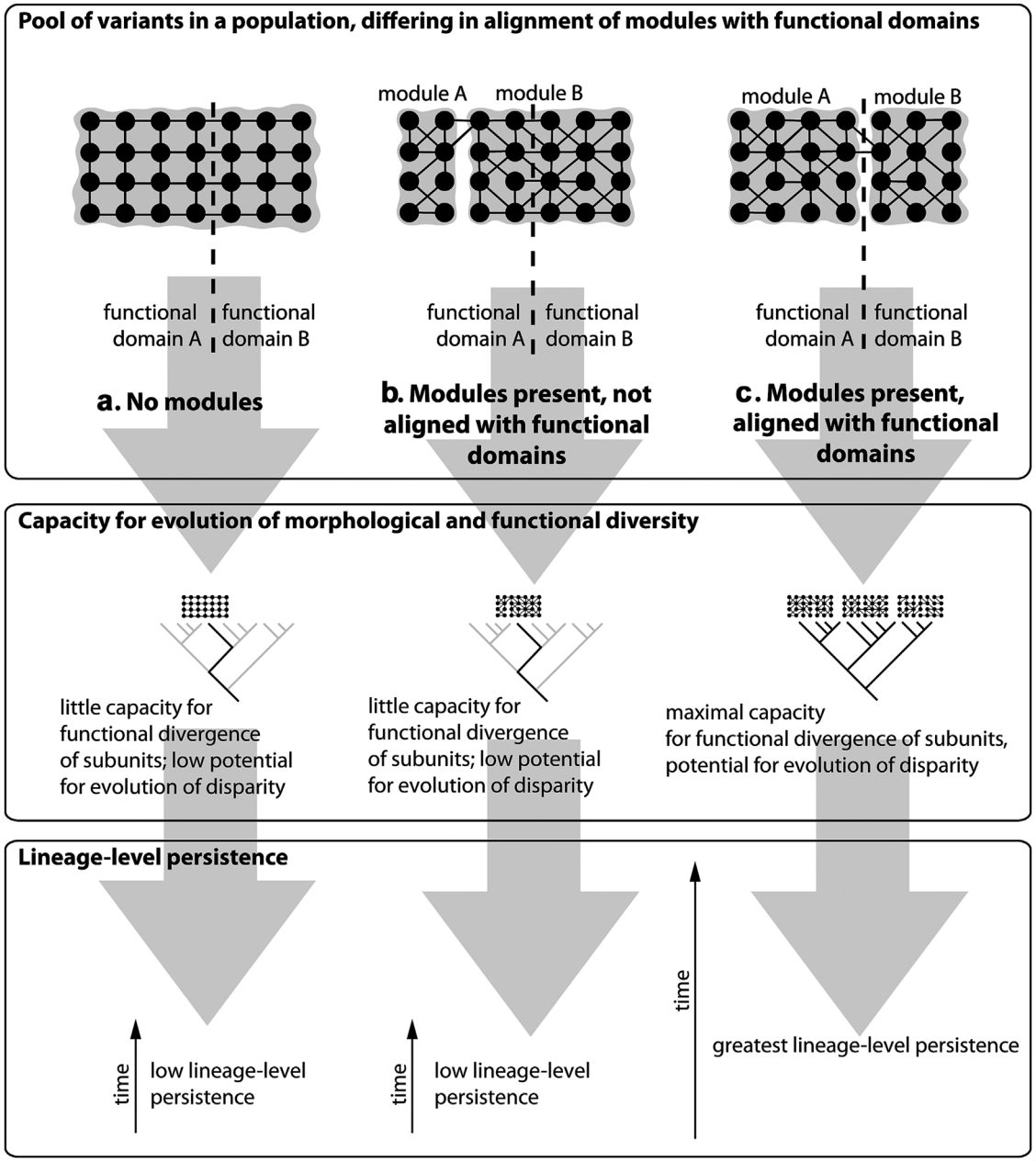

FIGURE 1 Evolution of modularity by natural selection. (a) Individuals with high connectivity across all parts and little capacity for modification of isolated parts. The possibility of natural selection to shape form–function relation is limited and offspring have little independent variation between parts. Lineage persistence in time low. (b) Heterogeneous connections between parts and modular structure not aligned with functional subunits gives little capacity for functional independence. Lineage persistence in time low. (c) Individuals with heterogeneous connections between parts and modular structure aligned with functional subunits gives high capacity for functional independence. Modular structure with maximal evolvability, potential for greater persistence than a and b, and greater probability to give rise to morphologically disparate species

mutual developmental independence (Kivell, Schmitt, & Wunderlich, 2010; Pellis & Pellis, 2012; Soligo, 2005; Wagner, 2014). However, the middle finger of the aye–aye is extremely different from neighboring digits. Such examples abound (see the Figure 1 of Frankino, Emlen, & Shingleton [2009] who show a series of mammal skulls, graphically illustrating the relative independence of the maxilla and orbit), showing that organisms are integrated, but some developmental independence between body sectors clearly exists.

Relative independence of character subsets is known as modularity, and the character subsets that are more strongly linked among one another than to other subsets are known as modules (Bolker, 2000; Breuker, Debat, & Klingenberg, 2006; Eble, 2005). Modularity allows parts to vary to an extent independently of one another in ontogeny, allowing

evolutionary change within modules without catastrophically altering function or structure of other modules (Breuker et al., 2006; Espinosa-Soto & Wagner, 2010; Wagner, 1996). As a result, modular organization is a key notion in thinking regarding morphological diversity, phenotypic evolution, and evolvability (Kirschner & Gerhart, 1998; Klingenberg, 2008; Schlosser & Wagner, 2004; Wagner, 1996; West-Eberhard, 2003; Wagner & Altenberg, 1996; Wagner & Mezey, 2004). By offering hope that organisms can be parsed into non-arbitrarily delimited subsets, the study of modularity may help to solve the problem of "arbitrary atomization" of organisms in studies of adaptation (Lewontin, 1978; Wagner, 2001; Wagner, Pavlicev, & Cheverud, 2007). Because of the importance of modularity in many crucial biological issues, it is important to understand how modularity arises evolutionarily.

The most prominent view regarding the evolution of modularity is that modules correspond to functional units whose boundaries are shaped by natural selection (Breuker et al., 2006; Müller, 2007; Wagner, 1996). The hypothesis regarding the shaping of modularity by natural selection is as follows. Individuals within a population vary heritably with respect to developmental connections between parts. Some combinations of connections confer greater or lesser degrees of evolutionary independence between these parts (Figure 1, Kemp, 2007; West-Eberhard, 2003). Individuals with high degrees of connectivity across all parts will have little ontogenetic independence between parts (Figure 1a). The ability of selection to hone the form-function relation on relatively isolated organismal subsets in lineages of such individuals would be limited. Some individuals might have highly heterogeneous connections between parts, with some sectors of the phenotype being highly connected and others less so (Figure 1b,c). Variants could arise in which low developmental connectivity coincides with the boundaries of functional units (Breuker et al., 2006; Kemp, 2007). Such variants would have maximal capacities for developmental and therefore evolutionary independence between parts. These would be likely to found lineages with maximal evolvability and, presumably, would come to predominate over less flexible competitors via greater persistence (lineage-level "survivorship") and perhaps the potential for generating morphologically disparate additional species (lineage-level "fecundity") (Figure 1c). From this point of view, the module boundaries observed in current populations are variants favored by selection, leading to the prediction that functions should coincide with module boundaries.

Here, we test the hypothesis that function should predict module boundaries (Wagner, 1996). We use a comparative approach focusing on the wood (=secondary xylem) of the species of a small clade of morphologically and ecologically diverse tropical trees. Wood is an ideal system in which to study developmental evolution because it has relatively few parts with relatively few functions, meaning that they can be studied comprehensively. Woody plant stems perform three main functions. Wood is made up of three main cell types, and for over a century each has been regarded as corresponding with one of the three functions (Baas, 1986; Bailey & Tupper, 1918; Carlquist, 1975; Carlquist, 2001; Cutler, Botha, & Stevenson, 2007; Esau, 1977; Mauseth, 1988; Rudall, 2007). The most abundant cell types are fibers, long, slender cells with thick walls and relatively little internal space or lumen. With their thick walls and abundant areas of overlap, these cells are thought to be the "skeletal elements" or support cells of most trees (Bailey & Tupper, 1918). Vessel elements are cylindrical cells that are dead at maturity and occur in strands running lengthwise in stems. Vessel elements lack endwalls, so these strands form long tubes connecting roots to leaves. Vessels form the water conduits of most flowering plants. Because they are largely made up of empty space filled with sap, they are thought to contribute little to mechanical support of stems. Being dead, they also contribute little to the storage of photosynthetic products. This storage is instead traditionally regarded as taking place in parenchyma cells (Morris et al., 2016). Parenchyma is made up of living cuboidal or rectangular cells, usually with relatively thin walls and communicated with one another via wide pits in the cell walls. These thin walls and wide pits make parenchyma cells likely contribute much less to resisting bending in stems than fibers do (though like any viable biological structure, they do offer some mechanical resistance, e.g., Burgert & Eckstein, 2001). The thin walls mean that abundant lumen space is available for storage, and parenchyma cells are often replete with starch grains (Baas, 1986; Carlquist, 2001; Esau, 1977). The xylem thus offers a system with a long tradition of assigning part-function relation with which to test hypotheses regarding the relationship between morphological modularity and function.

The xylem also offers a useful system for studying the evolution of modularity because its ontogenetic pathways are well understood. Wood is produced in concentric layers of cells by a meristem called the vascular cambium, a continually embryonic layer of cells just below the bark. In the cambium, spindle shaped cells called fusiform cambial initials proliferate such that meristem circumference keeps pace with the increase in girth of the stem. Occasionally, fusiform cambial initials differentiate into ray initials by division into many small cells (Figure 2). These embryonic initial cells, fusiform, and ray, give rise to all of the mature cells of the wood. The ray initials produce ray parenchyma, which usually have their long axes oriented radially in the stem, lying like spokes from the inside of the stem to the outside. The fusiform initials give rise to the other cells, vessel elements, axial parenchyma cells, and fibers (Figure 2). Because they are produced from the same initial cells, vessel elements, axial parenchyma cells, and fibers start life at the same size (Baas, 1986; Carlquist, 2001; Mauseth, 1988).

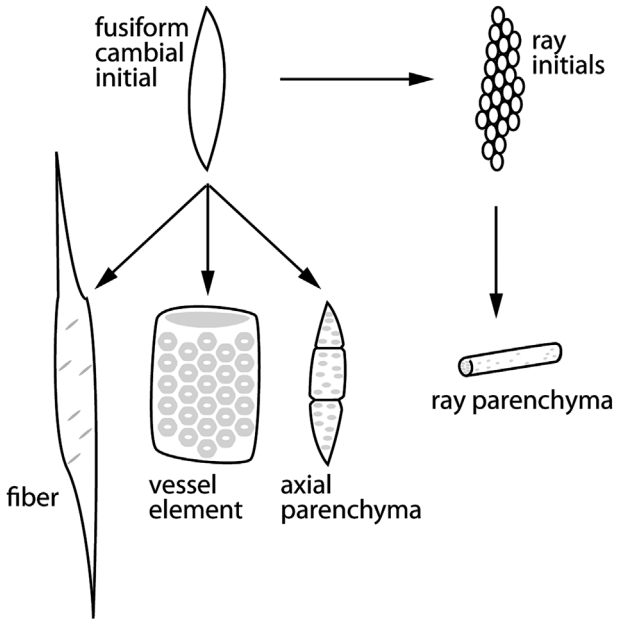

FIGURE 2 Ontogenetic pathways from the vascular cambium to mature secondary xylem (wood) cells. Fusiform cambial initials follow two routes to produce wood. In one route, fusiform cambial initials differentiate into ray initials by division into many small cells. The ray initials produce ray parenchyma, whose long axes are oriented radially in the stem. In the other route, fusiform initials give rise to vessel elements, axial parenchyma cells, and fibers oriented axially with respect to the stem. They are produced from the same initial cells, so vessel elements, axial parenchyma cells, and fibers start life at the same size. Fibers elongate as they mature, and axial parenchyma usually divides into short strands, whereas vessel elements tend to remain the same length as the fusiform initials that gave rise to them

Knowledge of potential developmental associations is essential for exploring the potential role of selection favoring functional independence in the evolution of modularity.

To explore the module-function relation we chose a group of species from the *simaruba* clade of the genus *Bursera* (Rosell et al., 2010; Rzedowski, Medina, & Calderón, 2005). This clade consists of 14 species and spans a range in size from trees 3 to over 30 m tall, including one species with lianescent branches and another an epiphyte (Rosell et al., 2010). These species span a very wide array of environments, from rainforests receiving over three meters of rain to dry forests receiving less than one. Using a clade as a study system ensures that all of the species began with the same ancestral condition. Any differences observed between species are therefore the result of recent divergence rather than simply inheriting these differences from distant, disparate ancestors. Moreover, using a clade that is ecologically diverse maximizes the chance of finding functionally divergent modules.

If selection favors modular configurations in such a way that autonomy of function is maximal (Breuker et al., 2006; Wagner, 1996), then secondary xylem should have three modules. Here, we test for the presence of modularity through morphological covariation sets from a comparative point of view. This approach invokes the notion that character independence detected at an interspecific level can only be produced by a degree of developmental independence (Eble, 2005). This necessary relationship between interspecific and developmental diversity means that a comparative approach is the only sure way of detecting developmental independence sufficient for the evolution of diversity (Frankino et al., 2009; Olson, 2012). We measured anatomical variables that should reflect different stem functions. For example, fiber wall thickness is often found to be positively associated with stem resistance to bending, whereas wider vessels conduct water more efficiently. Morphological features that affect a given function could be expected to be associated mainly with one another. Specifically, we would expect a conductivity module made up of vessel anatomical characters. A mechanical support module would be expected to be made up of fiber anatomical characters. Finally, a storage module would be made up of parenchyma cell characters. We use our results to highlight how the study of morphological covariation sets in a comparative context sheds light on the causes of character association. We conclude with comments on challenges inherent in the study of modularity.

2 | MATERIALS AND METHODS

2.1 | Plant material, anatomical techniques, and variables measured

One way of estimating modularity is provided by morphological covariation sets (Eble, 2005). The first step in identifying these sets involves measuring morphological variables thought to reflect important developmental and functional aspects. Many methods are available to explore the patterns of covariation between these variables, but all share similar ideas. A lack of covariation between two variables suggests that they are free to vary evolutionarily with respect to one another, that is, belong to different modules, whereas those that are closely associated could belong to the same module. We obtained anatomical data from the secondary xylem ("wood" sensu stricto, as distinguished from the primary xylem produced by the apical meristem in young shoots) of terminal branches. We collected a branch segment of approximately 1.5 cm in diameter from seven adult trees per species (nine species). We selected species so as to include diversity of life forms and environment. We used standard anatomical techniques to prepare sections and macerations for light microscopy (Olson & Carlquist, 2001). We measured anatomical variables that should reflect the three functions of storage, conduction, and mechanical

TABLE 1 Descriptive statistics of anatomical variables

Anatomical variable	Function	Abbrev.	Min.	Max.	Mean	Stand. Dev.	Var.
Ray % area	Storage	R	6.9	15.8	10.6	2.1	4.3
Ray cell lumen area (μm^2)	Storage	RCA	142	751.3	416.7	109.44	11,978
Axial parenchyma lumen area (μm^2)	Storage	APA	70.6	298.6	149	40	1,573
Axial parenchyma wall thickness (μm)	Storage	APWT	0.42	1.5	0.84	0.29	0.08
Vessel % area	Conduction	V	13.6	44.4	25.9	6.3	40
Vessel density (#vessels/mm^2)	Conduction	VD	39	132.2	75.9	18.7	350.6
Vessel lumen area (μm^2)	Conduction	VA	1763.2	6051.8	3583.9	874.9	765,557
Vessel element length (μm)	Conduction	VL	278.6	477.2	381.8	47	2,207
Vessel wall thickness (μm)	Conduction	VWT	1.4	2.7	1.9	0.2	0.06
Fiber % area	Mechanical support	F	43.1	76.9	63.5	6.8	46.7
Fiber lumen area (μm^2)	Mechanical support	FA	187	1619	366.6	159.1	25,302
Fiber length (μm)	Mechanical support	FL	371.6	732.5	581.7	73.7	5,439
Fiber wall thickness (μm)	Mechanical support	FWT	1.34	2.4	1.7	0.2	0.04

Abbrev, abbreviation; Min, minimum; Max, maximum; Stand. Dev., standard deviation; Var., variance.

support in the secondary xylem (Carlquist, 2001). Anatomical data were measured from the outermost wood. The variables measured are summarized in Table 1 and their putative functions in appendix 1. We calculated sample mean values based on 25 observations per variable for each branch (Carlquist & Hoekman, 1985). We $\log_{10}$ transformed data to normalize them (Sokal & Rohlf, 2012) and we analyzed data with R v. 3.2.1 (R Development Core Team, 2015).

2.2 | Covariation between anatomical variables

To identify the covariation patterns among anatomical characters we calculated correlation coefficients between variables (Eble, 2005; Goswami & Polly, 2010; Klingenberg, 2008). Significant character correlation is expected to be stronger or more numerous inside modules than with other modules (Mitteroecker & Bookstein, 2007), presumably due to functional and developmental relations (Eble, 2005; Hearn, 2013; Klingenberg, 2008; Martínez-Cabrera, Jones, Espino, & Schenk, 2009; Wagner, 1996). We tested for phylogenetic signal in the residuals of regression of anatomical variables on one another (Revell, 2012; Swenson, 2014) using the phylogeny of Rosell et al. (2010), pruning the tree to the nine species from which we collected data, and the phytools package in R (Freckleton, 2009; Revell, 2012). Some tests did show evidence of phylogenetic signal ($k > 1$, $p < 0.05$, $n = 63$, e.g., in R-VA, VWT-APWT residuals). We incorporated phylogeny in the calculation of correlation coefficients (Swenson, 2014) and generated a phylogenetic correlation coefficient matrix. Both correlation matrices, with and with out phylogenetic signal, were very similar, so we tested whether they were significantly different from one another using the test of Jennrich (1970). The matrices were not statistically different from one another ($X^2 = -32358.76$, $p = 1$, $n = 13$), so we proceeded with non-phylogenetically corrected analyses. In our analyses we identified correlations between paired variables as a first approximation to detect associations between anatomical variables by function (conduction, mechanical support, and storage).

2.3 | Cluster and factor analysis

Another approach we used to identify modules involved multivariate methods. Multivariate analysis organizes data in groups of variables correlated among one another and less correlated with other groups, exactly the concern of modularity studies (Goswami & Polly, 2010). Multivariate analyses often start with correlation matrices, in this case built from Pearson correlation coefficients (Goswami & Polly, 2010; Klingenberg, 2003a; Magwene, 2001; Manly, 2005; Tabachnick & Fidell, 2007). We used cluster and factor analysis to delimit morphological covariation sets. These multivariate methods were selected because they identify groups of covarying variables and are commonly used in modularity studies (Goswami & Polly, 2010; Mitteroecker & Bookstein, 2007).

First, we used cluster analysis, an approach that groups variables based on their similarity. We performed a hierarchical cluster analysis using Euclidean distance and the nearest neighbor method to form groups (Everitt, Landau, Leese, & Stahl, 2011; Kaufman & Rousseeuw, 2005). Groups represent morphological covariation subsets and we interpreted these morphological covariation subsets as modules (Eble, 2005).

In addition, we also used factor analysis to identify morphological covariation sets. The goal of factor analysis is to identify groups of correlated variables. Factors represent morphological covariation sets defined by the highest loading variables (García Jiménez, Gil Flores, & Rodríguez Gómez, 2000; Kline, 1994). We performed a factor analysis using principal components and varimax rotation to identify morphological modules. We interpreted covariaton of variables with the highest loadings as modules.

3 | RESULTS

3.1 | Plant material, anatomical techniques, and variables measured

We obtained 34,125 anatomical data from 105 terminal branches from 105 adult trees. Descriptive statistics of anatomical variables are given in Table 1.

3.2 | Covariation of anatomical variables

We tested the prediction that covariation patterns in the secondary xylem should reflect the three main functions of stems. We expected to find three clusters of covariation, for example, with variables relating to conduction covarying more closely with one another than with support or storage variables. However, the patterns of association between anatomical variables revealed a covariation network involving characters traditionally assigned to different functions (Figure 3). For instance, vessel element and fiber length, traditionally regarded as affecting conduction and mechanical support respectively, were significantly correlated with one another ($r = 0.76$, $p < 0.001$, $n = 105$). Our data thus highlighted correlations both within as well as across functions.

3.3 | Cluster and factor analysis

3.3.1 | Cluster analysis

Cluster analysis identified three morphological covariation sets (Table 2). One was made up of vessel (conduction) and fiber (mechanical support) wall thickness. Another was made up of vessel (conduction) and fiber (mechanical support) length. The third was made up of vessel lumen area (conduction), vessel density (conduction), percent area of the xylem occupied by rays (storage), fiber lumen area (mechanical support), axial parenchyma cell lumen area (storage), axial parenchyma wall thickness (storage), and ray cell lumen area (storage). These results suggested morphological modules made up of anatomical characters that perform different functions.

3.4 | Factor analysis

The first four factors explained 66% of the variance of the data (Table 3). These four factors were sufficient for explaining the correlation structure between anatomical variables because they had eigenvalues greater than 1 (Kaiser, 1960, Table 3). The first factor was determined by fiber lumen area (support), axial parenchyma wall thickness (storage), axial parenchyma lumen area (storage), and ray cell lumen area (storage). The second factor was made up of vessel element (conduction) and fiber (storage) length. The third factor was made up of percent area of the xylem occupied by rays (storage), vessel density (conduction), and vessel lumen area (conduction). The fourth factor was made up of fiber wall thickness (support) and vessel wall thickness (conduction, Table 4). Factors were made up of variables with different putative functions. As with results of the other techniques we employed, covariation of anatomical variables and cluster analysis, factor analysis showed covariation of attributes between as well as within functions across the *simaruba* clade.

4 | DISCUSSION

4.1 | The module-function relation

One of the most common explanations for the origin of modular covariation patterns in organisms is that modules represent functional units (Armbruster, Di Stilio, Tuxill, Flores, & Velásquez Runk, 1999; Breuker et al., 2006; Cheverud, 1996; Cheverud, Hartman, Richtsmeier, & Atchley, 1991; Eble, 2005; Hearn, 2013; Klingenberg, 2008, 2010; Mezey, Cheverud, & Wagner, 2000; Wagner, 1996; Wagner et al., 2007). In addition to function, our results highlight the diversity of factors that underlie covariation between cell attributes in *simaruba* clade secondary xylem. The patterns of covariation we recovered (Figure 3) show numerous connections between anatomical variables performing different functions (Hearn, 2013). Although exact results differed to an extent between methods, cross-function trait associations were recovered across all the methods we used. Modules were made up of conduction and mechanical support characters, of conduction, mechanical support, and storage characters, of mechanical support and storage characters, or of storage and conduction characters (Tables 2 and 4). For example, vessel element and fiber length were strongly correlated in all of the methods (Figure 3; Tables 2 and 4). Modules were found made up of cells representing the three putative functions of storage, mechanical support, and conduction (Table 2 group 3) or two putative functions of mechanical support and storage (Table 4 factor 1). These patterns of trait covariation raise the question of why traits covary.

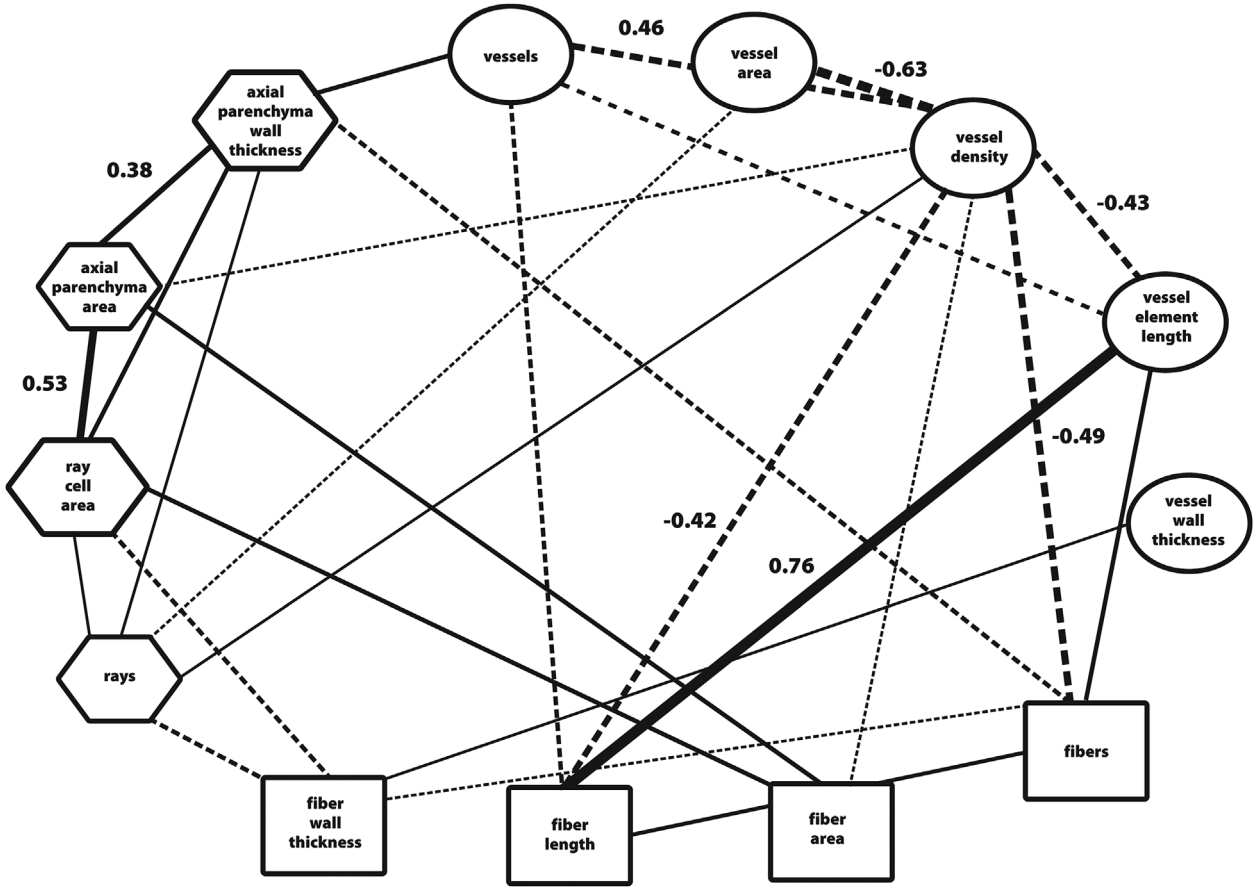

FIGURE 3 Anatomical character correlations across the 9 *simaruba* clade of *Bursera* species studied. Significant correlations are shown. Solid lines indicate positive correlations and dashed lines negative ones. Thicker lines indicate higher correlations. Vessel (ellipses), fiber (rectangles), and parenchyma (hexagons) anatomical characters. Anatomical variables and their functions are given in Table 1

4.2 | Covariation causes

The patterns of covariation recovered likely reflect several different causes, perhaps the most important of which are shared cell developmental pathways (Figure 2). The association between vessel element length and fiber length was one of the strongest correlations we documented (Figure 3 and Table 2 group 2 and Table 4 factor 2). Vessels are traditionally regarded as serving a conductive function whereas fibers a mechanical support one, so it would not have been surprising to find them falling in separate modules rather than covarying strongly. Because they are traditionally regarded as performing largely independent functions, if function drives modularity then selection would favor developmental independence between them. No functional relationship between fiber and vessel element length has ever been suggested in the literature, so there are no known adaptive reasons to expect their lengths to be correlated. However, vessels and fibers differentiate from the same mother cells, known as fusiform cambial initials. Cambial cells are found beneath the innermost layer of the bark. These continually embryonic cells produce vessels, fibers, and parenchyma to the inside of the trunk. In any given growing season, a single cambial cell can produce dozens or hundreds of fibers and several to dozens of vessel elements. Because both cell types originate from the same individual embryonic cells, they begin life at exactly the same size. This shared origin imposes the same minimum length for both cell types, with fibers elongating markedly as they mature whereas vessel elements do not (Baas, 1986; Carlquist, 2001; cf., Losos, 2011;

TABLE 2 Cluster analysis of anatomical variables

Group	Anatomical variables	Function
1	VWT, FWT	Conduction (VWT) and mechanical support (FWT)
2	VL, FL	Conduction (VL) and mechanical support (FL)
3	R, RCA, APWT, APA, VA, VD, FA	Storage (R, RCA, APWT, APA), conduction (VA, VD), and mechanical support (FA)

TABLE 3 Percentage of variance explained in factor analysis

Factor	1	2	3	4
Eigenvalues	2.543	2.175	1.375	1.161
Sum of squares loadings	2.23	1.93	1.75	1.34
Proportion variance	0.20	0.18	0.16	0.12
Cumulative variance	0.20	0.38	0.54	0.66
Proportion explained	0.31	0.27	0.24	0.18
Cumulative proportion	0.31	0.57	0.82	1.00

Mauseth, 1988; Olson, 2012). This lack of independence could potentially limit selection or facilitate it. Selection favoring, say, shorter vessel elements, often thought to provide greater safety against propagation of embolisms throughout a vessel, would likely be opposed by that favoring the greater mechanical support offered by longer fibers. If a positive correlation in their length were adaptive, for example, if longer fibers are favored in larger plants for their mechanical support, and longer vessel elements as well for their efficient conduction, then this shared developmental pathway would bias developmental outcomes in ways that are likely to be of high fitness. In this way, shared developmental pathways can bias evolution in ways that are potentially orthogonal as well as parallel to vectors of selection.

Another potential source of character covariation is competition for developmental resources (Rosell, 2010; Stearns, 1992). Because resources are finite, allocation to one character necessarily involves a reduction in the allocation to another (Stearns, 1992). Because a given area can be occupied by many narrow or few wide vessels, but not many wide ones, there is a tradeoff between vessel lumen area and density across the flowering plants (Rosell & Olson, 2014), and the pattern was also recovered here (Figure 3). Similarly, the negative relation between percent of the xylem occupied by fibers (mechanical support tissue) and vessel density (conduction tissue, Figure 3) could reflect a space tradeoff (Carlquist, 2001; Mauseth, 1988), with investment in conduction occupying stem transectional space that cannot be occupied by fibers. Tradeoffs therefore seem very likely factors that lead to lack of independence between xylem characteristics.

Characters can also covary evolutionarily as parts of functional complexes, in which traits are developmentally independent but selection favors certain combinations (Breuker et al., 2006; Klingenberg, 2003b; Wagner, 1996; Wagner et al., 2007). For instance, jaw characters covary more strongly with one another than with other characters from the skull, an association usually interpreted in terms of selected function (Cheverud, 2004; Klingenberg, 2003b; Perez, de Aguiar, & Guimarães Jr., 2009). In *simaruba* clade secondary xylem, we found some evidence for modularity delimited by function. For example, we found attributes of axial and ray parenchyma, both regarded as serving a storage function, to be significantly positively related to one another (Figure 3). These features included percent area of the xylem occupied by rays, the lumen area of axial and ray parenchyma cells, and axial parenchyma wall thickness. These features diverge from one another ontogenetically very early (Figure 2), so early that there is little developmental reason to expect them to resemble one another in their cell dimensions. That these ontogenetically very distant but functionally similar attributes should correlate significantly is congruent with the traditional expectation that they should

TABLE 4 Factor analysis with anatomical variables

Factor				
Latent factor	Storage and mechanical support	Conduction and mechanical support	Conduction and storage	Conduction and mechanical support
Anatomical variable	1	2	3	4
R	0.44	0.01	0.60	−0.17
VD	−0.19	−0.43	0.74	0.06
VA	0.15	0.03	−0.86	−0.06
VWT	0.06	−0.11	0.09	0.80
FWT	−0.31	0.04	−0.08	0.70
VL	−0.01	0.91	−0.15	0.01
FL	−0.01	0.94	−0.05	−0.07
FA	0.60	0.17	−0.16	0.34
APA	0.79	−0.03	−0.15	0.02
APWT	0.60	−0.03	0.08	−0.16
RCA	0.74	−0.01	0.00	−0.19

evolve in a coordinated way. The lumen area of axial and ray parenchyma cells also significantly covaried positively with fiber lumen area. Parenchyma is traditionally interpreted as serving as storage in secondary xylem whereas fibers are seen as providing mechanical support. However, fibers in *Bursera* often have starch in them, a storage product, and are water-filled (Durán Guerra, Quintanar Isaías, Villanueva Díaz, Jaramillo-Pérez, & Cerano Parede, 2014). As a result, fibers likely serve both a mechanical and a storage function. Modules made up of all three cell types (group 3, Table 2) strengthen the idea of a lack of independence between these parts.

Our results could be regarded as suggesting that, in general, selection in woody plants does not appear to have acted in such a way as to maximize developmental independence between parts. Alternatively, it could suggest that traditional functional ascriptions are incorrect. Xylem studies recently suggest that cell types can be functionally associated in ways different from the traditional perspective of vessels = conduction, fibers = support, and parenchyma = storage (e.g., Martínez-Cabrera et al., 2009, also Ziemińska, Butler, Gleason, Wright, & Westoby, 2013; Ziemińska, Westoby, & Wright, 2015). In addition to the likelihood that fibers sometimes participate in storage, there is some evidence that fibers also participate in resisting vessel deformation under the negative pressure under which plants conduct water (Hacke & Sperry, 2001; Hacke, Sperry, Pockman, Davis, & McCulloh, 2001; Jacobsen, Ewers, Brandon Pratt, Paddock III, & Stephen, 2005; Sperry & Hacke, 2004). Fibers thus might participate not only in mechanical support of the stem and in storage but in conduction as well. Congruent with this possibility is our observation of the relation between fiber and vessel wall thickness, a trait certainly important in cell mechanical integrity (Figure 3, Table 2 group 1, and Table 4 factor 4). Another example is the relation between axial parenchyma lumen area, axial parenchyma wall thickness, ray cell lumen area, and fiber lumen area, all potentially involved in storage (Figure 3 and Table 4 factor 1). Our results thus do seem to show correlation plausibly favored by selection, and also highlight the intervention of shared developmental pathways and tradeoffs in limiting independent variation. They also highlight the limits of methods available to study modularity.

4.3 | Methodological considerations

The different methods we used to analyze the same dataset led to slightly different module delimitations (Tables 2 and 4), a common result in studies of modularity (Goswami & Polly, 2010). It is not straightforward to decide which modular structure, obtained from different techniques, best represents the biology of the system in question (Adams, Cardini, Monteiro, O'Higgins, & Rohlf, 2011; Clune, Mouret, & Lipson, 2013; Goswami & Polly, 2010; Lipson, Pollack, & Suh, 2002; Magwene, 2001; Mitteroecker & Bookstein, 2007; Monteiro, Bonato, & dos Reis, 2005). Accordingly, there is no consensus regarding how modules are to be identified (Breuker et al., 2006; Klingenberg, 2008; Olson & Rosell, 2006; Roseman, Kenny-Hunt, & Cheverud, 2009; Wagner & Altenberg, 1996). For instance, different authors have reported different modular skull structures in mammals. Drake and Klingenberg (2010) proposed a two module models, Cheverud (1995) a six module model, and Goswami and Polly (2010) a different six module model (see Table 1 in Goswami & Polly, 2010). This lack of consensus seems likely due to modularity not being an either-or condition (Adams et al., 2011; Clune et al., 2013; Klingenberg, 2008; Klingenberg et al., 2003; Lipson et al., 2002; Monteiro et al., 2005; Wagner et al., 2007). All parts of organisms covary to one degree or another, so total character independence is impossible. However, developmental independence is such that morphological diversity can evolve. The key criterion for identifying modules, then, is delimiting the "parts" with *sufficient* evolutionary independence as to permit diversification. This independence is exactly the sort identified in comparative studies of taxic homology (Patterson, 1982).

4.4 | Final considerations

One explanation of the origin of modularity involves module composition being shaped by function (Breuker et al., 2006; Eble, 2005; Klingenberg, 2008; Wagner, 1996; Wagner et al., 2007), and such selection probably is involved to some degree in most cases of modularity. However, it is not clear how much developmental independence is necessary to allow for the evolution of morphological diversity. Whatever its developmental biases and correlations, the degree of developmental independence between parts in *simaruba* clade xylem has clearly been sufficient for the evolution of the marked morphological and functional diversity observed across the group. It is this observation, in the context of the scenario presented in Figure 1, that is the most important result from our study. We predicted much more developmental independence between functional domains than observed. This result would seem to offer a case in which relatively little developmental independence is nevertheless sufficient for marked functional and morphological divergence. Given that the number and phylogenetic position of extinct species in *Bursera* is unknown, it is difficult to generalize regarding the species-level emergent fitness that variation in modular structure might confer. What can be said is that *Bursera* does span a remarkable array of life forms, including succulent-stemmed dryland shrubs, dry forest trees of all shapes, hemiepiphytes, to giant rainforest emergents, from temperate deserts to some of the wettest tropical forests in the world,

diversity that is uncommon in groups of similar sizes and geographical extents. Our results seem to suggest that, insofar as the xylem is involved in this diversification, a relatively low degree of developmental independence is apparently sufficient to permit the evolution of diversity rivaling any clade of similar size. In this way, covariation between parts in the secondary xylem highlights the variety of factors that likely influence the evolution of organismal modularity and the evolution of morphological diversity.

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APPENDIX 1

Anatomical variables and their putative functions in secondary xylem

Anatomical variable	Definition	Function
Ray % area (R)	The percent of a linear transect perpendicular to the rays occupied by rays in transection, 25 transects per sample.	Water and starch storage[a,b]. A higher ray percentage should be associated with greater storage capacity.
Ray cell lumen area (RCA)	Ray parenchyma cell lumen available for storage as seen in transection. We measured widths and lengths of ray cell lumina as seen in transection to calculate the area. We calculated 25 RCA per sample.	Water and starch storage. Parenchyma cells with greater lumen area should have greater storage capacity.
Axial parenchyma lumen area (APA)	Axial parenchyma cell lumen as seen in transection. We measured widths and lengths of axial parenchyma lumina as seen in transection to calculate the area. We calculated 25 APA per sample.	Water and starch storage[a,b]. Larger areas imply greater storage capacity.
Axial parenchyma wall thickness (APWT)	Thickness of the wall of axial parenchyma cells as seen in transection. We measured wall thickness on the same cells for which we calculated APA.	Water and starch storage[a,b]. Thicker walls presumably reduce space available for storage.
Vessel % area (V)	Percentage of a given area of wood transection that is occupied by vessels. We measured with transects as for rays.	Water and solute conduction[a,b]. A higher vessel percentage could be associated with greater conductive capacity.
Vessel density (VD)	Number of vessels per mm^2 in transection. We measured 25 VD per sample.	Water and solute conduction[a,b]. All else being equal, more vessels per unit transection could be associated with greater conductive capacity.
Vessel lumen area (VA)	Vessel lumen available for water conduction[a,b] as seen in transection. We measured major and minor cell diameters of vessels to calculate vessel lumen area. We measured vessels that intersected with the transects in V. We calculated 25 VA per sample.	Water and solute conduction[a,b]. Wider areas imply greater conductive capacity.
Vessel element length (VL)	Axial tip-to-tip distance of a vessel element as seen in macerations. We measured 25 VL per sample.	Water and solute conduction[a,b]. Vessels made up of longer vessel elements are sometimes suggested to conduct water with less resistance than those made up of short ones[c,d].
Vessel wall thickness (VWT)	Thickness of the wall of a vessel element as seen in transection. We measured wall thickness in the vessel elements for which we calculated VA.	Water and solute conduction[a,b]. Thicker walls presumably resist vessel wall deformation but reduce lumen space available for conduction.
Fiber % area (F)	Percentage of a given area of wood transection that is occupied by fibers. We measured with transects as for rays.	Mechanical support of the whole plant[a,b]. Large amounts of axial parenchyma, rays, and vessels would presumably exclude fibers in secondary xylem and reduce support capacity[a].
Fiber lumen area (FA)	Fiber cell lumen area[a,b] as estimated from macerations. We measured widest distance of the same fibers in which we measured FL, and calculated transectional area assuming a circular cross section.	Fibers are regarded as serving in mechanical support of the whole plant[a,b]. Wider fibers in Bursera seem likely to provide less mechanical support than narrow ones because of the lower amount of rigid cell wall per unit transection.
Fiber length (FL)	Axial tip-to-tip distance of a fiber as seen in maceration. We measured 25 FL per sample.	Mechanical support of the plant[a,b]. Longer fibers are often associated with greater stem resistance to bending.
Fiber wall thickness (FWT)	Thickness of the wall of a fiber cell as seen in transection. We measured FWT adjacent to vessels for which we calculated VA.	Mechanical support of the plant[a,b]. Fibers with thicker walls are associated with greater resistance to bending of the stem.

[a]Mauseth (1988).
[b]Carlquist (2001).
[c]Carlquist (1996).
[d]Jacobsen et al. (2005).