Universität für Bodenkultur Wien

Wood Variation in Tropical Tree Species

How do wood functional traits explain evolutionary adaptations and life history strategies?

Thesis

Dissertation for obtaining a doctoral degree
at the University of Natural Resources and Applied Life Sciences, Vienna

Submitted by

M.Sc. Kanin Rungwattana

Supervisor: **Univ.Prof. Mag.Dr. Peter Hietz**

Institute of Botany
Department of Integrative Biology and Biodiversity Research
University of Natural Resources and Applied Life Sciences Vienna

Vienna, August 2018

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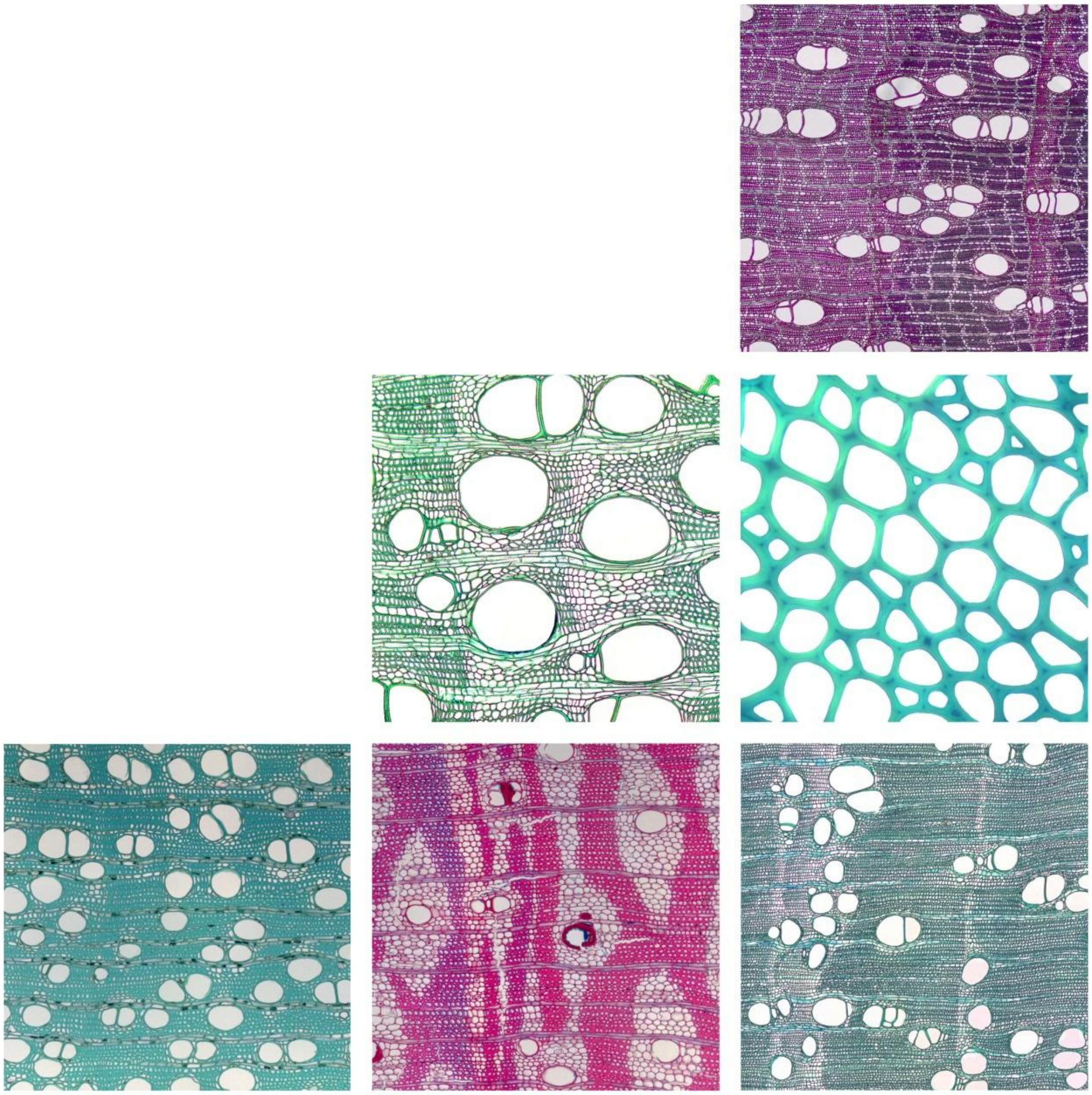

Trees are more than just the green leaves you see

and the hard wood you touch; welcome to the impressive world of trees

Kanin Rungwattana

Acknowledgements

It is a well-known fact that PhD students often face challenging situations. We feel the pressure that comes with the time constraints our projects imposes on us and more often than not we worry about the financial aspects and how to further the project along. I am fortunate to not have to cope with these issues on my own. I have my family, friends and supervisor to thank for their support during the difficult periods. Were it not for the unyielding encouragement and guidance I received during this time, I would have not completed my doctoral dissertation. Here in this text, I would like to express my gratitude to the people that accompanied me during this long journey.

First of all, I would like to express my sincere gratitude to my supervisor, Univ. Prof. Dr. Peter Hietz. He was the support pillar during my work and without him I would have not had success in my endeavor. Peter helped me deepen my knowledge in the area of wood biology and anatomy. My studies in this discipline started from the ground up. I am immensely grateful to the incredible amount of time you spent supervising my project. Thank you greatly for your massive support and advice, in particular with the help you offered me when it came to writing my research proposal. Your invitations to conferences, support in preparing fieldwork activities, collecting wood samples, advice in searching for additional grants and aid with completing my manuscripts were hugely educational and help me gain a deeper insight into the field of wood biology. Dear Assoc. Prof. Dr. Sabine Rosner, my co-supervisor, thank you so much for your guidance and suggestion during my work. Your advice when it came to technical literature has always been very helpful. I have always appreciated the fact that you inquired about my academic progress and I found that we developed a friendship during my time here.

In Thai there is a common phrase that loosely translates to “successful teamwork must lead to an achievement”. I would not have had success in my PhD program without being part of the wonderful plant-ecophysiology laboratory team at the Institute of Botany at the University of Natural Resources and Life Science Vienna. Dear colleagues Danny Tholen, Susanne Scheffknecht, Josef Gadermaier and other team members I apologize for not mentioning explicitly, thank you for being such great companions both as colleagues and as friends. All of you made our working environment feel like home. Susi, many thanks for helping and instructing me with all of the advanced laboratory equipment.

There are also people back home in Thailand whom I would like express my gratitude towards. I would like to thank Assoc. Prof. Dr. Poonpipope Kasemsap who gave me the opportunity to research intraspecific variation in wood structure and function of rubber trees. The starting point of the intraspecific variation in wood structure of rubber trees research initially seemed to be incidental, happening during an unofficial meeting in the time of a short business trip to Vienna. Building on our initial idea, we managed to write an academic paper on the topic “Trait evolution in topical rubber trees is related to dry season intensity”. I would also like to thank Thitaporn Phumichai who provided the phylogenetic information of the rubber clones, Ratchanee Rattanawong who allowed us to work on the Nong Khai rubber plantation and who took care of us during our stay there, Ornuma Duangngam and Dokkeaw Chura who helped me with wood sampling and coordination of the expedition.

Special thanks to my family for their enormous support throughout my entire life. Many thanks to my life partner and all of my lovely friends both in Thailand and inVienna, you made my stay here pleasant so much so that I almost never felt homesick.

Finally I am grateful to the Austrian government for organizing financial support for my PhD program through the OeAD (Austrian Agency for International Mobility and Cooperation in Education, Science and Research). It provided me with one of the greatest opportunities and experiences I have ever had and for that I will always be thankful.

Abstract

A suite of plant functional traits is considered to be of key importance for tree species in forests. The different combinations of traits come about as a result of different functional trade-offs. They reflect a substantial variation in life history strategies of different tree species. Wood performs several functions: mechanical stability, water transport and storage. These functions are provided by different specialized wood cells with a considerable amount of variation in their anatomy. This variation is to be found not only between tree belonging to different species, but also within individual trees belonging to the same species. To better understand the mechanism involved and help explain variation in the wood structure and function, an efficient tool for quantitative wood anatomy is important. In a first manuscript of this thesis a measurement approach is presented that uses a laser scanning microscopic technique, which speeds up sample preparation and provides reliable results, with a clear distinction between the cell lumina and walls.

Wood structure and hence function varies substantially among species, but also within species and individual trees, which is the topic of two main chapters of this thesis. Within-species variation can be due to phenotypic plasticity, ontogenetic variation or intraspecific genetic differences. The latter two factors are addressed in subsequent chapters. One tested how wood variations is affected by tree age or size, and how these ontogenetic trends in wood structures and functions relate to life history strategies of five dominant species from a natural monsoon forest in Thailand. Ontogenetic variations within individual trees are linked to differences in growth trajectories and shade tolerance among tree species. Average wood density (WD) and theoretical hydraulic conductivity (K_h) reflect the phenology, with deciduous and shade- intolerant *Toona* and *Melia* having low WD and high K_h, and shade-tolerant brevi-deciduous *Chukrasia* and evergreen *Neolitsea* having higher WD and low K_h. The radial gradients in WD and K_h also reflect within-species differences in growth rates during ontogeny. Across species, tree size had a stronger effect than age on most parameters.

The third manuscript investigates intra-specific variation of wood and leaf traits in *Hevea brasiliensis* originating from different locations in the Amazon basin planted in a provenance trail in Thailand. Tree size in Hevea was found to have a relatively small contribution to trait variation, while the genotype explained between 30% and 70% of the variation in traits. Rainfall in the driest quarter at the place of origin in Amazon was strongly related to leaf mass per area, carbon isotopic composition and area-based nitrogen content and weaker to wood traits (vessel density and vessel lumen fraction). All traits that were correlated with the climate of the place of origin in the Amazon, showed a significant phylogenetic signal. We found evidence of the trait spectrum and higher growth in trees from drier locations suggesting that

deciduous rubber trees have adapted via drought avoidance rather than tolerance. Giving a comprehensive quantification of intraspecific functional variability within species and within individual trees, these findings suggest that intraspecific functional variability are important to understand ecological strategies of trees and including them opens new opportunities to better understand and predict ecological patterns in a changing environment.

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Chapter 1

General introduction

Kanin Rungwattana

Understanding tropical trees via plant functional traits

Tropical forests are essential for life on earth. The functions of tropical forests can be productive (e.g. timber, fuelwood, rubber, and medicine), environmental (e.g. climate regulation, carbon sequestration and storage, reserve of biodiversity, and soil and water conservation), and social (e.g. subsistence for local population, culture, and education) (Montagnini & Jordan 2005). A vast majority of species that drive these principle productive and environmental services of tropical forests are tropical tree species. Accordingly, forest ecologists, plant physiologists and wood biologists have paid attention and made a great effort to better understand tropical trees. These have often initiated their studies by asking a simple question in the aspect of growth, reproduction, adaptation and interaction of tree species: How do trees grow?; Because a tree is sessile, how does it respond to an environmental variation or herbivores? How do trees compete with others?

Plant functional traits and life history strategies have been considered extensively over the past decades elucidate plant adaptive response to their biotic and abiotic environments (e.g. Thuiller et al. 2004, Wright et al. 2005). A group of functional traits is therefore considered as a key importance among forest trees; for example, maximum height, leaf area, seed mass and wood density. Traits that been suggested to reflect independent plant strategy axes representing fundamental principle of tree anatomy and physiology include tree size, seed size, leaf economics, and wood economics (e.g. Weiher et al. 1999, Westoby et al. 2002, Wright et al. 2004, Chave et al. 2009, Baraloto et al. 2010, Muller-Landau 2010). These traits also represent a trade-off related to competition for resources (e.g. shade tolerance or water and nutrient availability), dispersal, and resistance to pest or physical damage. For example, short-lived leaves require high nutrient concentration and low leaf mass per unit area which increase leaf vulnerability to herbivory and physical hazards, and require high photosynthetic rate per unit mass which drives fast growth. On the other hand, long-lived leaves require the robustness associated with high leaf mass per unit area and low palatability including chemical defenses (Wright *et al.* 2004). Species with dense wood tend to have slower stem growth rate but higher survival because of having higher mechanical stability than species with soft wood (Chave *et al.* 2009). This impacts fitness indirectly via the effects on growth, reproduction and survival, the three components of individual performance (Violle *et al.* 2007).

In this thesis, wood functional traits are mainly considered including wood density and wood anatomical features in particular. Wood density is one of the most frequently used traits. It correlates with tree growth and mortality rate, and it can be easily measured to be used as an indicator of tree life history strategy (Muller-Landau 2004). However, wood density should not be considered solely as predictive parameter in all cases. Ziemińska et al. (2015) stated that “Just as people with the same weight can have different body builds, woods with the same wood density can have different anatomies", implying that species with similar wood densities might actually have various anatomies. The magnitude of this wood anatomical variation might relate to potentially unexplored dimensions of ecological property and tree life history strategies.

What is wood and their functions?

Before the conceptual framework of wood variation is discussed, it is necessary to know just what wood is. Wood, or xylem, is "the hard fibrous substance beneath the bark in the stems and branches of trees and shrubs" (Webster & McKechnie 1980). Wood is a product of the cambium and it consists of cells or wood elements that have passed through various stages of development; all developmental stages of cellular division, differentiation, and maturation taken together constitute wood formation (Larson 1969).

Humans have used wood from the first hunting club or digging tool of ancient man to the great variety of industrial and decorative furniture of modern civilization. Through the ages, although some knowledge, research and experience how to properly use and control wood quality has been passed through from one generation to another generation, but the conceptual domain of this technology is still absolutely important key to shed light on an advancing great deal about wood quality of a manufactured product today and in the future. To improve wood quality (e.g. moduli of elasticity and rupture), ones needs to understand fundamental wood characteristics such as ring width, ring density and microfibril angle (Alteyrac *et al.* 2006). Wood characteristics are basically derived from a biological process occurring within a living tree, whereas wood quality is the somewhat arbitrary evaluation of an isolated piece of wood or wood derivative (Larson 1969). Wood characteristics are the cause, and wood quality is the result. Thus, wood quality can only be modified via wood characteristics, and a better understanding of wood characteristics, including its structure and function, opens the potential to modify wood quality.

Regardless of wood quality and utilization, to understand trees, wood biologists ask how trees benefit from its own wood. Basically, wood performs several functions in plants, including mechanical stability supporting aboveground tissue (Rowe & Speck 2005), transporting sap from roots to leaves (Sperry, Meinzer & McCulloh 2008), and storing water and other essential compounds (Harmon *et al.* 1986; Kozlowski 1992). Because wood needs to balance these different functions, there might be trade-offs among the many potential roles that the wood can play (Chave *et al.* 2009). For example, species with low hydraulic capacitance and denser wood experienced greater daily maximum xylem tension and structural adaptation to avoid embolism at these tension, whereas species with higher capacitance and less dense wood exploit stored water to avoid the transient high maximum xylem tensions (Meinzer *et al.* 2009). How trees optimize the functional trade-offs responding to their needs has been explored by many studies, and these showed that wood anatomy is of a key importance to the diversity of trees across a range of ecological settings (Feild *et al.* 2000; Choat, Cobb & Jansen 2008).

Angiosperm wood is built up of three main cell types that serve different functional roles: fibres provide primarily mechanical strength; vessels provide longitudinal water transport; and parenchyma, as the only living cells, produces defence reactions, stores carbohydrates and other essential compounds and serves for local radial transport. Additionally, the alignment, number and size to these cell elements might be subject to different functional trade-offs. Not only the stem cross-sectional area occupied by vessels affects hydraulic conductance, but also the size and number of these vessels are related. Wilder vessels conduct more efficiently (Sperry, Hacke & Pittermann 2006), whereas narrower vessels imbedded in a matrix of dense tissue contribute to higher hydraulic safety due to less risk of vessel implosion (Hacke *et al.* 2001). For this conceptual framework, quantitative wood anatomy therefore plays an important role in addressing research questions related to plant functioning, growth, and environment.

Wood variation

Wood is very variable, with difference occurring among species and genera, among geographic sources within a species, among trees within a geographic source as well as within each individual tree (Zobel & Van Buijtenen 1989). The great need for research into the variations in wood characteristics is emphasized by nearly every wood scientist. Sometimes wood variation brings benefit to tree breeder, and a range of wood characteristics are of interest for selective breeding programs to improve wood quality (Louzada & Fonseca 2002). On the other hand, less variation or greater uniformity will help avoid inefficiency and variation in the quality of the end product of wood processing (Zobel & Van Buijtenen 1989). Independent of wood utilization, ecologists are paying attention to wood variations to better understand the success or failure of plants responding to an environment (e.g. Falster and Westoby 2005, Jacobsen et al. 2007, Poorter et al. 2010).

Interspecific variation

The distribution of wood characteristics among species and genera is theoretically expected to vary considerably and relate to variation in strategies to survive in different environments. An example of interspecific variation in wood anatomical structure for tropical tree species is illustrated in Fig. 1. Differences in the structure of xylem affect interspecific variation in the ability of woody plants to survive under an extreme environment, in particular drought, by embolism resistance (Maherali, Pockman & Jackson 2004; Sperry *et al.* 2006). Drier regions were expected to favor more drought-prone species with more resistance to drought-induced cavitation, whereas in well-irrigated zones the moisture preference species exhibit higher hydraulic conductance but less cavitation-resistance (Brodribb, T. 1999; Tissier *et al.* 2004). However the relationship between embolism resistance and habitat preference are not always present; for example, some species growing in drier environments have low embolism resistance, similar to species growing in areas of high rainfall (Choat *et al.* 2012). If this association appears weak, further understanding of evolutionary adaptation and ecological significance for a large number of tree species may provide a better understanding. Clearly, embolism resistance and wood characteristics are not the only traits relevant for successfully living under dry conditions.

Intraspecific variation

Functional trait-based analyses generally assume that differences in trait values are much larger for interspecific than for intraspecific comparison (Reich *et al.* 1999; McGill *et al.* 2006), assuming that species can be reasonably characterized by their unique trait values and consistent and meaningful species rankings (Garnier *et al.* 2001; Al Haj Khaled *et al.* 2005). However many studies demonstrate that intraspecific trait variation of plants in response to environmental factors is greater than previously assumed (Jung *et al.* 2010; Messier, McGill & Lechowicz 2010; Albert *et al.* 2010b). If intraspecific trait variation is large but often lower than interspecific variability (Albert *et al.* 2010a), it is likely to have important ecological consequences and should not be neglected. Variation in wood can also occur among geographic sources of seed or locations where the trees are grown. Sometimes the wood produced differs among sites within a location. Until recently, wood ecologists have paid attention to intraspecific variation in wood structure across climatic zones or along environmental gradients to make a better understanding of evolutionary adaptation in tree species (e.g. Eilmann et al. 2014, Hajek et al. 2016). Fig. 2 illustrates the variation in wood anatomical structure for *Hevea brasiliensis* originating from different provenances along environmental gradient (see chapter 3 for details). There is also growing evidence that intraspecific functional variation can have significant effects on community dynamic and ecosystem functioning (Boege & Dirzo 2004; Crutsinger *et al.* 2006; Lecerf & Chauvet 2008) and the rapid adaptation of species to novel environment (Urbanski *et al.* 2012). Integrating intraspecific trait variation for trait-based research in community ecology thus has the potential to strengthen our understanding of processes operating at the community and ecosystem levels.

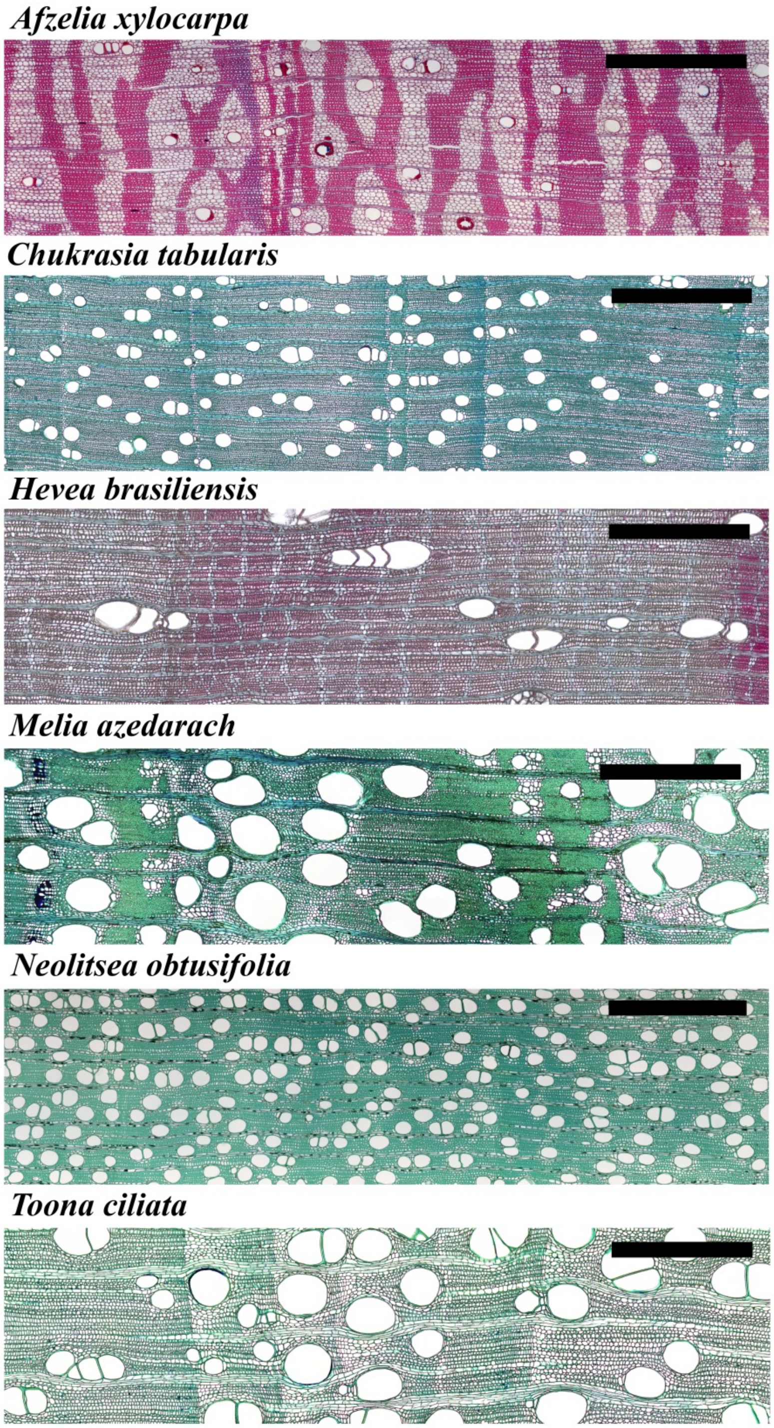


Fig. 1 Examples of interspecific variation in wood anatomy of six tropical tree species: *Afzelia xylocarpa* (with high wood density, very thick-walled fibres, low vessel density and paratracheal axial parenchyma), *Chukrasia tabularis* (with medium wood density, medium vessel density, axial parenchyma band), *Hevea brasiliensis* (with medium wood density, low vessel density and axial parenchyma in narrow band), *Melia azedarach* (semi-ring porous wood, medium wood density and wild vessels), *Neolitsea obtusifolia* (with medium wood density, high vessel density and small vessels), and *Toona ciliata* (low wood density, very thin-walled fibres and wild vessels). Bars, 1 mm.

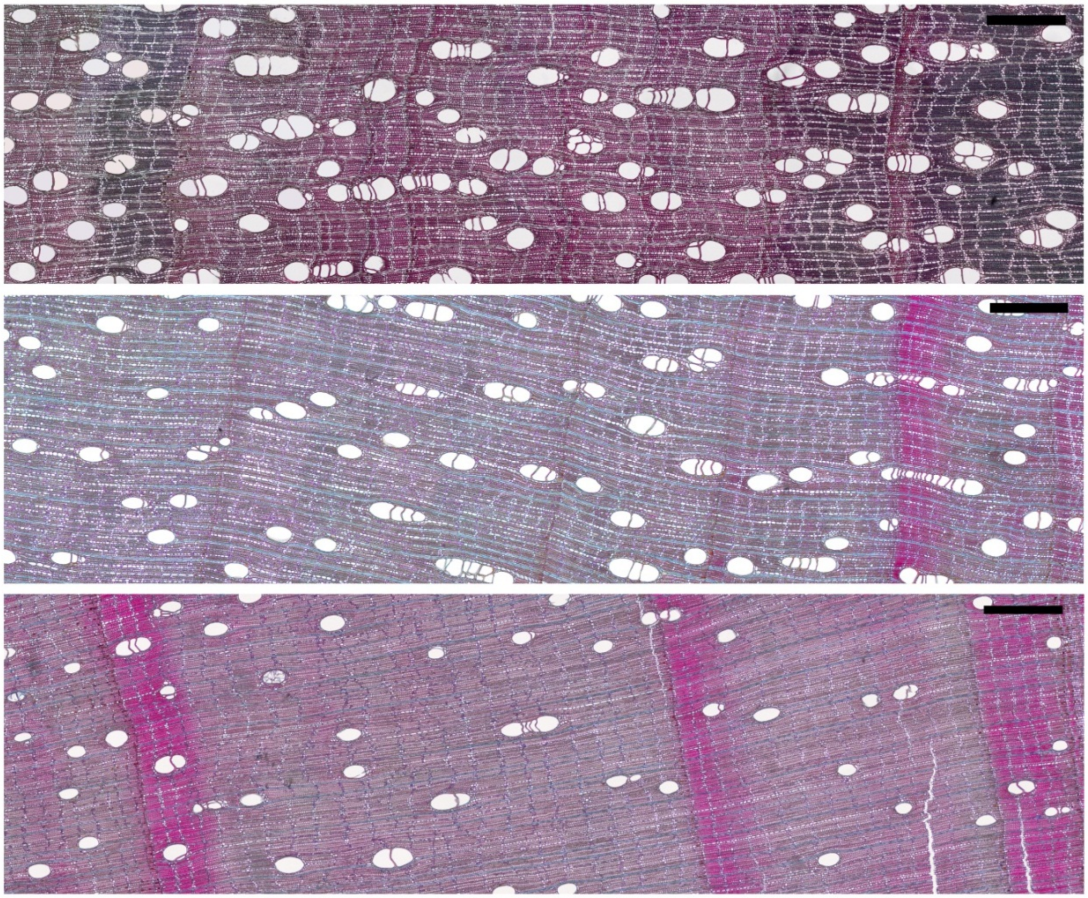


Fig. 2 Example of intraspecific variation in wood anatomy for *Hevea brasiliensis* originating from different provenances. Vessel density (i.e. number of vessels per area) can vary greatly among trees of the same species and exactly at the same age. To the top, wood represents high vessel density (4.46 mm^{-2}), in the center (3.18), and to the bottom (1.48). Although vessel sizes for all three woods show fairly similar, the different values for vessel will affect hydraulic conductivity. Bars, 1 mm.

Ontogenetic variation within individual trees

Wood records a tree's entire ecologically relevant history. As trees are long-living organisms, they are confronted with natural disturbances (such as fire, flooding or drought events) and a long series of climate change, which can sometimes be thousands of years. This environmental variation causes a considerable variation in wood anatomical, chemical and physical properties not only between different plant parts (such as stems, branches, and roots), but also within any given plant part. The well known and most studied within-tree variability in wood is the progressive change from the pith (the tree center) to the bark, which is frequently referred to as "the core/outerwood or juvenile/mature wood pattern" or "the typical radial pattern” (Lachenbruch, Moore & Evans 2011b). These patterns are largely independent on the effect of environmental variation. In conifers, growth rings near the pith usually consist of a large proportion of early tracheids, which have larger diameter and thinner cell walls than latewood tracheids, that gradually transitions to a larger proportion of latewood tracheids with increasing ring number from the pith (Lachenbruch *et al.* 2011b). There is no universal position within a stem to define a transition zone from corewood to outerwood because wood properties gradually change depending on individual trees and locations (Cown, McConchie & Young 1991; Fabris 2000). An example of the radial gradient of wood anatomical structure for, e.g. *Toona ciliata* is illustrated in Fig. 3 (see chapter 4 for detail).

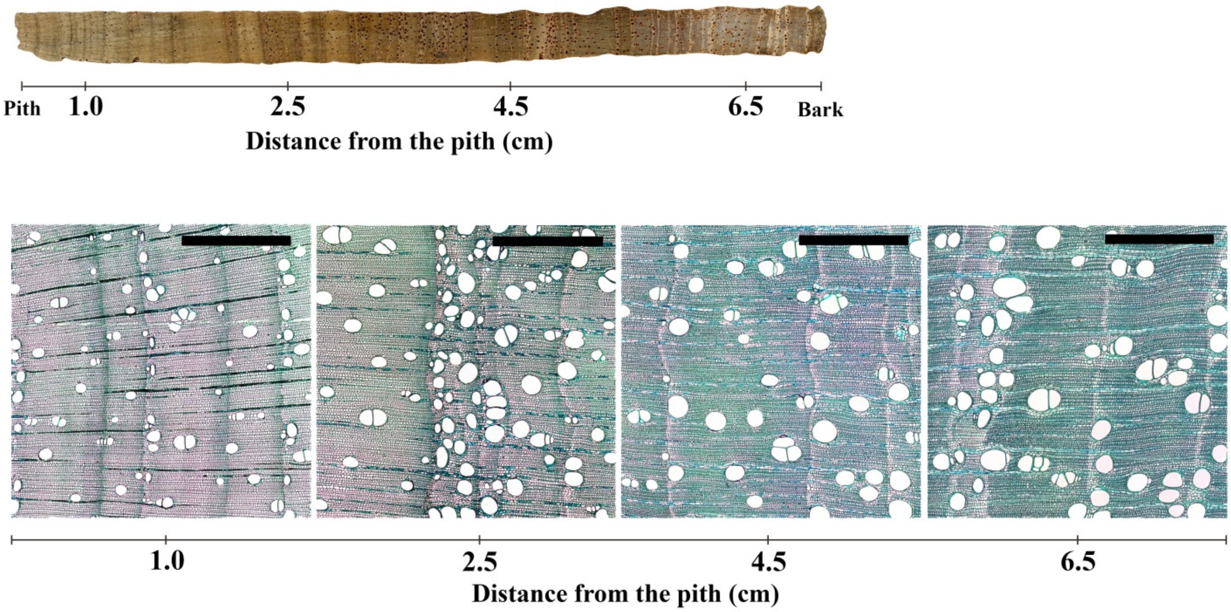


Fig. 3 Example of wood radial variation for increment core from the pith to the bark of *Toona ciliata*. The juvenile wood at the far-left being close to the pith center has smaller vessels and very thin-walled fibres, whereas the mature wood at the far-right has wilder vessels and thicker-walled fibres. Along this radial gradient, wood anatomy appears to change gradually to serve an optimal balance for a variety of potential functions during tree ontogeny. Bars, 1 mm.

Wood properties at a specific location within a tree represent adaptive solutions to ensure an optimal balance among the potentially competing functions of support, transport and storage, and responds to internal (e.g. larger crown to sustain and more leaves to supply with water) and/or changing environmental (e.g. from humid shade to dry light of crown or roots in shallow vs. deep soil) influences throughout their lives of a tree. Lachenbruch *et al.* (2011) proposed three hypotheses for why trees develop radial changes in wood properties (Fig. 4): (a) developmental constraint, (b) the different water transport requirements in small and large trees, and (c) the optimal mechanical construction of trees of different sizes. The hypothesis of developmental constraint states that the pattern of radial change is controlled by the age of the cambium. This suggests that a developmental constraint prevents young cambium from producing the optimal wood from the early stage of life and allows mature cambium to produce adaptive wood (e.g. wider lumen diameter, thicker cell wall, and low microfibril angle) for optimization of the tree. Several studies reported that the transition from corewood to outerwood was more strongly correlated to cambial age for WD in two species of southern pines (Clark & Saucier 1989) and for tracheid length in two species of spruce (Yang 1994). The hypothesis of hydraulic constraint states that the pattern of radial change allows juvenile wood to have higher resistance to hydraulic failure (Domec & Gartner, 2002; Rosner *et al.*, 2008, 2009; Domec *et al.*, 2009) and permits mature wood to have higher specific hydraulic conductivity (Spicer & Gartner 2001; Domec & Gartner 2002). In young trees xylem water potential tends to be more negative because they have less readily available stored water and lower uptake capacity due to their small root system. Older trees require higher hydraulic conductivity to compensate for the increased resistance caused by the longer conduit path. For example in Norway spruce, higher resistance to embolism was pronounced in corewood (Rosner 2006) and was related to small pit apertures, low pit frequencies and narrow tracheids (Pittermann & Sperry 2003; Domec, Lachenbruch & Meinzer 2006). Lastly, the hypothesis of mechanical constraint states that the pattern of radial change enables a tree to shift its strategy to mechanically adjust in response to different loads in various part of a tree. Corewood has more flexibility to reduce the associated drag in response to wind and snow (Bertram 1989), whereas outerwood has more rigidity needed to maintain adequate mechanical stability (King 2011) for their self-weight as well as from applied loads. The flexibility of small stems and branches was substantially related to structural features such as high microfibril angle, low fibres wall thickness, and low wood density (Cave & Walker 1994; Barnett & Bonham 2004; Lachenbruch *et al.* 2010). For example in Norway spruce, the variation in microfibril angles provides an optimum combination of extensibility (high angle in corewood) and stiffness (low angle in outerwood) in response to the actual mechanical demands on each ontogenetic stage of the tree (Reiterer, H. Lichtenegger, S. Tsche 1999).

If these wood properties reflect adaptations to the tree's needs, the change in wood structure and function along tree-ring sequences can be used to elucidate how individual trees and species respond to a changing environment, where environment refers to all external factors including climate, latitude, soil properties etc. and also internal influences, at different stages of their lives.

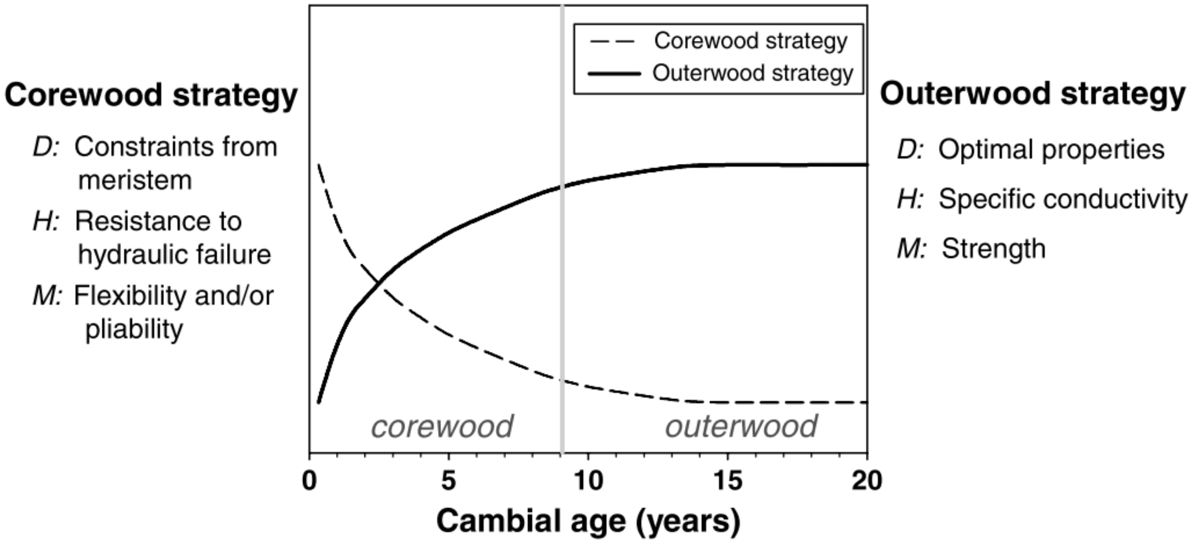


Fig. 4 Schematic diagram of three possible hypotheses why trees develop radial change in wood properties: developmental *(D)*, hydraulic *(H)*, and mechanical *(D)* constraints. The changing strategy for corewood is represented as dashed lines and left axis, whereas the strategy for outerwood is represented as solid line and right axis. (Credit: Lachenbruch *et al.* 2011b)

Causes and consequences of wood variation

In assessing the extent and role of intraspecific trait variation in evolutionary adaptation, ecological significance and life history strategy, it is important to recognize that trait variation arises from multiple mechanisms, including heritable genetic variation, phenotypic plasticity, tree age and tree size.

Genetic variation and phenotypic plasticity

The mechanisms of phenotypic adaptation to a changing environment are well-known to be Darwinian evolution by natural selection on genetic variation, and phenotypic plasticity through environmental influence on individual development. Phenotypic plasticity provides a mechanism by which species can tolerate wide environmental heterogeneity without genetic change (Via 1994). Species with a high degree of phenotypic plasticity are more likely to enhance the potential of their ecological success and their impact across ecosystems (Daehler 2003; Hulme 2008). Plasticity can also evolve by natural selection (Via *et al.* 1995) and genetic variation contributes to the degree of plasticity (Falconer 1990). Genetic diversity as well as phenotypic plasticity may contribute to the potential of a species expanding to a novel environment (e.g. Sexton et al. 2002, Lande 2009).

The ability of an introduced species to successfully tolerate and colonize over a broad range of geographic areas has been basically related to two main mechanisms: (1) broad environmental tolerance, that is, successful non-native species possess life history strategies that confer superior colonizing ability and/or phenotypic plasticity allowing acclimation to a wide range of habitats; (2) local adaptation, that is, successful non-native species rapidly adapt to new local selective pressures (Schlichting 1986; Williams & Black 1993) such as drought, low or high temperature, and elevation. A schematic diagram incorporating phenotypic plasticity and local adaptation of introduced species to a novel environment is illustrated in Fig. 5. Sexton et al. (2002) summarized the conceptual framework from the ability of phenotypic plasticity in an individual genotype to the local adaptation at the population level. Initially, phenotypic plasticity allows introduced species the environmental tolerance to become naturalized across a range of habitats (Baker 1974). Instantly the species is naturalized, a subset of the introduced genotypes may be favored by local selection. Eventually, a combination of gene flow, mutation, and recombination of genetic variation among introduced genotypes selected by given local selective pressure can provide a range of heritable phenotypes that are closer to the optimum for the novel habitat and produce offspring with higher fitness (Ellstrand & Schierenbeck 2000). The strong selection during the naturalization phase may provide an adequate genetic variation to create a locally adapted population (Antonovics 1976). Although species introductions often involve population bottlenecks, non-native species often represent astonishingly high degree of genetic variation (Barrett & Richardson 1986). With redundant genetic diversity, an evolutionary adaptation has been predicted to be an important mechanism for the rapid expansion across environmental heterogeneity.

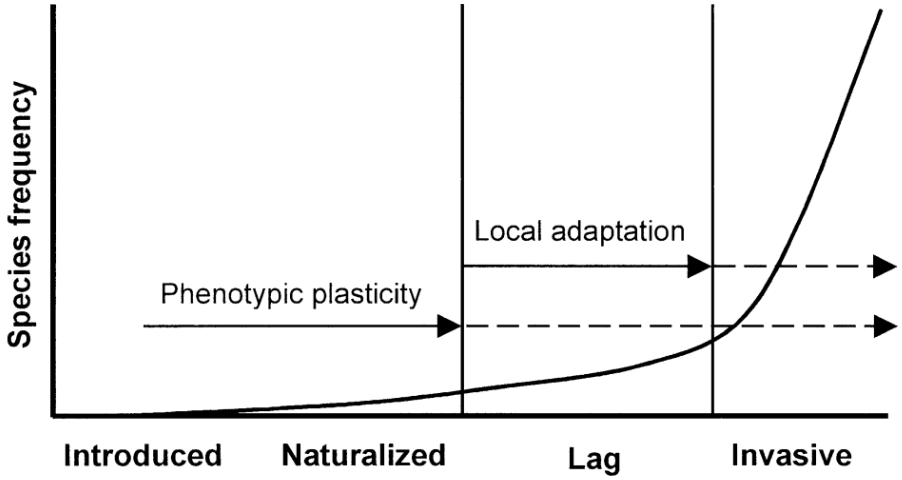


Fig. 5 Schematic diagram of species introduction. Introduced species tolerate a large range of environmental heterogeneity via phenotypic plasticity and colonize over a novel habitat via local adaptation. Since individuals are translocated to a novel environment, a subset of these species potentially acclimates to this condition through phenotypic plasticity. Once the introduced genotypes are fully naturalized, they reproduce offspring with higher fitness and create locally adapted population, which fully flourish into the novel habitat. Sometimes these species may not only survive in their new habitat, but become invasive, dominating the new community and even causing the extinction of native species through rampant growth and/or indirect effects. (Credit: Sexton *et al.* 2002)

As local adaptation results from various processes with genotypic and phenotypic responses being the main drivers, scientists were searching for an appropriate method to define which factor is responsible for the trait adaption. A common garden experiment tests for the local adaptation signals in traits of interest since it enables to identify the genetic basis of complex phenotypes across various populations without the confounding effects of environmental variation (Kawakami *et al.* 2011; Brachi *et al.* 2013; Villemereuil, Gaggiotti & Mouterde 2016). Recently, few studies have identified the intraspecific variation in local adaptation of tree species (Eilmann *et al.* 2014; McKown *et al.* 2014; Hajek *et al.* 2016) and the contributions of genetic predisposition *vs.* phenotypic plasticity (Schreiber, Hacke & Hamann 2015). The results showed differences in wood anatomical traits between drought-susceptible provenances and drought-tolerant provenances, with the latter group having an efficient water transport system to cope with dry condition during severe drought events (Eilmann *et al.* 2014). This certainly supports the hypothesis of genetic control of these features. However, the genotype does not control all wood traits; only three ecophysiological traits investigated (vessel density, the xylem pressure causing 88% loss of hydraulic conductance and mean leaf size) out of 22 traits showed significant genetic differentiations between provenances (Hajek *et al.* 2016).

If wood anatomical traits show no significant differences among provenances, this theoretically translates into no adaptation, but in reality this does not mean that traits had no evolutionary adaptation to local environment. The alternative reason can be that the high variance within a given provenance dominated inter-population variability (e.g. found in δ^{13}C Gornall & Guy 2007; Hajek *et al.* 2016). Explanations for a high degree of intra-population divergence of ecophysiological traits within a provenance might include a large environmental heterogeneity over short geographical distances (Brousseau *et al.* 2013). These suggest a high phenotypic plasticity to cope with climate change across environmental gradients.

Tree age vs. Tree size

Trees have evolved wood that functions appropriately for stresses encountered at each stage of the growth. Apart from genetic control and phenotypic plasticity, wood properties along radial changes from pith to bark are simultaneously controlled by the other mechanisms. These mechanisms can either be controlled by age of the cambium or controlled by factors that vary as tree increases in diameter or height. In wood biology, this fixed and plastic mechanisms are discussed as the age vs. size effects. Elucidating whether tree age or tree size is more related to wood properties along the radial change helps to understand the factors that influence wood structure and function. A size effect could be explained by the changing demand for mechanic and hydraulic functions of wood, by contrast an age effect could point to an intrinsic control of wood development such as the number of dormancy periods experienced (Lachenbruch *et al.* 2011b). To distinguish age vs. size-related effects, one can use either plant material with a common age that differs in size or trees of the same size that differ in age. In a recent report, the radial changes in tropical trees of different size but the same age was investigated. Some studies found wood specific gravity more closely related to age than to size (de Castro, Williamson & Jesus 1993; Williamson & Wiemann 2010). Also Kojima et al (2009) tested a study testing age vs. size dependence for fibres length by selecting trees in each species to represent a range of diameters at breast height but the same age, namely 14 year-old plantation for Eucalyptus grandis and 11 year-old plantation for Acacia auricuriformus. The results showed that fibres length was more closely related to age than to size in *Eucalyptus grandis*, whereas in *Acacia auricuriformus* it depends on tree size, not cambial age (Fig. 6). In the case of age-dependence, a time-related metric such as the number of dormancy periods experienced would trigger the cambium to produce wood in *Eucalyptus grandis*; on the other hand in *Acacia*, a size-related algorithm such as number of periclinal divisions that the cambium has undergone would determine wood structure and function in *Acacia auricuriformus.*

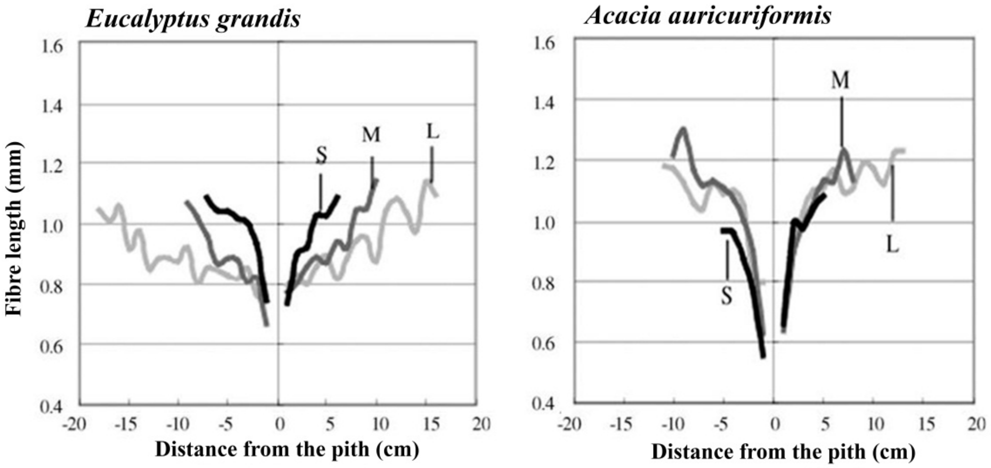


Fig. 6 Typical patterns of radial change of fibre length from three different size classes of diameter trees: small (S), medium (M) and large (L), but at the same age. *Eucalyptus grandis* showed dependence of fiber length on cambial age, not tree size, whereas *Acacia auricuriformis* showed dependence of fiber length on tree size, not cambial age. (Credit: Kojima *et al.* 2009)

However in many cases, distinguishing age vs. size-related factors for wood structure and functions is difficult because age and size are correlated with one another (Lachenbruch, Meinzer & Dawson 2011a). And in tropical trees that do not form annual tree rings, tree age is impossible to assess (Affan Abdul Azim & Okada 2014). Although a number of studies reported growth rings in tropical trees and their potential for tropical dendrochronology have been reported (e.g. Roig et al. 2005, Maingi 2006, Worbes 2011), knowledge of tree age of tropical trees and its relation to wood structure is poorly studied.

Life history strategy

Over the course of evolution, wood structures initiate different potential functions across a huge range of ecological settings (Sperry *et al.* 2008; Chave *et al.* 2009; Carlquist 2013), reflecting a substantial variation in life history strategies of tree species. The variation in life history strategies within communities contributes to the maintenance of diversity (Loehle 2000; Wright & Wright 2002) resulting in a high potential capacity to respond to natural selection pressures imposed by the abiotic and biotic environment. Across a diversity of life history strategies, shade tolerance of juvenile tree is one the most important for tropical tree species, in closed forest in particular (Poorter, Bongers & Bongers 2006). Different degrees of shade tolerance allow tree species to partition the horizontal light gradient in the forest understory (Kitajima & Poorter 2008). Several studies in wood functional traits found wood density related to growth and morality (Muller-Landau 2004; Wright *et al.* 2010) and vessel traits to growth (Poorter *et al.* 2010; Rungwattana & Hietz 2018). Wood density and vessel traits therefore appear to be critical component of tree performance and have been used in relation to life history strategies of tropical tree species. Fast-growing and light-demanding species tend to have low wood density (Nock *et al.* 2009; Poorter *et al.* 2010), facilitating rapid canopy ascension because of cheap volumetric construction costs of the wood, and wide vessels reflecting high hydraulic conductance (Castro-Díez *et al.* 1998). By contrast, shade-tolerant species have higher wood density, contributing to slower growth but higher survival because of greater biomechanical and hydraulic safety (Hacke *et al.* 2001).

Main objectives

Main objectives of my research projects were the following:

(a) To assess the usefulness for quantitative wood anatomy, we tested a method using an advanced laser scanning microscope to distinguish cell walls from lumina and shorten the processing time needed, while still obtaining accurate results. (b) Because intraspecific trait variation is essential to the fundamental process of evolutionary adaptation, I investigated which mechanisms (genetic relatedness, phenotypic plasticity, tree age, and/or tree size) control intraspecific trait variation and which climatic factor (temperature, precipitation, drought intensity) shaped intraspecific adaptation. (c) To determine the relationship among plant functional traits for the intraspecific variability and compare them with interspecific variability. (d) To assess the radial variation from pith to bark within individual tree species. Such wood radial variation analysis is needed for a better understanding of the patterns of critical wood functions (i.e. mechanical support, water conductance, and storage) and differences in the species' growth strategies during different stages of their lives. (e) I tested whether radial variations more controlled by tree age or tree size to understand a trees' potential to respond to their demand.

Study tree species

In this thesis, wood variation was accessed by using the six different tree species all collected from Thailand. Five are native to Thailand and were collected from a natural forest (Huai Kha Khaeng Wildlife Sanctuary). *Afzelia xylocarpa*, *Chukrasia tabularis*, *Melia azedarach*, *Neolitsea obtusifolia* and *Toona ciliata* are dominant forest trees (based on basal area or frequency Baker et al. 2005) The sixth (rubber tree *Hevea brasiliensis*) was planted in a provenance trial in north-eastern Thailand (Nong Khai Rubber

Research Center) with clones originally collected from the Amazon basin in Brazil. Studied tree species are shown in Fig. 7. The six tree species here belong to different families and represent a variety of light-demanding requirements and leaf phenology characteristics: from shade-tolerant evergreen to very shade-intolerant deciduous species (Table 1).

Afzelia xylocarpa (Kurz) Craib is a shade-intolerant deciduous tree with a large stem diameter and broad spreading crown. It commonly grows up to 25 m tall. The bole divides characteristically between 4-8 m height into two to four main spreading branches. The leaves are leathery. Fruit falls in August and the tough persistent pods are obvious on the ground much of the year. Typically it is found along streams in dry forests. It is very poorly regenerate in a natural habitat. (Bunyavejchewin *et al.* 2009)

Chukrasia tabularis A. Juss. is moderate shade-tolerant brevi-deciduous tree. Tree commonly grows up to 100 cm of the diameter at breast height (dbh) and 40 m tall. The bole is cylindrical and flared at the base with short rounded buttresses. The bark is dark brown and black with deeply furrowed. The inner bark is red and white with neither sap nor odor. The leaves are paripinnate. The leaflets are mostly subopposite with glabrous, dark green. It is superficially similar to *Toona ciliata*, but differs in fine venation. (Bunyavejchewin *et al.* 2009)

Hevea brasiliensis (Willd. Ex A. Juss.) Müll. Arg. is a fast-growing, shade-intolerant deciduous tree. A tree may grow to over 40 m and live for over 100 years in native habitat; however, it rarely exceeds 25 m in plantation because growth is reduced by tapping for rubber and because it is usually replanted after 25-35 years when yields fall to an uneconomic level. The trunk is cylindrical, but frequently swollen towards the base, and the bark is pale to dark brown with a smooth surface and the inner bark pale brown with abundant white or cream colored latex. The leaves are in spirals and with three leaflets. The flowers are small with no petals, bright or cream-yellow in color and extremely pungent. They are either male or female but both are found in the same inflorescence. The mature fruit is a large 3-lobed capsule, 3-5 cm in diameter, having a woody endocarp and a thin, leathery mesocarp and containing 3 seeds. (Baulkwill & Webster 1989)

Hevea brasiliensis is native to the Amazon basin (Pires, Secco & Gomes 2002). It mainly occupies the region south of the Amazon, extending to Acre, Matto Grosso and Parana areas of Brazil and into parts of Bolivia and Peru. Rubber tree is planted widely in present-day in a number of South-East Asian countries as a plantation crop for natural rubber production. Tapping of rubber trees starts in the fifth to seventh year after planting and then continues for 25 to 35 years. After 35 years when latex production of trees declines, trees are logged and used for wood as secondary product of interest.

Melia azedarach L. is a large fast-growing, very shade-intolerant deciduous tree. The bole is cylindrical. The bark is bright brown, with sharp deep fissures. The leaves are compound with tripinnate. (Bunyavejchewin *et al.* 2009)

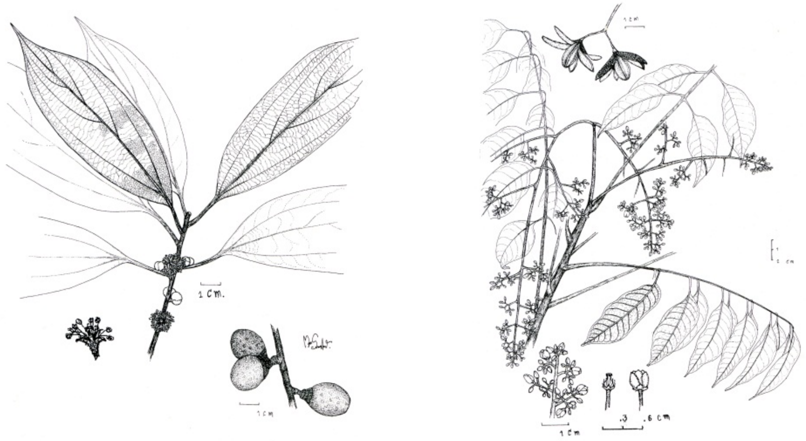
Neolitsea obtusifolia Merrill is shade-tolerant evergreen tree with a large stem diameter. It can grow up to 90 cm dbh with not very tall stem. The bark is variable with flakey grey-brown to very broad and shallowly furrowed. The leaves are in upturned clusters with a bit glaucous at the abaxial surface (Bunyavejchewin *et al.* 2009)

Toona ciliata M. Roem. is fast-growing, shade-intolerant deciduous tree. Tree commonly grows up to 1 m dbh. The bark is black with slightly furrowed, thick and flakey. The inner bark is red and white with strong cedar-like scent. The fruit is narrow capsules with about 2 cm by 1 cm in size. (Bunyavejchewin *et al.* 2009)

It was hoped that this variation of selected tree species will provide insight into wood ecology and a better understanding of the different life history strategies through their variations in wood structure and functions.

Afzelia xylocarpa   ***Chukrasia tabularis***

Hevea brasiliensis   ***Melia azedarach***



Neolitsea obtusifolia   ***Toona ciliata***

Fig. 7 The botanical illustrations of six studied tree species: *Afzelia xylocarpa*, *Chukrasia* tabularis, *Melia azedarach*, *Neolitsea obtusifolia*, *Toona ciliata* (photo credit: Bunyavejchewin et al. 2009) and *Hevea brasiliensis* (Credit: Baulkwill and Webster 1989)

Table 1 Characteristics of the six studied tree species with their light demand(SI, shade-intolerant; MST, moderately shade-tolerant; VSI, very shade-intolerant, ST, shade-tolerant), leaf phenology (D, deciduous; B, brevi-deciduous, E, evergreen), the diameter of breast height (dbh), tree age, and chapters in which they are included.

Species	Family	Light-demand[1]	Phenology[2]	DBH (cm)	Age (year)	Chapter
Afzelia xylocarpa	Fabaceae	SI	D	37-166	25-276	4
Chukrasia tabularis	Meliaceae	MST	B	9-80	25-123	2,4
Hevea brasiliensis	Euphorbiaceae	SI	D	15-38	23	3
Melia azedarach	Meliaceae	VSI	D	28-94	6-84	2,4
Neolitsea obtusifolia	Lauraceae	ST	E	22-70	na[3]	4
Toona ciliata	Meliaceae	SI	D	16-74	24-121	2,4

[1]Light-demanding requirements for all tree species (Baker *et al.* 2005), except for *Hevea brasiliensis* (Mostacedo & Fredericksen 1999); [2]Leaf phenology for all species (Williams, Bunyavejchewin & Baker 2008), except for *Hevea brasiliensis* (Chen *et al.* 2009); [3] *Neolitsea obtusifolia* had anatomically indistinct annual growth rings, tree age therefore could not be calculated in species.

Thesis outline

This thesis consists of four chapters: a general introduction (Chapter 1), three research chapters (Chapter 2-4) and a general conclusion.

Chapter 2, has been nearly submitted to International Association of Wood Anatomists (IAWA journal) with the title of "An alternative method for quantitative wood anatomy using laser-scanning profilometry". Producing high-quality thin sections for quantitative analysis of wood can be challenging and time-consuming. We thus presented a technique using a laser scanning microscope to visualize and measured anatomical feature, in particular cell walls and lumina. We compared measurements based on surface images taken with a laser scanning microscope with thin sections based on conventional method and analyzed images either with mostly automated image analysis or by manually correcting imprecisions. Comparing these two methods was based on accuracy and efficiency in terms of image quality and the time required. This technique allows to obtain reliable quantitative data of xylem anatomy. If this method is more efficient or precise than thin sections depends on the anatomical details measured.

Chapter 3 has been accepted for publication in *Functional Ecology* (Rungwattana K, Kasemsap P, Phumichai T, Kanpanon N, Rattanawong R and Hietz P. Trait evolution in topical rubber (*Hevea brasiliensis*) trees is related to dry season intensity). We used a provenance trial of rubber trees (*Hevea brasiliensis*) to control for potentially confounding effects of phenotypic plasticity, tree age and size, and tested intraspecific evolutionary adaptation for rubber tree clones originating from 15 different locations in the Amazon basin in Brazil. Relating trait variation to the phylogenetic relationship among the clones and the climate at their places of origin, provides insight into local genetic adaptation, particularly to drought.

Chapter 4 has been published in *Functional Ecology (*Rungwattana K & Hietz P, 2018. Radial variation of wood functional traits reflect size-related adaptations of tree mechanics and hydraulics. Funct Ecol. 32:260–272.). While the previous chapter looked at trait variability within species, this chapter investigates trait variation within trees on a basis of radial variation within individual trees. We quantified wood density and wood anatomical feature for five canopy tree species in western Thailand to examine the pattern of wood function together with differences in the species' growth trajectories during ontogeny. We took advantage of the species that formed distinct annual rings to test whether tree age or tree size mainly controlled wood properties. These frameworks helps to better understand the variation in life history strategies of different tree species.

Chapter 2

An alternative method for quantitative wood anatomy using laser-scanning profilometry

Kanin Rungwattana, Susanne Scheffknecht**,** Peter Hietz

to be submitted to IAWA Journal

An alternative method for quantitative wood anatomy using laser-scanning profilometry

Kanin Rungwattana, Susanne Scheffknecht and **Peter Hietz**

Institute of Botany, University of Natural Resources and Life Sciences Vienna, 33 Gregor-Mendel Strasse, Vienna 1180, Austria

Abstract

Producing high-quality thin sections for the quantitative analysis of wood can be challenging and time-consuming. We here describe an alternative technique to visualize and measure cell walls and lumina using a confocal laser scanning microscope. Measuring the distance between the lense and the object at high resolution in the xy-plane, we obtain a 3D image that permits a clear distinction between cell walls and lumina. Anatomical images can be obtained using the strength of the laser signal, the distance in the z-axis or a combination of both. This method still requires a clean cut with a microtome, but no thin section and no staining and embedding of samples. We measured vessel area, fibre lumen percentage and fibre wall thickness in three hardwood species. We compared measurements based on surface images taken with a laser scanning microscope and with thin sections and analyzed images either with mostly automated image analysis or by manually correcting imprecisions. Comparing the accuracy and efficiency of both methods shows that the laser scanning technique produces reliable result and can safe time.

Keywords

Automated image analysis, Cell structure, Fibre characteristics, Surface profile, Vessel characteristics

Introduction

The quantitative analysis of wood cell lumina and walls is essential for studies in comparative wood anatomy, tree ring research or tree physiology. For instance a phylogenetic analysis revealed that smaller and safer conduits (vessels or tracheids) were an essential adaptation for woody, evergreen angiosperms to freezing temperatures (Zanne *et al.* 2014). As the environment influences cell sizes and wall thickness during wood formation, anatomical details other than ring width can be used in dendrochronology to reconstruct climate or other environmental changes (Fonti *et al.* 2010). Although conducting elements in wood do not really implode, the theoretical implosion resistance, estimated from wall thickness and conduit diameter, has been shown to be a good proxy for xylem vulnerability to cavitation, an important component of drought resistance in plants (Rosner *et al.* 2016).

Cell sizes or other features on wood anatomical images can be measured manually by tracing cells on an image, or automated using image analysis software, which requires a clear distinction between cell walls and lumina. Various algorithms for image analysis have been developed for wood anatomical studies, often tuned to the needs of specific projects. Automated image analysis may miss-interpret features, so some control appears warranted. Brunel *et al.* (Brunel *et al.* 2014) presented an image analysis algorithm that is much faster than manual measurement and also includes a reliability indicator that highlights potentially problematic areas on the image. A method with a 99% accuracy of automated vessel identification was also developed for birch and poplar wood (Chen & Evans 2010) and 98% of all vessels of a root were correctly identified through a root xylem analysis system (Von Arx & Dietz 2005). Image analysis solutions for the analysis of wood features are typically plugins or macros for flexible general purpose image analysis software such as ImageJ (e.g. Gurau *et al.* 2013) or Image Pro Plus (Media Cybernetics, Rockville, USA; e.g.Von Arx & Dietz 2005).

Whether for automated or manual measurements, images are usually produced with transmission light microscopes from thin sections. For any image analysis approach, but particularly for automated measurements, a clear distinction between cell walls and lumina is essential. This can be challenging for wood that is difficult to cut thin, or if cell dimensions are small, because any wall not parallel to the optical axis will fail to produce a sharp wall-lumen boundary, which often results in walls appearing too thick. Most automated wood anatomical measurements measure features that are large (vessels) or samples that are relatively easy to cut (softwood), but not, for instance, small and thick-walled hardwood fibres. There are many ways to improve the quality of sections and images (e.g. Barbosa *et al.* 2010; Gärtner & Schweingruber 2013) and ultra-thin sections generally yield high-quality images. However, all of these increase the time required, so that there is a trade-off between the time invested in sample preparation, which increases for difficult specimens, and the quality of images.

Confocal laser scanning microscopy (CLSM) allows optical sectioning to obtain images from near the surface of wood blocks. CLSM requires a clean cut surface, but no thin section and clearly contrasts cell walls from lumina. CLSM was first used for wood anatomy to obtain images of xylem details similar to those by scanning electron microscopy (Knebel & Schnepf 1991), but not for quantitative image analysis. A quantitative image analysis of transmission light microscope (TLM) and CLSM found that CLSM produced thinner walls and larger lumina in conifer tracheids (Donaldson & Lausberg 1998). This was explained by the out-of-focus blur in thicker specimens (sections were 20 µm) with TLM and the inclusion of lateral ray walls within the 20 µm thick sections. CLSM can be used to obtain images below the surface, but in this case light scattering within the sample also produces biased images with too thick cell walls (Donaldson & Lausberg 1998).

By scanning objects at various distances and storing the distance (z-axis score) with the highest laser intensity of each pixel in the x-y planes, CLSM can also be used to measure a three-dimensional surface profile of an object. This permits to differentiate cell walls from lumina based on the distance from the plane rather than the laser intensity at a given plane. For a smoothly cut wood surface where the cut cell walls are in one plane and the axial cell walls are generally perpendicular to this plane, this produces a very sharp border between cell walls and lumina.

To assess the usefulness of this alternative use of CLSM for quantitative wood anatomy, we compared the image quality and ease of measurement using laser scanning and transmission light microscopy with different hardwood species, quantifying vessel and fibre lumina and fibre wall thickness. We compared results obtained with both methods from images that were either manually coded or with automated cell recognition based on size and colour thresholds. Our main objective was to shorten the processing time needed to obtain accurate measurements.

Materials and Methods

Plant materials

Samples were collected from a 50-ha forest dynamics plot established in seasonally dry evergreen forests in the Huai Kha Khaeng Wildlife Sanctuary in west-central Thailand located at 15º40´N, 99º10´E (Baker *et al.* 2005) in 2007. Wood from dominant trees was collected at breast height with 5-mm increment corers. Here, we selected three dominant canopy species namely *Toona ciliata* M. Roem., *Melia azedarach* L. and *Chukrasia tabularis* A. Juss. , all belonging to the Meliaceae, for comparing different measurement techniques.

Sample preparation

One-cm core sections were softened with 10% ethylenediamine (Kukachka 1977) overnight at 60ºC prior to cutting with a WSL core-microtome (Gärtner & Nievergelt 2010). Several thin sections of 20 µm were cut, stained with methylene blue to improve the contrast between cell wall and lumen, dehydrated with alcohol series and embedded in euparal to produce permanent slides. The remaining cut surface was used for the laser scanning microscopy.

Image acquisition

Images of thin sections were captured with a DM5500B transmission light microscope (TLM; Leica, Germany; software: LAS version 4.6.1). Images for vessel analysis were taken with an objective with 5-fold magnification, and for fibre analysis with a 63x objective. Image resolution was 860 and 10860 pixel per mm for 5x and 63x objective, respectively. For low-resolution images, an area of 25.22 mm^2 (7070 x 2757 pixels) was photographed for each sample. This image is composed of 14 individual frames with a 25% overlap that were merged into one image per section with Photoshop CS6 (Adobe Systems, U.S.A.), which performed better than the image merging module in the LAS software. Fibre size and wall thickness can be quite variable within one sample. To characterize the average fibre size, we randomly selected five points within each section and took image of fibres covering areas of 0.03 mm^2 (2048 x 1536 pixels) with high magnification.

Surface profiles were obtained with a VK-X100 confocal laser scanning microscope (Keyence, Osaka, Japan). As the laser (658 nm) scans a horizontal (xy) plane, the light reflected from the focus plane is recorded by a photomultiplier and stored. The laser then moves successive steps in the z-direction, scanning the xy-plane at each distance. The final image stores the distance, at which the laser intensity is maximal, which is the surface, and the laser intensity of this distance. Thus one image of the reflected laser intensity and one of the distance is stored. In addition, a white light produces a reflected colour image of the surface. Scanning time and file size depend on the number of layers scanned, which is determined by the distance between layers (z resolution) times the total height range (the difference between maximum and minimum distance). Since the height range is smallest when the surface of the sample is in one focal plane, a tilted sample surface increases scanning time, but does not much affect image quality. We used automatic height adjustment to identify the highest and lowest position for each frame, automatic double scan for high quality, and set the z-pitch to 6 µm for the 10x and 20x objective and to 3 µm for the 100x objective.

For the analysis of vessels we used a 10x objective for *Toona* and *Melia* and a 20x objective for *Chukrasia*, which has smaller vessels (Fig. 1). For fibres we used a 100x objective for all species. The resolution of the images was 720, 1440 and 7138 pixel per mm for the 10x, 20x and 100x objectives, respectively. Each laser scanning image for vessels analysis was composed of 14 single images covering a total area of 24.65 mm^2 (9081 x 1407 pixels). For fibres we took five pictures, each composed of two adjacent frames covering 0.05 mm^2 (3831 x 1538 pixels). The VK-X100 software (VK-Analyzer, Keyence, Osaka, Japan) produces four different seamlessly merged images: an optical image, an image showing the intensity of the reflected laser signal, a combined laser plus optical image, and a colour-coded height image (Fig. 2). We used the height image for the analysis of vessels, and the laser image for fibres (Fig. 3a). For the height image, first a possible tilt is corrected so that the entire cut surface is at the same height (or colour). When the laser signal is weak, the distance measurement is imprecise. In the case of the cut wood surface, the reflected laser signal is weak when the laser is directed into holes, i.e. lumina, or when the lasers beam hits a surface nearly parallel to the beam, normally axial cell walls. We therefore first filtered all areas with weak laser signals and then used the height information from the strong laser signals to distinguish flat cut cell walls from vessel lumina. For fibres with small lumina and high resolution images, the distinction between cell walls and lumina was better using the laser signal strength. The optical image (Fig. 2a) from the reflected white light yielded no additional benefit.

Because we wanted to compare the methods cell-by-cell, surface scans covered the same area as the thin section images. For high-resolution images used to measure fibres, care was taken that the thin section used was the one immediately adjacent to the surface rather than one of several cut from the block. This was because in the rather short and thin fibres, tapering might affect lumen area over a shorter distance than in the larger vessels, where one out of several sections cut was used to compare with the surface scan.

Image analysis

Ideally, cell dimensions can be analyzed automatically by image analysis using an appropriate algorithm. In practice, this may not always avoid errors due to imperfect sample preparations such as broken cell walls, or because the algorithm cannot distinguish between cell types that can readily be identified by a trained anatomist. In this comparative analysis we either used image analysis where vessels were identified on the height images by selecting the colour representing the cut plane (Fig. 2f) and fibres by identifying an intensity threshold to distinguish cell walls from lumina. Vessels were selected by their

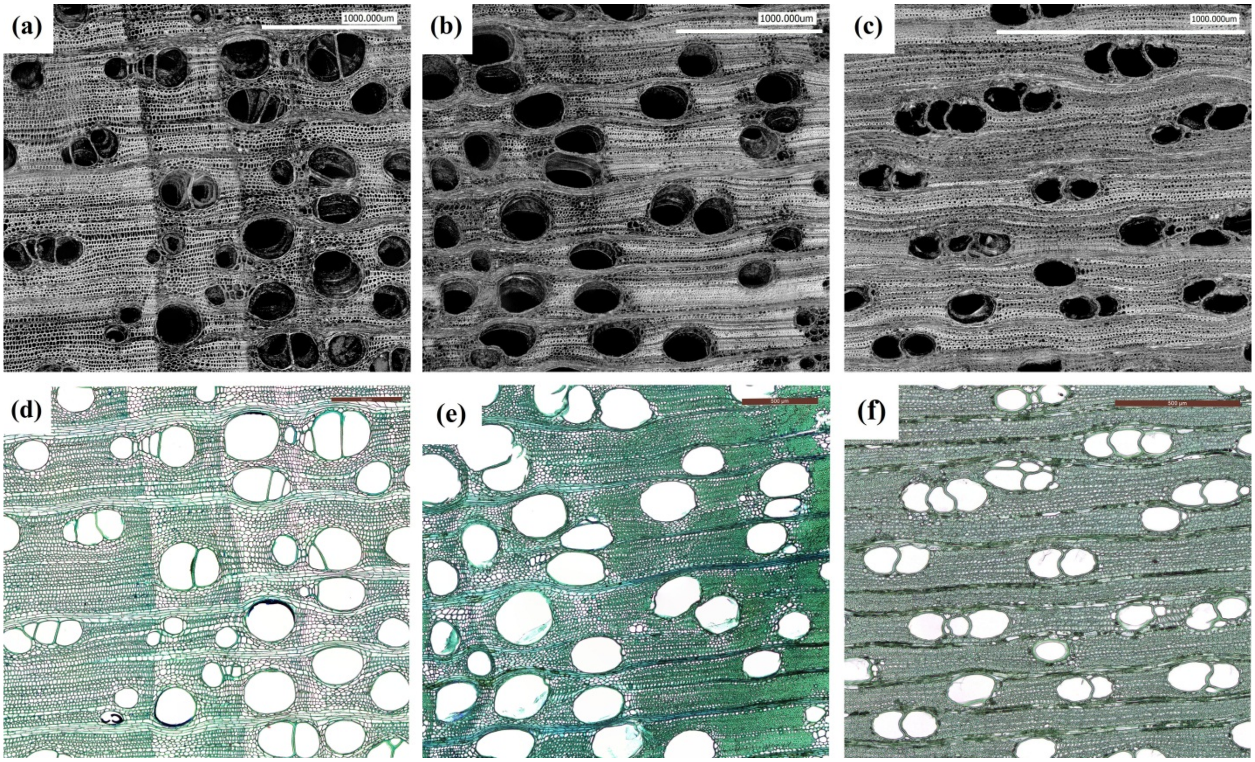


Fig. 1. Wood cross sections of *Toona* (a, d), *Melia* (b, e) and *Chukrasia* (c, f) obtained with a laser scanning microscopy (top row) or thin sections and a transmission light microscope (bottom row) *Chukrasia*, which has smaller vessels (note scale bar) was scanned with a 20x lense, *Toona* and *Melia* with a 10x lense.

size and circularity (4π x area / perimeter^2) with ImageJ 1.49o (http://imagej.nih.gov/ij). Minimum size to qualify as a vessel was set at 2700, 6750, and 1200 μm^2 for *Toona*, *Melia* and *Chukrasia* respectively and circularity was 0.2-1. For the automatic measurement of fibres, the minimum lumen size was set to 3.5 μm^2 in all species and no threshold was set for circularity. Prior to setting a threshold and counting particles, image noise was reduced by “erode particles”, followed by “despecle” and “dilate” in ImageJ. On inspection, four vessels that were below the size threshold were later added in *Toona* and two vessels in *Chukrasia*. Alternative to the automatic detection of cells, cells were individually identified, colour-filled using the bucket-tool in Photoshop and inaccuracies of the latter step were corrected by tracing the lumen outline using a graphic tablet. Cells on these edited images were also measured with ImageJ, but there was no need to reduce noise or for a size or shape threshold.

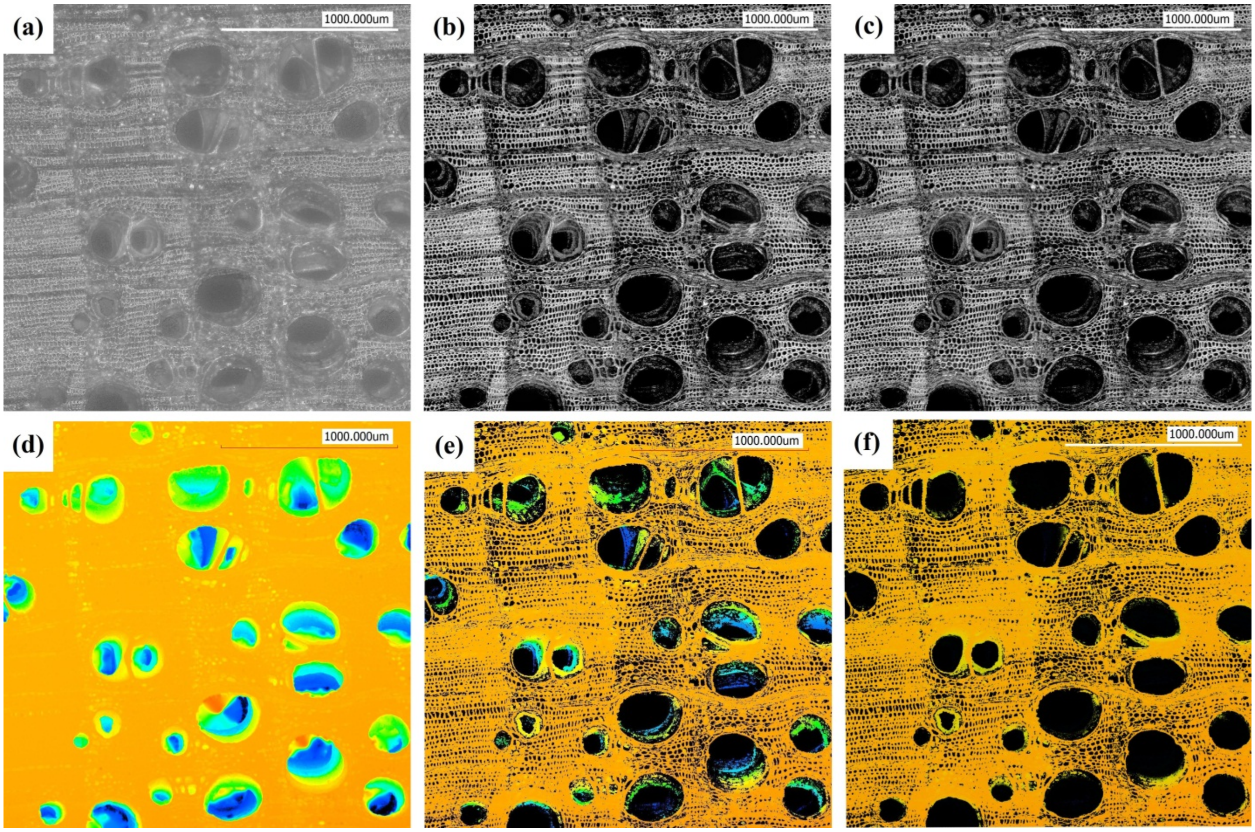


Fig. 2. Surface scans of a *Toona* cross-section with 10x lense and image processing with the VK-X software. The VK-X produces four different images: an optical image (a), a laser image (b), a combined laser plus optical image (c), and a colour-coded height image (d). (e) is the image after removing weak laser signals, which still leaves some signals from the inner vessel walls (yellow to blue). These are eliminated in (f) using a colour filter.

After all individual lumina had been identified either manually or by automatic detection, the size and number of individual elements as well as the size of the entire region of interest (ROI) analyzed was measured automatically with ImageJ.

Fibre wall thickness is not homogeneous and tends to be higher in the corners where three or more cells meet. With the area of the ROI (A), the total lumen area (AFL), and the number of fibres (n) per image, we calculated the average fibre area (Af = A/n; lumen plus wall) and the average lumen area (Afl = AFL/n) and estimated fibre wall thickness (Tfw) as the distance between the radius of a whole fibre cell and fibre lumen.

$$Tfw = sqrt((A-AFL)/n*pi) – sqrt(AFL/n*pi)$$

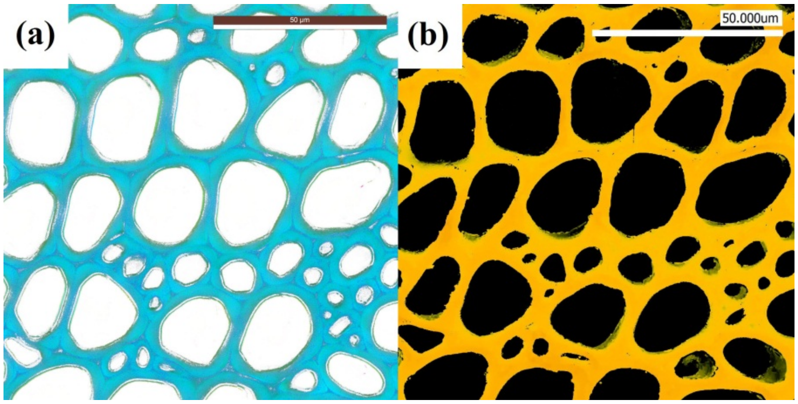


Fig. 3. High-magnification images of a *Toona* cross-section to analyze fibres with either -- a: thin section with 63x lense. or -- b: height image with 100x lense. -- scale bar = 50 µm in all images.

Results and Discussion

The CLSM produces an image quality from prepared surfaces similar to the TLM with thin sections (Fig. 1,3). Although in CLSM images the cut surface of the cell wall is readily distinguished from lumina, walls lining the lumina but not parallel to the laser beam will also reflect light and make the distinction using the laser strength alone difficult (Fig. 1). This was much less of a problem for fibres (Fig. 3). We therefore filtered weak laser signals, which typically come from laser beams hitting a cell wall at a flat angle (Fig. 2e). After that, the image can be filtered by height (i.e., the z-score) using either a colour filter or by saving the height information in a greyscale image. The processed image (Fig. 2f) was directly analyzed by ImageJ.

Table 1 summarizes vessel and fibre measurements of three hardwood species obtained from thin sections with a transmission light microscope and from surface scans using a laser scanning microscope. Images obtained by either technique were either manually coded to identify wall/lumen boundaries and vessels or analyzed largely automatically with ImageJ. Analyzing identical areas of the sections, the same numbers of vessels were identified. When vessels were traced manually, the area of individual vessels as well as the vessel lumen fraction was also nearly identical. Automatic image analysis produced vessel areas nearly identical to manually coded images in *Chukrasia* and *Melia*, but gave a somewhat lower mean vessel area in *Toona* measured with the LSM. This appears mainly due to an imprecise distinction between walls and lumina in some vessels (Fig. 4).

Table 1. Vessel and fibre characteristics determined by manual and automated measurements in comparison between thin sections with a transmission light microscope (TLM) and surface sections with a confocal laser scanning microscope (CLSM) in *Toona*, *Milia* and *Chukrasia*.

Species	Image analysis	Micro scope	NO. of vessel	Vessel area (mm^2)	Vessel lumen (% area)	NO. of fibre	Fibre lumen (% area)	Fibre lumen area (µm^2)	Fibre wall thickness (µm)
Toona	Manual	TLM	56	0.039	19.56	79	54.10	143.85	2.43
		CLSM	56	0.041	20.4	76	56.98	127.75	2.07
	Automated	TLM	56	0.038	19.78	78	50.37	135.64	2.69
		CLSM	56	0.034	17.24	77	54.40	120.38	2.2
Melia	Manual	TLM	28	0.045	21.75	96	42.35	69.78	2.53
		CLSM	28	0.044	21.15	94	40.77	61.61	2.51
	Automated	TLM	28	0.043	20.72	93	42.18	71.75	2.58
		CLSM	28	0.041	19.56	94	38.89	58.77	2.61
Chukrasia	Manual	TLM	89	0.010	16.33	98	22.44	29.83	3.42
		CLSM	89	0.011	17.28	97	22.84	21.26	2.84
	Automated	TLM	89	0.010	16.95	91	14.50	20.76	4.18
		CLSM	89	0.010	15.66	93	19.40	18.83	3.11

TLM and CLSM produced very similar images of fibre walls/lumina at high resolution (Fig. 3). However, the edge in the latter images was clearer, which translates to an easier and more reproducible selection of the brightness threshold. In *Toona* and *Chukrasia* fibre walls were somewhat thinner and fibre lumen areas smaller in the CLSM compared to the TLM images (Table 1). In *Melia*, fibre lumen area was also somewhat smaller with CLSM compared to TLM, but fibre wall thickness was nearly identical.

To evaluate the accuracy of the automated measurement we also compared the areas of individual vessel measured with either TLM or LSM (56, 28 and 89 in *Toona*, *Melia* and *Chukrasia*, respectively) Fig. 5.The coefficient of determination (r^2) was 0.9 for *Toona* and *Melia* and 0.7 for *Chukrasia.* The average vessel lumen area was about four times larger in both *Toona* and *Melia* than in *Chukrasia*, which may have resulted in higher inaccuracies in *Chukrasia*. Note that while the same areas of the samples were compared, we took one out of several 20 µm-sections made for the TLM image and the remaining sample for the LSM image. Thus the two images may represent cross-sections up to c. 100 µm apart along the axis, while the same individual vessels could be identified, the images cannot be expected to be perfectly congruent. The image quality and accuracy of the LSM is clearly affected by the magnification and smaller elements such as fibres cannot be accurately measured (or even distinguished) with low

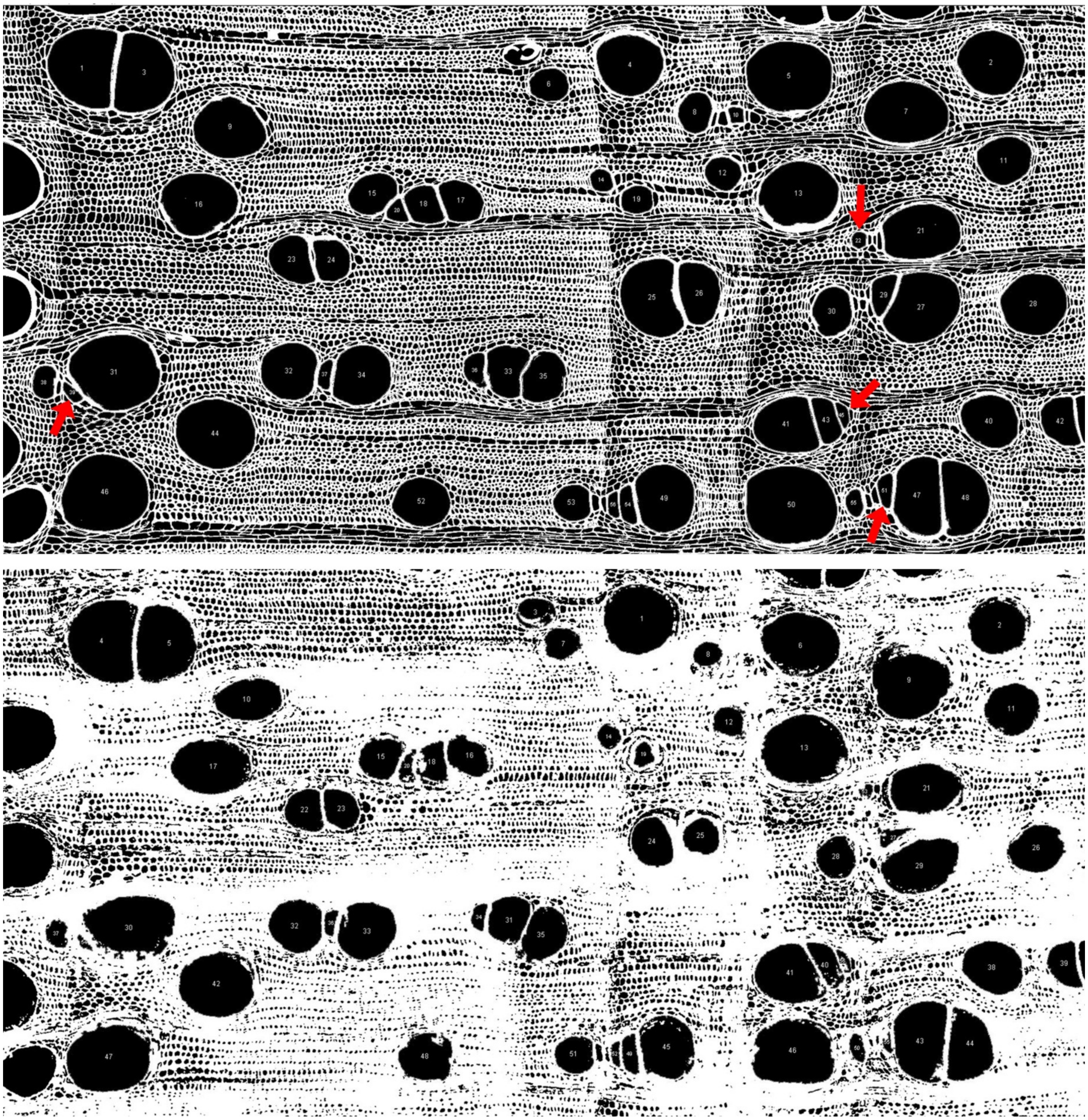


Fig. 4. Images processed for automatic vessel detection in a cross-section of *Toona* wood obtained from a thin section and a transmission light microscope (top) and a cut surface with a confocal laser scanning microscope (bottom). Vessels were identified, numbered and measured with ImageJ. Arrows in the top image point to vessels that were not automatically detected in the laser scanning image.

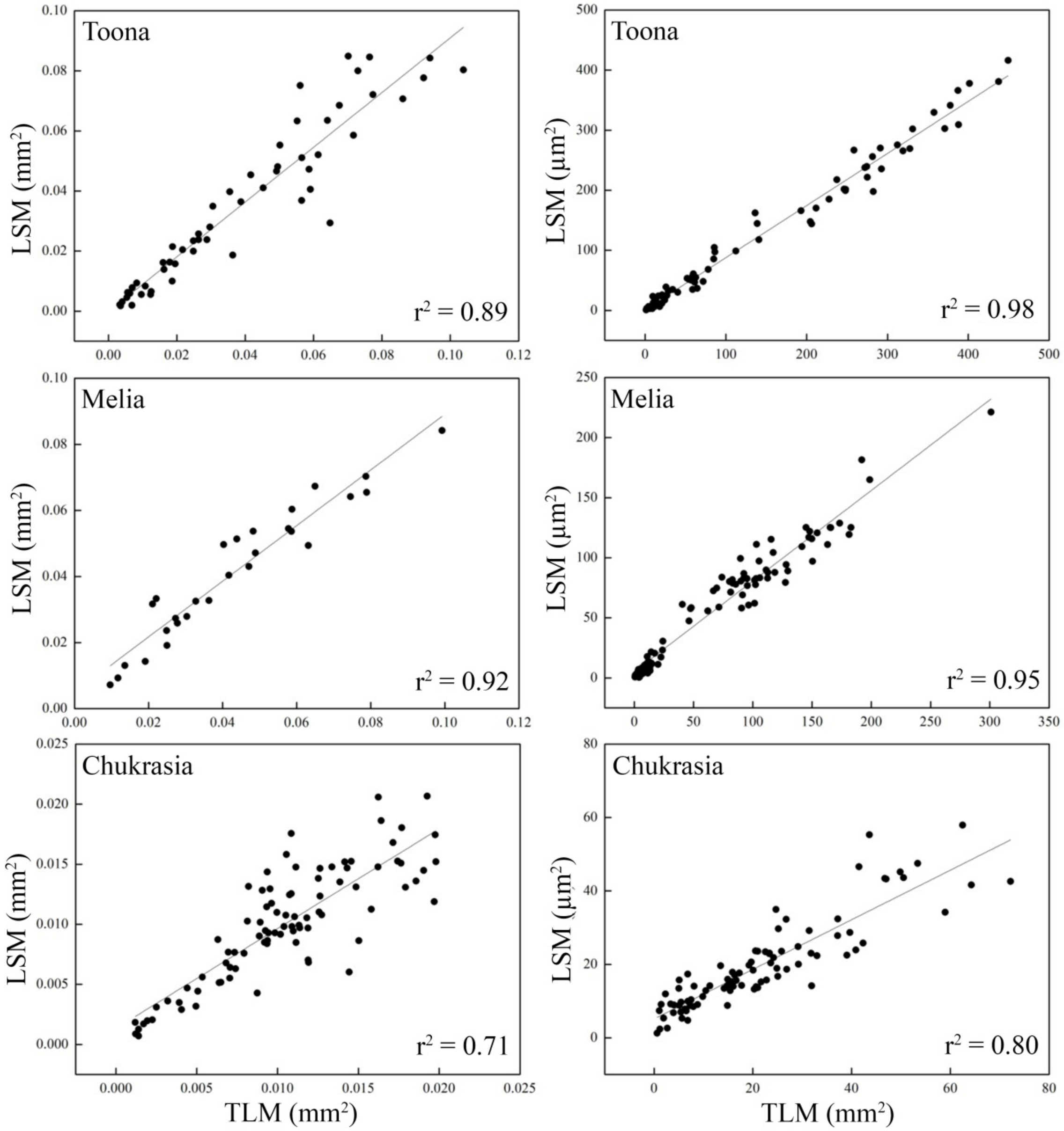


Fig. 5. Area of individual vessels (left panel) and fibres (right panel) obtained with either thin sections and a transmission light microscope (TLM) or a cut surface and a laser scanning microscope (LSM) for samples of three different species

magnification. For individual fibres measured by either method, r^2 was 0.80 for *Chukrasia*, 0.95 for *Melia* and 0.98 for *Toona*. Here, care was taken to take the section next to the scanned surface, which likely caused the higher agreement between the two methods even though fibre lumina are much smaller than vessels. Image quality of the LSM also depends on the quality (sharpness) of the cut, but, in contrast to TLM, not on the thickness of the section.

For quantitative wood anatomy, the preparation of good samples is an essential, if time-consuming step to produce correct measurements. For permanent slides, this includes sectioning, staining and embedding. Additionally, wood samples often need to be softened and the blade or cutting angle of the microtome has to be optimized for the type of wood (Barbosa *et al.* 2010). For the surface scan we still need high quality surface cuts, but no thin-sections and samples do not need to be stained and embedded. Sample preparation for thin-sections took c. 40 min., in contrast to 5 min. for samples to be scanned with the CLSM. Image acquisition is slower with the CLSM, typically 15 min for a stack of 45 layers along the z-axis with each layer a combined image merged from 14 single image frames (and a size of c 12 m pixels). Image acquisition time of with the CLSM, depends on several settings. Double scan increases sharpness somewhat and approximately doubles scan time and a high z-resolution increases the image layers to be scanned. Apart from the total image size, the alignment of the sample, which affects the number of layers needed to cover the cut surface in the z-axis, strongly affects image acquisition time. The VKX software merges individual frames seamlessly, but we were not satisfied with the stitching of the LAS software, and merged individual frames of the TLM with Photoshop instead. The time for image analysis, if done manually, depends strongly on image size, image quality, the number of elements to be marked and the accuracy with which the individual elements are traced. The latter may also depend on the purpose of the project. We put rather high emphasis on accuracy and corrected individual vessels when the vessel wall was broken or the vessel lumen was not completely free. Thereby image quality also affects processing time. We found it somewhat more challenging to produce high-quality images for the laser scans because debris may have fallen into vessels or small bits of the cut cell wall protrude into the lumen (Fig. 3a). For an automated analysis that distinguishes vessels based on size and shape, image size has little effect on processing time, but the quality of the data is strongly affected by image quality. Algorithms for a fully automated analysis of wood images have been developed (Von Arx & Dietz 2005) that permit multiple images to be analyzed without supervision, but setting the optimal parameters for this will mostly differ among species, so we did not try this when working with wood from different species.

The aim of our study was to test a new measurement approach that might speed up sample preparation and still procedure reliable results, by a clear distinction between cell lumina and walls, for quantitative wood anatomy. We use a different approach for CLSM using a combination of laser signal strength and the surface profile of the cut wood surface to obtain high-quality images both under low magnification for vessels and high magnification for fibres. The faster sample preparation for surface scans compared to microscopic slides can save time overall, even if the scanning is slower. As with thin sections, a sharp cut is crucial for image quality, but with a flat and sharp cut, the contrast between cell walls and lumina of axially extended cells is sharp and does not depend on the thickness of the section. At high resolution, image quality is similar to that of good thin sections and suitable for automated image analysis. However, it may be impractical to measure large and small elements on the same image. At low resolution fibre lumina are not properly distinguished, and scanning an area large enough to include a sufficient number of vessels with a resolution high enough to measure fibre lumina would take very long.

Acknowledgement

We thank the Thai Royal Forest Department for permission and help to sample wood. K.R. is supported by a grant from the Austrian Federal Ministry of Science, Research and Economy (BMWFW) within the framework of the ASEA UNINET.

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Chapter 3

Trait evolution in topical rubber (*Hevea brasiliensis*) trees is related to dry season intensity

Kanin Rungwattana, Poonpipope Kasemsap, Thitaporn Phumichai, Nicha Kanpanon,
Ratchanee Rattanawong, Peter Hietz

Published in Functional Ecology

Trait evolution in topical rubber (*Hevea brasiliensis*) trees is related to dry season intensity

Kanin Rungwattana[1], Poonpipope Kasemsap[2,3], Thitaporn Phumichai[4], Nicha Kanpanon[3,5] Ratchanee Rattanawong[6] and Peter Hietz[*,1]

[1]Institute of Botany, University of Natural Resources and Life Sciences, 33 Gregor Mendel Straße, Vienna 1180, Austria;

[2]Hevea Research Platform in Partnership, DORAS Centre, Kasetsart University, Bangkok, 10900, Thailand;

[3]Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand;

[4]Rubber Research Institute of Thailand, Bangkok 10900, Thailand;

[5]UMR 1137, Ecologie et Ecophysiologie Forestières, Faculté des Sciences, Université de Lorraine, F-54506 Vandoeure-les-Nancy, France

[6]Nong Khai Rubber Research Center, Rubber Research Institute of Thailand, Rattanawapi District, Nong Khai, 43120, Thailand.

* Author for correspondence, email: peter.hietz@boku.ac.at, tel: +43-1-47654-83110, fax: +43-1-47654-3180

Abstract

1. Drought shapes the distribution and survival of trees even in tropical wet forests, and the wood and leaf trait spectra are used to understand drought adaptations. However, trait variation may result from ontogenetic adjustment or be related to tree size, and not reflect evolutionary adaptations.
2. Intraspecific variation in adaptations to drought can be an important factor in a species' distribution and response to climate change, but excluding potentially confounding factors and proving adaptive evolution is challenging. Provenance trials can identify hereditary variability.
3. We analysed wood and leaf traits in rubber (*Hevea brasiliensis*) tree clones from 15 locations in the Amazon basin that were planted in northern Thailand, controlled for tree size, tested for genetic relatedness and the phylogenetic signal in traits, and compared trait variations with the climate at the location of origin.
4. Correlations between traits and tree size were low. Intra-specific trait variation was similar to relationships in published among-species comparisons and correlations among wood traits and correlations among leaf traits were stronger than between wood and leaf traits. Genotype explained 30 – 70% of the trait variation, and traits differed in how much of this variation was controlled by location or the relatedness among clones.
5. There was no correlation with mean temperature or total annual rainfall. However, rainfall in the driest quarter (19 – 199 mm) was strongly related to leaf mass per area, carbon isotopic composition and area-based nitrogen content($r^2 = 0.54 - 0.70$) and weaker to wood traits (vessel density and vessel lumen fraction). Trees from locations with a stronger dry season also had higher growth rates in Thailand.
6. All traits correlating with climate showed a significant phylogenetic signal. We found no evidence of increased drought tolerance, but the trait spectrum and higher growth in trees from drier locations suggests that deciduous rubber trees have adapted via drought avoidance rather than tolerance. Our study also underlines the importance of looking at a suite of traits rather than individual ones to understand adaptive strategies.

Keywords: drought resistance, evolutionary adaptation, intraspecific trait variation; provenance trail, tropical tree

Introduction

Drought is a main driver in tropical forest composition (Engelbrecht *et al.* 2007) and even in evergreen tropical rainforests such as the Amazon affects tree survival and the carbon balance (Phillips *et al.* 2009). Parts of the tropics and particularly the Amazon basin are expected to become drier with climate change (Davidson *et al.* 2012). Therefore, predicting the effect of drought on these forests and understanding the effects on and adaptations by individual tree species is important.

A few manipulative experiments with rainfall exclosure have shown that drought mortality is species-specific, related to tree size and can be explained by specific traits that provide drought tolerance (Rowland *et al.* 2015). The functional trait approach to understand species distribution or demographic rates is attractive because some traits provide a direct functional explanation and some traits are easy to measure and available for many species (Escudero & Valladares 2016), although these two groups of traits are not necessarily the same. The possible adaptive benefit of functional traits can be inferred from distribution, performance or functional relationships with traits that provide a clear benefit. For instance cavitation resistance is a direct measure of the functionality of the water transport system at different levels of drought stress (Choat *et al.* 2018) but is notoriously tricky to measure (Cochard *et al.* 2013). Wood density also relates to drought stress, but the relationship in this case is likely indirect, possibly due to the correlation between WD and cavitation resistance (Nardini, Battistuzzo & Savi 2013), even though there may be no direct causal link and the statistical link is sometimes weak (Lachenbruch & McCulloh 2014). Leaf mass per area (LMA, a measure of sclerophylly) has also been suggested as a predictor of drought tolerance and at least in evergreen species is related to rainfall (Niinemets 2001; Wright *et al.* 2005). Functionally, this may be explained by its correlation with turgor loss point (Bartlett *et al.* 2012), which is a good predictor of ecological drought tolerance (Bartlett, Scoffoni & Sack 2012). High WD and LMA were also found to be correlated with survival under drought (Greenwood *et al.* 2017; O'Brien *et al.* 2017).

Fewer multi-species studies of tropical trees looked at drought resistance and wood traits other than WD. WD, sapwood-specific hydraulic conductivity and vessel length scaled negatively with drought tolerance as measured by minimum leaf water potential (Markesteijn *et al.* 2011a) or cavitation resistance (Markesteijn *et al.* 2011b). Wood density is also related to sapwood water content, capacitance and turgor loss point (Santiago *et al.* 2018). However, looking at a single trait provides an incomplete and sometimes biased picture. A global dataset found species from high rainfall areas having low cavitation resistance, but species from areas with low rainfall range from very high to very low xylem vulnerability (Choat *et al.* 2012). Even acknowledging that mean annual precipitation is not a perfect measure of drought stress, xylem vulnerability - while clearly significant for drought resistance - is not the only adaptation to drought or the best predictor of drought resistance.

Comparing the relationship between individual traits and species distribution or drought resistance can also be misleading. Average hydraulic conductivity and maximum photosynthesis were higher in dry forest species across a rainfall gradient in Panama (Brenes-Arguedas, Roddy & Kursar 2013), which appears to contradict other studies on traits related to drought resistance. Some of these species have adapted to drought by employing desiccation avoidance (high investment in root system) or desiccation delay (deciduousness) strategies rather than desiccation tolerance. Similarly, trees in a Mexican forest with a long dry season scale along a gradient from drought avoiding species, which tend to shed leaves early in the dry season, have low wood density and cavitation resistance to drought-tolerant species, which are evergreen (Méndez-Alonzo *et al.* 2012). Thus single traits, even with a clear functional importance, can be rather poor predictors of species distribution or survival.

Ignoring other factors may also lead to wrong interpretations. Particularly wood traits are strongly related to tree size (Lachenbruch, Moore & Evans 2011) and trees tend to grow larger in wetter forests. When differences in stem diameter were accounted for, vessel sizes did not differ between angiosperms communities of differing water availability (Olson & Rosell 2013), which challenges previous interpretations on the functional advantage of smaller vessels. Therefore, if tree size, for whatever reason, is related to the environment, then interpreting traits that scale with the environment and tree size as adaptive is questionable, unless tree size is accounted for.

Trait variation can reflect evolutionary adaptations or the environmental imprint on individuals and the contribution of inherited factors in trait variation can be studied in provenance trials (Alberto *et al.* 2013). When populations from places representing an ecological gradient are genetically adapted to the local environment, we can expect to see a cline, which is a gradual variation in a character that is related to the environment the plants originate from, when grown in a common garden (Alberto *et al.* 2013).

Multi-species comparisons are useful to understand adaptations and ecological strategies, but selection works on the levels of individuals and genotypes, not species. Thus to understand the effect of a changing environment it is important to also understand the variation within species and the adaptive significance of this.

Within species, potentially adaptive traits differ strongly in the extent they are either genetically controlled or can be adjusted phenotypically (Alberto *et al.* 2013; Hajek *et al.* 2016). Distinguishing between the two is important for theoretical and applied questions, from selecting resistant genotypes for reforestation to understanding and predicting the effects of climate change (Aitken *et al.* 2008; Valladares *et al.* 2014). Most provenance trials of trees have been conducted with northern temperate species across latitudinal or altitudinal gradients where the temperature, growing season length or day length of the place of origin tend to be the main climate factors driving genetic adaptations (Alberto *et al.* 2013).

As high water use efficiency (WUE, generally expressed as carbon uptake by water transpired, which scales with the carbon isotope signal δ^{13}C, Farquhar, Ehleringer & Hubick 1989) is seen as an adaptation to drought, trees from more arid regions might be expected to have higher WUE under the same climate conditions. While studies often find differences in δ^{13}C among populations, several studies that related WUE with the climate of the place of origin found no correlation with water availability (Peuke, Gessler & Rennenberg 2006; Premoli & Brewer 2007; Klein *et al.* 2013), or a correlation that co-varies with a temperature gradient and a higher effect of the latter (e.g., Gornall & Guy 2007; Aranda *et al.* 2010; Dounavi *et al.* 2016). A provenance trial of 13 *Nothofagus* species found a strong positive correlation between δ^{13}C and rainfall at the site of origin was (Read & Farquhar 1991). This means a lower WUE in species from drier areas, which the authors suggest resulted from mechanisms that permit these species to maintain stomata more open and photosynthesize during periods of mild water deficit. However, δ^{13}C in that study also strongly correlated with temperature, which co-varied with rainfall. Also leaf mass per area (LMA) and photosynthesis were related primarily to altitude or temperature rather than rainfall or drought indices (Cordell *et al.* 1998; Aranda *et al.* 2010).

Compared to leaves, still fewer studies looked at intraspecific adaptations of wood traits to drought in provenance trials. Variation in cavitation resistance across the range of *Fagus sylvatica* or *Pinus pinaster* was very low (Lamy *et al.* 2011; Aranda *et al.* 2015), but substantially higher and related to precipitation in *Pinus canariensis* (López *et al.* 2013). Wood density was not related to the climate of origin in a beech trial (Aranda *et al.* 2015), but another study on beech found a strong positive correlation between vessel size or theoretical hydraulic conductivity with a forest aridity index of the place of origin, and a negative correlation between wood δ^{13}C and aridity (Hajek *et al.* 2016). The latter is surprising as it suggests a lower WUE in plants from drier locations, although these correlations might be affected by an unexplored collinearity with temperature in that study. Also, results from this provenance trial contrast with an increase in vessel size and xylem vulnerability with rainfall in a field study of beech (Schuldt *et al.* 2016).

In sum, common garden experiments, which largely eliminate the effect of the growing environment to identify genetic differences in trait adaptation, provide limited and sometimes contrasting evidence of intraspecific adaptations to drought. The few provenance trials of tropical trees mainly looked at potential tree growth for plantations and did not investigated potentially adaptive traits (Evans & Turnbull 2004).

Using a *Hevea* trial of clones originating from a large area in Brazil, we first ask how high the intraspecific variation in leaf and wood traits is, and to what extent the variation is controlled by clone, the place of origin of the clone and tree size. We expected tree size to be related to many traits and prepared to control for size for the analysis of trait : climate correlations. We quantified the correlations among wood and leaf traits and looked for functional correlations and trade-offs. Overall, we expected the leaf and wood economics spectrum in *Hevea* to be similar to inter-specific trait patterns, but with lower correlations because trait values span a smaller range within species than among species. We tested which traits were related to the climate the clones came from. Given that this climate spans a substantial rainfall but a very small temperature gradient, we were expecting to find correlations between traits and rainfall, but not with temperature. Specifically, we expected clones from drier regions to have higher wood density, lower hydraulic conductance, higher LMA and higher $\delta^{13}C$ values, consistent with a drought-tolerance or conservative resource use strategy. Finally, we constructed a phylogenetic tree based on single nucleotide polymorphisms (SNPs) and analysed the phylogenetic signal in traits by comparing the similarity in trait values between clones to their genetic relatedness. If populations have adapted to variation in water availability through the evolution of traits, we expected that these traits show a phylogenetic signal.

Materials and Methods

Study site, plant materials, and sample collection

The rubber tree provenance trial is located in Nong Khai, Thailand at the Nong Khai Rubber Research Center (18º09'30"N, 103º09'31"E). Average temperature at the site is 27.5 °C and mean annual rainfall is approximately 1600 mm. The dry season between November and April receives less than 100 mm per month, and the total of the driest three months, December to February, is 60 mm (Thai Meteorological Department, 2017). Parent material (known as the International Rubber Research and Development Board IRRDB’81 collection) had originally been sampled from wild populations in forests in the Brazilian Amazon basin in the states of Acre, Rondonia, and Mato Grosso, Fig. 1) and were distributed via collections from Malaysia and Cote d’Ivoire. Between one and 18 individual trees had been collected from different municipalities (subsequently called “locations”) and clones from these trees were planted in 1994 with five replicates per clone in a spacing of 3 x 7 m. Trees were tapped regularly for latex (see Chanroj *et al.* 2017 for details) and fertilized twice a year with 500 g N:P:K (30:5:18) fertilizer per tree, applied in the middle between tree rows.

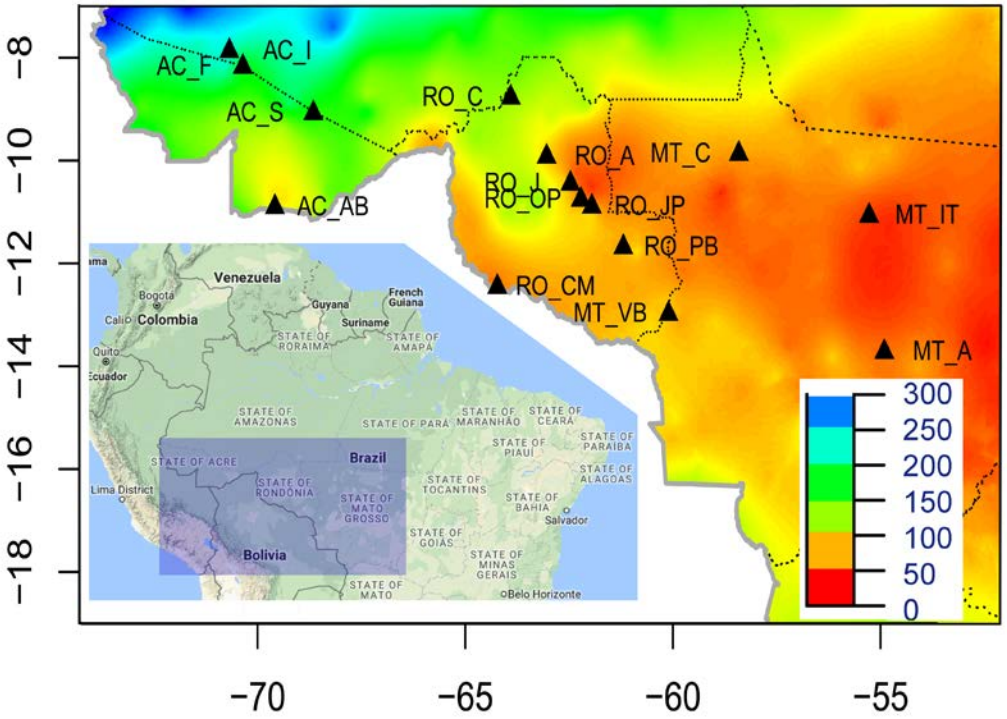

Fig. 1. Location of the origin of *Hevea* clones from the Brazilian states of Acre (AC), Rondonia (RO) and Mato Grosso (MT). The enlarged map corresponds to the shaded are on the overview map. The colour indicates the precipitation (mm) in the driest quarter of the year. See Table 1 for location codes.

We extracted climate data for the locations from WorldClim (www.worldclim.org) with 30 arc-sec (c. 1 km) resolution. Annual mean temperature (T) ranged between 23.8 and 26 °C, annual precipitation (P) between 1354 and 2215 mm, precipitation of driest quarter (Pq) between 19 and 156 mm and the coefficient of variation in monthly precipitation (Pcv) between 52 and 75% (Table 1).

Table 1. Geographic origin and habitat characteristics of *Hevea brasiliensis* populations. T: annual mean temperature (°C); P: annual precipitation (mm); Pcv: precipitation seasonality (CV); Pq: precipitation of driest quarter (mm) and n: number of clones analysed.

State	Municipality	Code	lat	lon	T	P	Pcv	Pq	number of clones
Acre	Assis Brasil	AC_AB	-10.89	-69.58	24.6	1640	61	66	1
Acre	Feijo	AC_F	-8.16	-70.35	25.9	2205	52	152	2
Acre	Ortet	AC_I	-7.86	-70.68	25.8	2254	47	199	5
Acre	Sena Madureira	AC_S	-9.07	-68.66	24.8	2017	53	140	6
Mato Grosso	Aracatuba	MT_A	-15.05	-58.29	23.6	1721	76	33	1
Mato Grosso	Cartriquaçu	MT_C	-9.86	-58.41	24.8	1980	73	46	18
Mato Grosso	Itauba	MT_IT	-11.06	-55.28	25.4	1885	75	19	16
Mato Grosso	Villa Bela	MT_VB	-12.97	-60.11	21.4	1943	70	57	6
Rondonia	Ariquemenes	RO_A	-9.91	-63.04	25.4	2184	64	62	18
Rondonia	Calama	RO_C	-8.76	-63.90	26	2096	60	100	3
Rondonia	Costa Marques	RO_CM	-12.45	-64.23	26	1501	69	35	15
Rondonia	Jaru	RO_J	-10.44	-62.47	24.9	1989	70	47	9
Rondonia	Jiparana	RO_JP	-10.89	-61.95	24.5	1951	70	51	1
Rondonia	Ouro Preto	RO_OP	-10.75	-62.22	24.4	1924	72	42	1
Rondonia	Pimenta Bueno	RO_PB	-11.67	-61.19	23.8	1922	70	51	16

In September 2013, sun-exposed leaves were collected from the upper crown of all remaining trees (3 – 5 per clone, as some had died) of 49 clones to analyse within-clone variation and for one tree per clone for another 101 clones to analyse coordination of traits among clones and correlations with climate. We collected one leaf from four branches per tree and used two leaflets per leaf to measure leaf mass per area (LMA) as dry weight / fresh leaf area. The eight leaflets per tree were pooled, ground to a fine powder and leaf nitrogen (N_{mass}) and carbon (C) concentrations and the carbon isotope signal ($\delta^{13}C$) were measured with a elemental analyser (vario ISOTOPE cube coupled to the IsoPrime100, IsoPrime Ltd, Cheadle, UK) at INRA-Nancy, France. The standard deviation of the isotope analyses for repeated measurements of the internal standard during each sample run was below 0.1‰. Some data on the $\delta^{13}C$ variation in 49 clones were presented previously (Kanpanon *et al.* 2017). Leaf $\delta^{13}C$ can be used to calculate air-to-leaf carbon isotope discrimination ($\Delta^{13}C$) and intrinsic water use efficiency (iWUE), which is photosynthesis per stomatal conductance (Osmond, Björkman & Anderson 1980). When the atmospheric CO_2 concentration and $\delta^{13}CO_2$ are constant, which we assume they were as the leaves were sampled during a short time interval and from the same canopy layer, $\delta^{13}C$, $\Delta^{13}C$ and iWUE will be linearly related and we therefore use the $\delta^{13}C$ signal as a proxy for time-integrated iWUE. Nitrogen per leaf area (N_{area}) was calculated as N_{mass} x LMA.

Wood traits

In April 2017, wood samples were collected from 607 trees of 153 clones at c. 195 cm height, which is above the height the bark had been cut for latex collection. Using a 5.15-mm-diameter increment borer (SUUNTO, Vantaa, Finland), 3-cm-long samples of the outer sapwood were extracted and sealed immediately into vials to avoid moisture loss. After cutting off the bark, sample length was measured with a digital calliper to 0.1 mm and volume was calculated as a cylinder from length and the borer diameter. Dry weight was measured after oven drying at 100 ºC for 72 h with a digital balance to 0.1 mg and wood density (WD) was calculated as oven dry weight divided by fresh volume. Tree circumference was measured at 170 cm height with a tape and diameter was calculated as circumference / π. Since all trees were planted in the same year, we can use diameter as a measure of tree growth rates.

For wood anatomy, we analysed all trees available (mean 4.3) for 70 clones to evaluate within-clone variation and one tree per clone for the other 83 clones for correlations among clones and with climate. We took the outermost 1-cm segments of the samples to ensure that this represented the most recent wood produced in all trees. Samples were softened with ethylene diamine at 60 ºC prior to cutting of 30 µm thick transverse sections with a core-microtome (WSL, Switzerland). Sections were double stained with safranin and astra-blue (Carl Roth, Germany), dehydrated with a graded alcohol series and mounted on slides embedded in Euparal (Carl Roth). Photographs of sections, each covering an area of 62.7 mm^2, were captured with a resolution of 864 pixels/mm, using a DM5500B transmission light microscope with a DMC2900 camera (Leica, Germany).

Vessel lumina (mean 109 per section) were color-coded manually and then measured automatically with ImageJ 1.49o (http://imagej.nih.gov/ij). We calculated the mean area of individual vessels (VA), vessel density per mm^2 (VD), the fraction of the cross-section occupied by vessel lumina (F), and the theoretical hydraulic conductivity (K_h) from vessel size and densities according to the Hagen–Poiseuille law (as in Sterck *et al.* 2008):

$$K_h = (\pi\rho_w/128\eta) \text{ x VD x } D_h^4,$$

where η is the dynamic viscosity of water ($1.002 \text{ x } 10^{-3}$ Pa·s at 20 ºC), ρ_w is the density of water (998.2 $kg{\cdot}m^{-3}$ at 20 ºC), VD is the vessel density (m^{-2}) and D_h is the hydraulically weighted vessel diameter (m), which was the average of major and minor axes of the diameter of individual vessels.

Data analysis

We tested the effects of provenance (location), clone and tree size on functional traits with analysis of variance (ANOVA) where clone was nested in provenance. For this test we used only clones with at least three replicated trees, i.e. 137 clones for wood density and tree size, 70 clones for wood anatomical traits and 49 clones for leaf traits. We calculated the proportion of the variance explained by location, clone within location, tree size and random effects as $\sigma^2_{effect} / \sigma^2_{total}$. Since “location” and “clone within location” represent the cumulative effect of the genetic variation, we can use $(\sigma^2_{location} + \sigma^2_{clone/location})/ \sigma^2_{total}$ as a measure of broad-sense heritability (H^2), which is the proportion of the total phenotypic variance attributable to genetic variation among individuals (McKown *et al.* 2014).

We calculated Pearson correlations to characterize the relationships among traits using the mean trait value per clone, i.e. we regard clones as independent data points. Scatterplots did not suggest any of these correlations to be non-linear. For correlations between traits and climate, we used the mean trait values of all clones collected from one location and weighed the correlation by the number of clones per location (Table 1), i.e. regard locations as independent data points. Since diameter was related to some climate parameters, we also tested the relationship between traits and climate in liner models that include tree diameter.

We calculated a principal component analysis (PCA) to evaluate associations among traits and climate to see how the trait spectrum is related to potentially important climatic drivers. Because the climate variables, which are clearly related to the place of origin, will affect the PCA and might result in a stronger clustering by regions, we calculated a second PCA with trait variables only, to see if clones from different states cluster by their trait composition. We then correlated the scores along the first two axes of this second PCA with climate to test if the combination of multiple traits shows a stronger correlation with climate than individual traits. For PCAs data that were mean-centred and scaled to unit variance

We used 1820 SNPs from DNA extracted from leaves to construct a phylogenetic tree with SplitsTree (www.splitstree.org) using the neighbour-joining algorithm to estimate relationships among clones (Fig. 2). These SNPs were filtered from 14155 SNPs from a published dataset of the same clones (Chanroj *et al.* 2017) using a linkage disequilibrium (r^2 threshold 0.4) and a Hardy-Weinberg equilibrium of $p < 0.05$.

We tested for a phylogenetic signal in traits using Pagel's λ (Pagel 1999), Blomberg's K (Blomberg *et al.* 2003) and Abouheif's C_{mean} (Abouheif 1999). λ and K compare the pattern of traits across a phylogeny to a Brownian motion model, the most common model for trait evolution (Revell, Harmon & Collar 2008; Münkemüller *et al.* 2012). λ scales between zero, when traits are not related to the phylogenetic distance among branch tips, and an expected value of 1.0, when the relationship follows Brownian motion. K is also under Brownian motion evolution, but will be > 1 if relatives are more similar than expected under Brownian motion. In contrast to λ and K, C_{mean} is an autocorrelation index that is not based on an evolutionary model and does not include branch length. We used the R package adephylo (Jombart, Balloux & Dray 2010) to calculated C_{mean} and phytools (Revell 2012) for λ and K.

All variables were checked for normal distribution with QQ-plots, and VA, VD, F, LMA and K_h were log-transformed. All statistical analyses were calculated with R 3.4.1 (www.r-project.org).

Results

Trait variation and heritability

The coefficient of variation was highest for wood anatomical traits (20 – 36% of the mean), somewhat lower for LMA, N_{mass}, N_{area} and diameter (14 – 20%), and lowest (<7%) for WD, C content and $\delta^{13}C$ (Table 2).

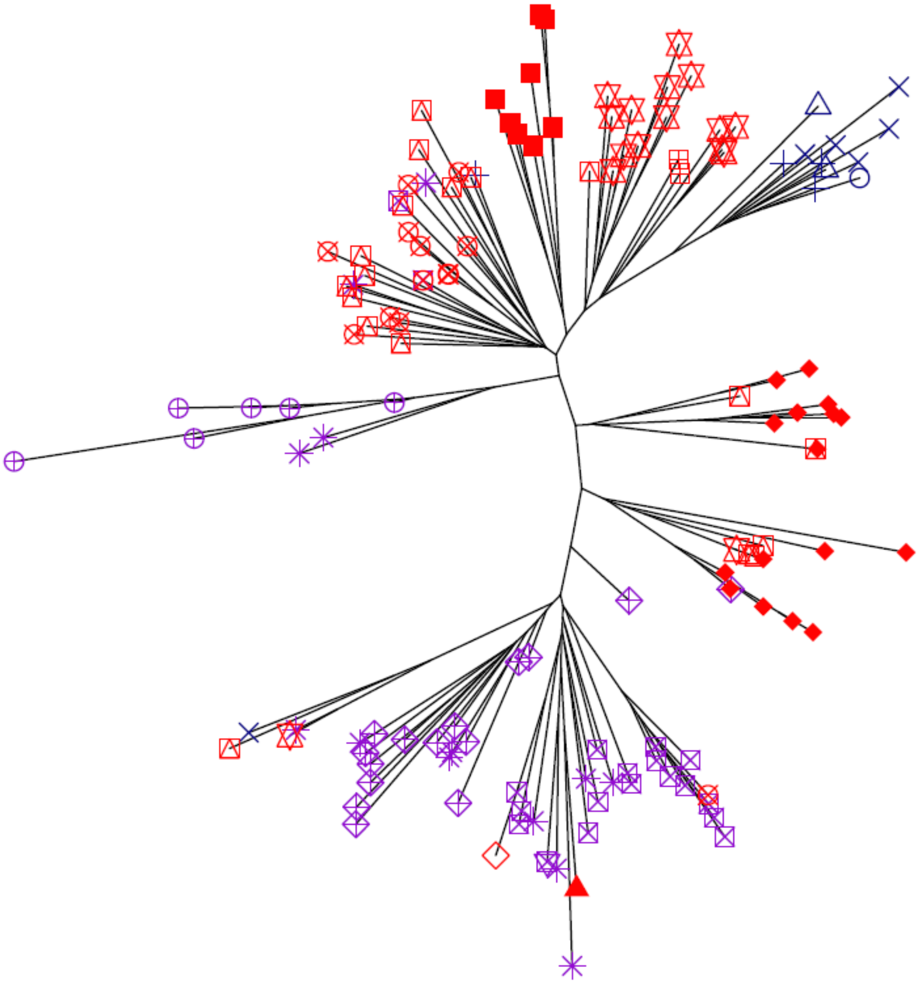

Fig. 2. Phylogenetic relationship among *Hevea brasiliensis* clones originating from Brazil. Symbol colour indicates clones from Acre (blue), Rondonia (red) or Mato Grosso (violet), different symbols denote different populations (municipalities where trees were sampled, see Table 1).

Heritability (H^2, since “clone” was nested within “location”, this is the variance explained by “clone” plus “location” in Fig. 3) explained 51 – 72% of the variation in traits, except for leaf C, N_{mass} and N_{area}, where < 42% were explained. Most of the remaining variation was unexplained with very little (< 6%) explained by tree diameter. Traits differed substantially in the extent they were explained by clone (nested in location) versus location. The variation in diameter and vessel size was controlled by clone, but not much by location, while leaf $\delta^{13}C$ and LMA were controlled about equally by clone and location, suggesting a high degree of inter-population variation for these traits. Even if the proportion of the variation explained was low, the effect of clone was significant for all traits except leaf N_{mass}, that of diameter for all except F, $\delta^{13}C$, N_{area} and C (Table 3).

All measures of phylogenetic signal were significant for K_h, LMA and $\delta^{13}C$. λ and C_{mean} were both also significant for VD, F and N_{area}, λ and K for VA, and C_{mean} for diameter and WD (Table 3). λ was significantly correlated (p = 0.009) with the variance explained by clone + location, while C_{mean} and K were significantly (p = 0.006 and 0.13, respectively) correlated with the variance explained by location alone, but none was related to the variance explained by clone within location.

Table 2. Mean, range, standard deviation and coefficient of variation (SD / mean * 100) of *Hevea* clones grown in a provenance trial in Thailand. WD: wood density; VA: mean area of individual vessels; VD: vessel density; F: the fraction of the cross-section occupied by vessel lumina; K_h: theoretical hydraulic conductivity; LMA: leaf dry mass per area; $\delta^{13}C$: leaf stable carbon isotope signal; N_{mass}: leaf nitrogen concentration, N_{area}: N per leaf area; C: leaf carbon content.

Trait	Unit	Mean	Max	Min	SD	CV (%)
Diameter	cm	24.60	37.59	14.56	4.20	17.06
WD	g/cm^3	0.55	0.66	0.45	0.03	6.07
VA	μm^2	20443	35899	9439	4750	23.23
VD	mm^{-2}	2.74	5.77	1.41	0.72	26.41
F	%	5.39	11.13	2.64	1.33	24.68
K_h	$kg.m.s^{-1}.MPa^{-1}$ x10^5	9.49	24.75	3.48	3.36	35.41
LMA	g/m^2	69.65	117.65	47.90	14.34	20.58
$\delta^{13}C$	‰	-30.63	-26.36	-33.33	1.25	-4.08
N_{mass}	% dry weight	2.81	3.93	1.64	0.42	14.78
N_{area}	g/m^2	1.93	3.15	1.23	0.37	19.02
C	% dry weight	48.25	53.30	43.88	1.49	3.09

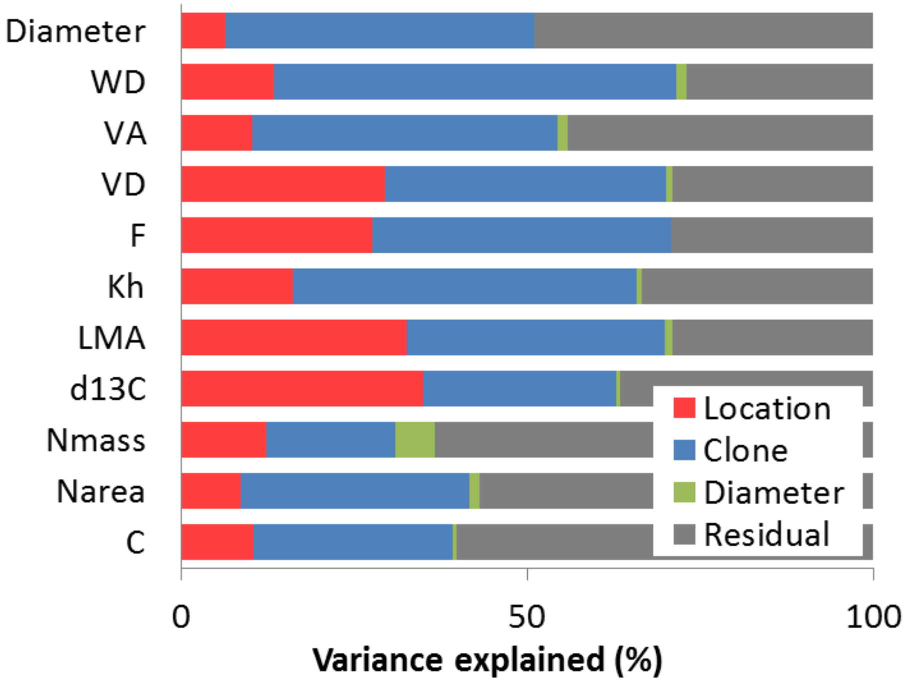

Fig. 3. Variance explained by location (the municipality in Brazil the germplasm was collected from), clone (nested in location), tree diameter and residual variance.

Table 3. ANOVA results (left) showing the significance of the effect of location, clone (nested in location) and tree diameter on the variation in tree diameter, wood and leaf properties in *Hevea* clones. n is the number of clones with 3 – 5 replicates tested. Phylogenetic signal (right; Pagel's λ, Abouheif's C_{mean} and Blomberg's K) for *Hevea* tree diameter, wood and leaf traits. p: significance. Traits with a significant phylogenetic signal ($p < 0.05$) are printed in bold. The phylogenetic signal was computed for all clones, the ANOVA for those with replicate measurements (n). See Table 2 for abbreviation of traits.

	ANOVA				phylogenetic signal					
	Location	Clone	Diameter	n	λ	p (λ)	C_{mean}	p (C_{mean})	K	p (K)
Diameter	**8.9E-06**	**3.1E-20**	NA	137	0.000	1.000	**0.131**	0.026	0.062	0.298
WD	**2.6E-28**	**1.6E-57**	**2.4E-06**	137	0.660	0.071	**0.133**	0.016	0.071	0.233
VA	**9.0E-06**	**2.5E-14**	**0.006**	70	**0.837**	7.9E-11	0.108	0.064	**0.180**	0.017
VD	**1.8E-27**	**1.1E-21**	**0.008**	70	**0.806**	9.4E-09	**0.196**	0.002	**0.150**	0.028
F	**7.0E-26**	**4.9E-23**	0.966	70	**0.592**	2.4E-04	**0.184**	0.004	0.105	0.067
Kh	**1.8E-13**	**3.5E-23**	**0.026**	70	**0.796**	4.1E-09	**0.140**	0.018	**0.168**	0.015
LMA	**1.9E-17**	**2.8E-11**	**0.024**	49	**0.685**	2.7E-15	**0.414**	0.001	**0.223**	0.006
$\delta^{13}C$	**2.4E-15**	**1.0E-05**	0.124	49	**0.827**	7.9E-25	**0.468**	0.001	**0.458**	0.002
N_{mass}	**6.6E-03**	3.6E-01	**0.001**	49	0.160	0.065	0.032	0.584	0.038	0.729
N_{area}	**3.9E-02**	**1.1E-03**	0.066	49	**0.410**	6.1E-06	**0.201**	0.002	0.076	0.257
C	**1.6E-02**	**1.1E-02**	0.267	49	0.000	1.000	-0.102	0.084	0.029	0.865

Associations among traits and climate

We found strong correlations among wood traits and among leaf traits, but few between wood and leaf traits (Fig. 4). The strongest correlation between VD, VA, F and K_h were to be expected because these parameters are partially calculated from each other. WD was negatively and weakly correlated with VA and K_h and positively with VD. LMA was strongly positively correlated with δ^{13}C and N_{area} and negatively with N_{mass}. LMA and N_{area} were the only leaf trait weakly related to wood traits.

LMA (r = 0.68), δ^{13}C (r = 0.70) and N_{area} (r = 0.55) showed a strong positive correlation with rainfall in the dry season (Pq), N_{mass} showed a weaker negative (r = -0.26), and VD (r = 0.42) and F (r = 0.39) weak positive correlations with Pq. Rainfall seasonality (Pcv) was strongly negatively correlated with Pq, and hence showed the opposite relationship with wood and leaf traits, except for K_h, where the correlation with Pcv was weakly significant but that with Pq was not. Relationships between traits and climate were very similar when tree diameter was included in models, but the significant relationship between dry season intensity and VD was lost and that with F was weaker (Table 4). No trait was related to total rainfall or mean temperature. Trees from locations with a stronger dry season had grown faster in Thailand (Fig. 4).

Rainfall seasonality scaled strongly positively and dry season rainfall negatively on the first PCA axis along with δ^{13}C, LMA, N_{area} and VD. WD, VA and K_h mainly scaled on the second PCA axis and mean temperature weakly along with F (Fig. 5a). When climate variables were omitted from the PCA the relationships among plant traits remained very similar (Fig. 5b). Pcv and Pq were strongly correlated with the scores of the first axis and weakly with the second axis of the PCA, P was weakly correlated with the first PCA axis and T was not correlated with the PCA of functional traits (Table 5). However, even the strongest of these correlations (between Pcv and PCA1, r^2 = 0.469) explained less than the correlation between the individual traits LMA, N_{area} or δ^{13}C and Pq or Pcv (r^2 = 0.53 – 0.70).

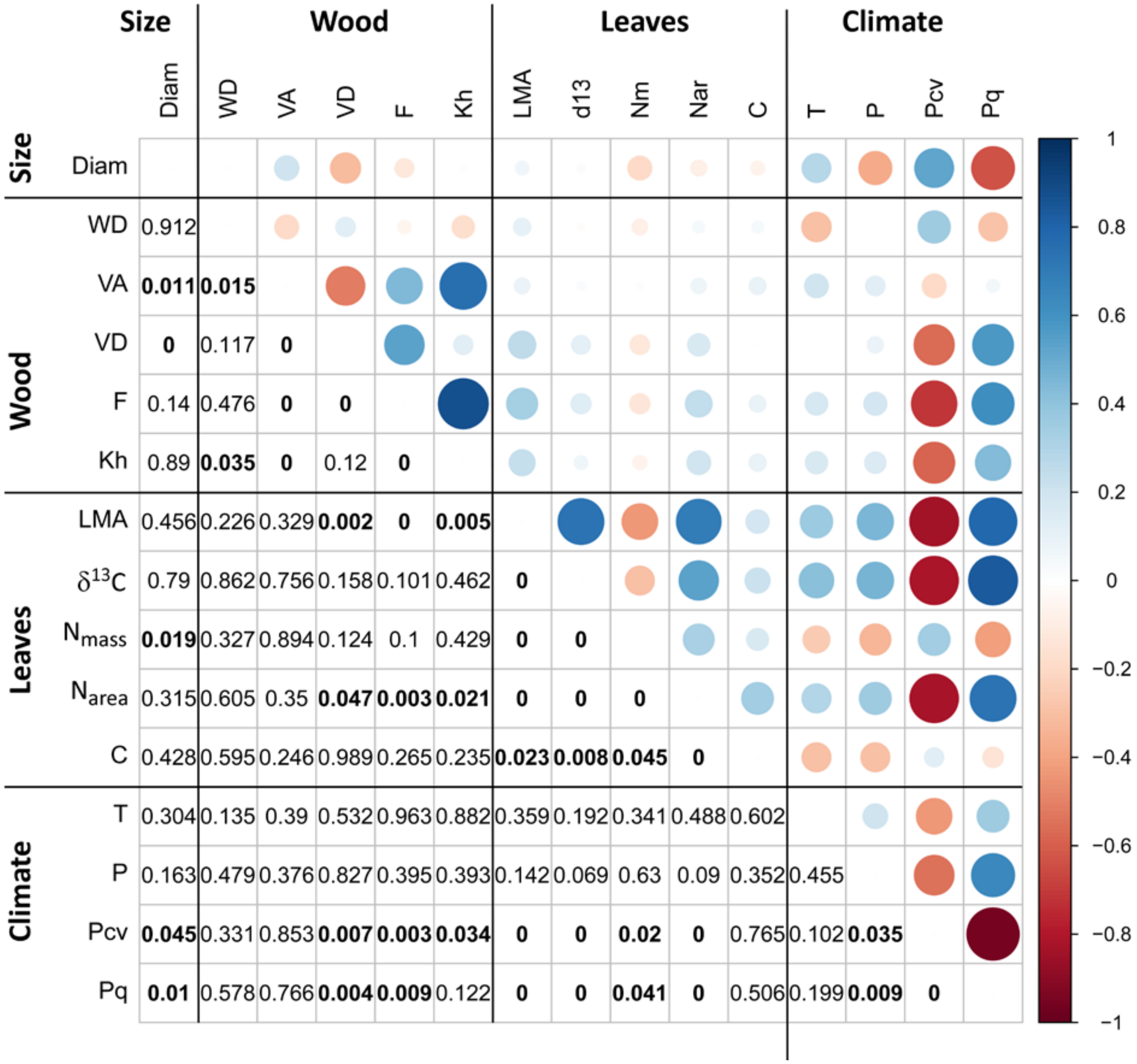

Fig. 4. Correlations among tree size (Diam), wood (WD – Kh) and leaf (LMA – C) traits, and climate (T – Pq) at the place of origin of Brazilian *Hevea* clones. For correlations among traits mean trait values per clone were used, for clone-wise correlations with climate, data were means per location weighed by the number of clones per location. Colour intensity and the size of the circle are proportional to the correlation coefficient with blue showing positive and red negative correlations. P-values of Pearson correlations are given in the lower triangle with values <0.05 printed in bold and $p < 0.001$ indicated as 0.

Table 4. Significance of the climate term in a linear model of the form trait ~ climate + tree diameter. See Table 1 for abbreviation of climate parameters and Table 2 for traits.

	T		P		Pcv		Pq	
	p	r	p	r	p	r	p	r
WD	0.172	-0.304	0.613	-0.029	0.142	0.342	0.254	-0.267
VA	**0.045**	**0.549**	0.311	0.186	**0.026**	**-0.249**	0.087	0.083
VD	0.362	-0.295	0.651	-0.003	0.375	-0.524	0.313	0.551
F	0.677	0.141	0.866	0.110	**0.038**	**-0.634**	0.060	0.536
Kh	0.574	0.263	0.735	0.130	**0.029**	**-0.534**	0.068	0.388
LMA	0.355	0.377	0.135	0.492	**7E-07**	**-0.798**	**4E-05**	**0.762**
δ^{13}C	0.192	0.419	0.071	0.458	**2E-04**	**-0.826**	**5E-05**	**0.839**
N_{mass}	0.347	-0.242	0.654	-0.289	**0.017**	**0.389**	**0.038**	**-0.441**
Narea	0.480	0.307	0.081	0.411	**1E-05**	**-0.770**	**1E-04**	**0.694**
C	0.596	-0.296	0.378	-0.311	0.728	0.092	0.487	-0.123

Table 5. Correlation between climate of place of origin and scores of the first two PCA axes of the traits-PCA. T: annual mean temperature, P: annual precipitation, Pcv: coefficient of variation of monthly precipitation, Pq: precipitation in the driest quarter.

	PCA1		PCA2	
	p	r^2	p	r^2
T	0.169	0.017	0.642	0.002
P	3.0E-04	0.113	0.648	0.002
Pcv	8.7E-17	0.469	0.006	0.067
Pq	7.8E-15	0.424	0.001	0.101

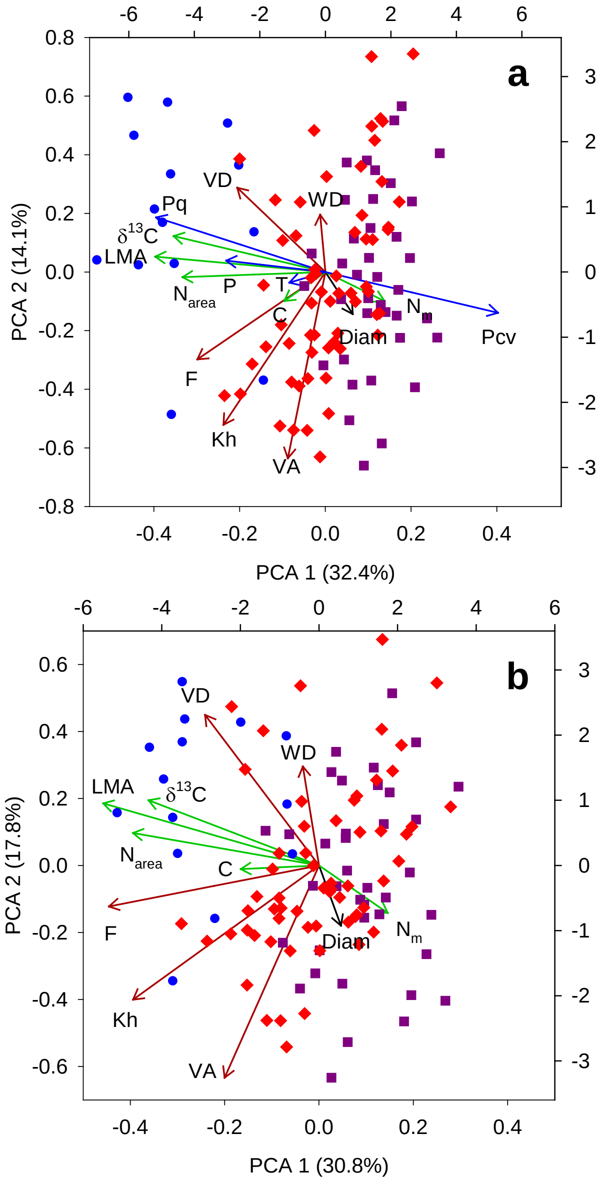

Fig. 5 Principal component analysis of traits and climate at the location of origin of *Hevea* traits. Symbol colour indicates clones originate from Acre (blue circles), Rondonia (red diamonds) or Mato Grosso (violet squares). Arrows represent the ordination of traits along the first two principal component axes with green arrows for leaf traits, brown arrows for wood traits and blue arrows for climate variables. a: PCA of traits and climate variable to show the relationship with climate, b: PCA with traits only to show to what extent genotypes from different states differ by their trait composition. This PCA without climate was used to correlate the axes with climate variables (Table 5).

Discussion

Looking for traits that may have evolved in adaptation to drought within a species, we sought to avoid or control for potentially confounding factors that would make an interpretation difficult. The common garden experiment avoids the effect of phenotypic adaptations to different environments. Tree were of the same age and, while size did have a significant effect on some traits, these effects were small and were accounted for in models looking for correlations between traits and the climate of origin. We report strong phylogenetic signals in several wood and leaf traits and particularly in these traits strong clinal variation with dry season intensity and finally discuss the mechanisms of drought adaptations.

Correlations among traits

Although many correlations with size were significant, diameter contributed at the most 6% to trait variation. Consequently, differences in tree size mostly had little effect on the relationships between traits and climate, which was verified by including tree size in models (Table 4). A larger range of tree sizes would likely have resulted in stronger correlations with traits, but for the purpose of our study, tree size was included mainly to avoid a potential size-related bias and not to study the size effect per se.

The pattern of intraspecific trait relationships was similar to that seen in studies investigating trait correlations among species. Correlations among wood traits and among leaf traits were generally stronger than between wood and leaf traits (Fig. 4), although leaf and wood traits were not unrelated (or decoupled) as seen in a large inter-specific comparison of tropical trees (Baraloto *et al.* 2010). Trait variations were lower than in interspecific comparisons because of a greater genetic relatedness of the *Hevea* clones and because phenotypic variation should play a minor role as trees were growing under uniform conditions and were the same age. Consequently correlations among traits were weaker. For instance, while the relationship between VA and VD was significant, it was much weaker in *Hevea* clones ($r^2 = 0.27$) than in interspecific comparisons ($r^2 = 0.81$ in Zanne *et al.* 2010, 0.88 in Hietz *et al.* 2017). Similarly, the negative correlation between LMA and N_{mass} was much weaker within *Hevea* (Fig. 4, $r^2 = 0.19$) than across species ($r^2 = 0.57$; Wright *et al.* 2004).

We found no correlation between WD and size in trees of the same age, which means that WD is not related to growth rates. This contrasts with across-species comparisons that found WD related to growth and mortality, which is seen as a trade-off between investment in either fast and cheap growth by producing light wood, or greater longevity at the cost of slower growth with heavy wood (Wright *et al.* 2010). While some studies did find negative WD : growth relationships also within species (Schuldt *et al.* 2016), the forestry literature is full of examples that found no such relationship, although this literature tends to be biased towards conifers (Saranpää 2003). The lack of such a correlation in spite of considerable variation of both is interesting as it suggests that there is no or little trade-off, which raises the question what if any disadvantages investing in high wood density might have in the evolution of a tree species. It is also of some practical relevance as it allows to select trees that produce high volumes of high-density wood.

Genetic variation

The differences in heritability were substantial and ranged from 31% of the variation in N_{mass} explained by clone (including the variation within locations) to c. 70% for WD, VD, F and LMA. Wood anatomy and LMA are thus under strong genetic control also within species. These values are similar to previous reports from temperate trees (e.g., McKown *et al.* 2014), but comparing H^2 between studies and also among traits is somewhat problematic. In theory, there should be no variation in traits measured in genetically identical individuals growing under identical conditions. Therefore, in our and similar studies, H^2 and thus the variance apparently not inherited will strongly depend on how well factors including growing conditions, size, sampling and measurement errors are controlled.

Phylogeny-based statistical methods are rarely used for intraspecific variation because populations are not genetically isolated and migration between populations is possible (Stone, Nee & Felsenstein 2011). The applicability of phylogenetic comparative methods for intraspecific variation strongly depends on the adequate resolution of the phylogenetic tree, a low migration rate between populations and the number of contrasts (Stone, Nee & Felsenstein 2011). One study found that the phylogenetic signal, its significance and the effect of sample size were similar for intra- and interspecific contrasts (Ashton 2004). Our study test the usefulness of intraspecific phylogenetic signal and compares it to heritability and published inter-specific signals for the same traits. Since various processes may result in a low phylogenetic signal, its interpretation is not straightforward and some caution against the use of phylogenetic signals as a measure of evolutionary rates or interpreting the underlying mechanisms (Revell, Harmon & Collar 2008, Blomberg *et al.* 2003). A strong phylogenetic in any case means that a trait has changed through the phylogeny, whatever the model or rate of evolution. While a phylogenetic signal does not prove that trait evolution is adaptive (the reference evolutionary model is random Brownian motion) we note that for all traits that showed a significant cline, at least one, and for those with the strongest correlations with climate (LAM and $\delta^{13}C$) all measures of phylogenetic signal were significant.

Spatially related clones collected from the same municipality or state also tended to be phylogenetically related (Fig. 2). All indices showed a significant phylogenetic signal for LMA and $\delta^{13}C$, and λ and C_{mean} also for VA and N_{area} (Table 3). The variance explained by location or clone plus location was correlated with at least one of the phylogenetic signals tested, but not the variance explained by clone within location. Although there is substantial genetic variation within locations (Fig. 3), individuals grow under the same local climate may face no clear evolutionary pressure to adjust (at least the traits we looked at) and hence a phylogenetic signal is not related to trait variation within locations.

Interestingly, two studies comparing >300 phylogenetically diverse species (Hietz *et al.* 2017 for wood and Valverde-Barrantes *et al.* 2017 for leaf traits) report λ very similar to our intraspecific study for WD, VA and VD (0.7 – 0.8), F (0.5) and K_h (0.55) and LMA (0.76), but much higher for leaf N_{mass} (0.67; vs 0.16 for *Hevea*). This suggests that, at least for our study system, phylogenetic signals are useful to study intraspecific trait evolution, and that the evolution of these traits follows similar patterns across vastly different scales of time and relatedness.

Evolution of adaptive traits

No trait was significantly correlated with the annual mean temperature of the place of origin, which was expected given that the temperature range was small. Also no trait was related to annual rainfall (range: 1354 – 2215 mm), but we found strong correlations with dry season rainfall (19 – 199 mm) and rainfall variability. Leaf traits, particularly N_{area}, LMA and $\delta^{13}C$ showed the strongest correlations with Pcv and Pq ($r^2 = 0.54 – 0.70$). Wood VD and F were also weakly related to both measures of dry season intensity ($r^2 = 0.34 – 0.37$), though the correlation with Pq may have been biased by tree diameter (VD and F were still significantly correlated with Pcv, Table 4). In two large-scale drought experiments in the Amazon rainforest, where water availability in the control and drought-stressed variants was similar to the wet and dry extreme of the *Hevea* sample locations, mortality was related to wood and leaf traits including xylem vulnerability and turgor loss point (Powell *et al.* 2017), which are functionally related to the traits we studied (see introduction). It is therefore likely that traits that confer drought resistance have been selected variably across the range of *Hevea*.

Our study minimized the effect of phenotypic plasticity by using trees from a common garden experiment; excluded an effect of age and accounted for a potential effect of tree size; used clones from locations that differed little in a potentially confounding temperature gradient; and found a significant phylogenetic signal in all traits strongly related to dry season intensity. These results provide strong evidence for intraspecific evolutionary adaptation of leaf traits to drought and weaker evidence for wood anatomical traits. However, the trait variation was not what we expected.

Mechanisms of drought resistance

We expected trees from more intense dry seasons to have higher WD and possibly smaller vessels and lower K_h. We also expected these trees to have higher LMA and water use efficiency as well as lower growth rate as these are related to a conservative resource use and also drought tolerance. However, WD was not related to any climate parameter and K_h only weakly and negatively to Pcv. We have no explanation why trees from a less intense dry season should have a higher vessel density and vessel fraction while vessel size remains constant. This may, however, be the effect of an even small correlation with diameter (these effects were mostly non-significant when diameter was included in models, Table 4), which highlights the importance of accounting for tree size when interpreting wood traits (Hietz *et al.* 2017), even when the size effect is quite low (Fig. 3).

LMA and $\delta^{13}C$ both decreased with dry season intensity. These correlations were the strongest of any trait with climate and showed a strong phylogenetic signal. Although few intraspecific provenance studies looked at drought resistance, some do report similar findings. *Pinus contorta* from more mesic locations had higher WUE (based on $\delta^{13}C$) than trees from xeric locations (Guy & Holowachuk 2001), but these provenances also represent an altitudinal (and hence temperature) gradient and the physiological basis as well as the adaptive function of differences in WUE remained unresolved. In a common garden trial of 22 *Nothofagus* species, $\delta^{13}C$ was also strongly positively related to total rainfall at the places of origin, but again precipitation strongly co-varied with temperature and temperature also correlated strongly with $\delta^{13}C$ (Read & Farquhar 1991). *Castanea sativa* from drier sites in Turkey had thicker leaves, higher protein per leaf area, stomatal conductance and assimilation rates than provenances from wetter sites (Lauteri *et al.* 1997). In that study, the higher $\delta^{13}C$ from wetter sites was explained by lower mesophyll resistance and photosynthetic capacity. In addition, an experiment in *Betula pendula* found that genotypes from regions with low rainfall had higher stomatal conductance than genotypes from high rainfall when grown in a common garden (Aspelmeier & Leuschner 2004). We did not measure gas exchange, but similar responses may explain the lower $\delta^{13}C$ and higher growth we found in *Hevea* from stronger dry seasons.

While many trees in seasonally dry tropical forests drop their leaves in dry season (Borchert 1994), evergreen as well as deciduous trees in Amazonian floodplain forests tend to drop their leaves at the height of the flooding season (Schöngart *et al.* 2002). *Hevea* grows in non-flooded terra firme and the higher levels of floodplain forests (varzeas) in the central Amazon as well as the seasonally dry south-east. In varzeas (Freitas, Oliveira & Barros 1997) as well as terra firme (Schöngart, pers.comm) it sheds leaves at the beginning of the dry season, but this coincides with high water tables. The forest type of the provenances has not been recorded, but varzeas are less extensive in the southern region of the Amazon basin compared to the central and eastern Amazon. Thus few if any of the original populations are likely from strongly flooded sites, whereas at least those from the eastern collection sites would experience substantial drought (Fig. 2). All provenances drop their leaves in the dry season at the Thai site as do rubber trees in plantations throughout the tropics (Priyadarshan, Sasikumar & Gonçalves 2001). That said, *Hevea* and other tropical deciduous trees might drop their leaves opportunistically whenever stress levels (by flooding or drought) are high. Few if any provenances we studied were likely from strongly flooded forests and we generally found correlations with dry season intensity (Fig. 4) and not with total rainfall, which would have been a measure of potential flooding stress for trees growing close to rivers. We therefore believe that clinal traits reflect adaptations to drought, and not flooding stress, although deciduousness might serve as an adaptation to both.

We can thus understand the strong relationship between dry season intensity and traits interspecific variation in drought-avoidance rather than tolerance. In seedlings of the tropical dry forest species *Quercus oleoides* LMA increased with the moisture of the location of origin, but plants from drier locations tended to drop their leaves faster in response to drought (Ramírez-Valiente & Cavender-Bares 2017). Adaptations to drought need not be at the leaf level. *Pinus pinaster* seedlings from a drier region in Morocco invested more in roots than plants from more mesic climates and consequently experienced less drought stress (low $\delta^{13}C$) under a low soil water regime (Aranda *et al.* 2010). Because *Hevea* from drought-prone areas have adapted to avoid drought, which likely includes traits in addition to those investigated, when grown under the same conditions they also experience less drought stress and can realize higher growth rates (Fig. 4).

Drought is a major limiting factor for tree growth and distribution, has become a driver of increased tree mortality and will likely increase in many rainforest areas. This calls for a better understanding of drought responses and intra-specific variation in tropical trees. To our knowledge, this is the first study of inter-specific trait variation of a tropical wet forest trees from across much of the species range to identify inherited component of traits. *Hevea* is untypical among rainforest trees in being deciduous, which is a strategy of drought-avoidance. Drought-avoiding trees do not have to tolerate negative xylem water potential by high cavitation resistance, associated with wood anatomical traits (Hoffmann *et al.* 2011), thus finding strong adaptations in wood traits in *Hevea* may be unlikely. Instead, drought avoidance is typically associated with acquisitive traits, low water use efficiency and higher growth, which was seen in *Hevea* from drier regions, although lower wood density is also common in drought avoiders (Méndez-Alonzo *et al.* 2012). Our study thus provides strong evidence of interspecific evolutionary adaptation to drought in trees and suggests that *Hevea* adapts by drought-avoidance rather than drought-tolerance. In other rainforest trees evolution likely leads to different patterns of intraspecific drought adaptations, which remains to be investigated. Drought-avoidance is only a rough classification of the trees' strategies. A more detailed picture might emerge from directly measuring physiological parameter instead of interpreting functional traits as proxies, although this is challenging for a large number of tall trees.

Conclusions and future directions

To assess a species' capacity to adjust to climate change, researcher have largely looked at traits conferring drought tolerance (Moran *et al.* 2017) and often focussed on specific traits such as cavitation resistance rather than the trait spectrum. Trees can survive drought with different strategies and failing to identify drought tolerance, particularly in wood traits, should not lead to the conclusion that species are not drought resistant or do not have the genetic diversity to adjust to drought. Drought avoiding traits that should be looked for are an efficient system of water uptake, seen in the root/shoot ratio or root morphology, and a conservative or efficient control of water loss, seen in stomatal control and leaf phenology. *Hevea brasiliensis* is especially attractive because it is one of the few trees with a completely sequenced genome (Tang *et al.* 2016), which enables to search for specific genes that are selected and test the association of genetic polymorphisms with adaptive traits (Moran *et al.* 2017). While sequencing is getting faster and cheaper, few tropical species are grown in established provenance trials of mature trees, although smaller trials with seedlings can more easily be established. The high genetic variation in drought resistance should provide *Hevea* with adaptations to cope with a changing climate. However, this will also depend on the potential gene flow across large distances, which might be impaired by an increasing isolation of remaining forest areas.

Acknowledgement

We thank the Rubber Research Institute of Thailand for on-site support, Ornuma Duangngam and Dokkeaw Chura for help during wood sampling, and Sabine Rosner and Daniel Tholen for helpful comments on the manuscript.

Author contribution

PK. and NK. contributed data on leaf traits, TP. contributed data on SNPs, RR. is managing the provenance trial, KR. analysed wood samples, PH. and KR. sampled wood traits, analysed the data and wrote the manuscript

Data accessibility

Data are deposited in the Dryad Digital Repository.

Funding

K.R. is supported by a grant from the Austrian Federal Ministry of Science, Research and Economy (BMWFW) within the framework of the ASEA UNINET. Field sampling of wood was supported by a ASEA UNINET grant to P.H.

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Chapter 4

Radial variation of wood functional traits reflect size-related adaptations of tree mechanics and hydraulics

Kanin Rungwattana, Peter Hietz

Published in Functional Ecology 2018;32: 260-272

Received: 21 March 2017 | Accepted: 28 July 2017

DOI: 10.1111/1365-2435.12970

RESEARCH ARTICLE

Functional Ecology BRITISH ECOLOGICAL SOCIETY

Radial variation of wood functional traits reflect size-related adaptations of tree mechanics and hydraulics

Kanin Rungwattana | **Peter Hietz**

Institute of Botany, University of Natural Resources and Applied Life Sciences, Vienna, Austria

Correspondence
Peter Hietz
Email: peter.hietz@boku.ac.at

Funding information
Austrian Federal Ministry of Science, Research and Economy

Handling Editor: Markku Larjavaara

Abstract

1. Wood serves for mechanical support, water transport and storage. These functions are provided for by different cells with a large variation in wood anatomy among species but also within individual trees. The latter often reflects ontogenetic adjustments, related to tree size or age, which can be studied by looking at patterns of radial variation in wood.
2. We quantified radial variation in wood density (WD) and wood anatomy and ask how ontogenetic changes of wood functions are controlled in five canopy tree species in western Thailand. We ask if there are trade-offs between these main functions of wood, how ontogenetic trends are linked to differences in growth trajectories and shade tolerance among tree species and if wood properties are mainly controlled by tree age or by size.
3. In all species studied, vessel fraction, vessel size, theoretical hydraulic conductivity (K_h) and fibre wall thickness significantly increased with tree diameter. While the ray fraction also increased in all species except *Neolitsea*, axial parenchyma changed significantly only in *Afzelia*, the species with by far the largest axial parenchyma fraction. The average WD and K_h reflect the phenology, with deciduous and shade-intolerant *Toona* and *Melia* having low WD and high K_h, and shade-tolerant brevideciduous *Chukrasia* and evergreen *Neolitsea* having higher WD and low K_h. Deciduous *Afzelia*, however, had the lowest K_h and second-highest WD. The radial gradients in WD and K_h also reflect within-species differences in growth rates during ontogeny.
4. The relationship between WD and its underlying anatomical components varied substantially among species. Modulating fibre wall thickness and vessel size enables growing trees to increase water transport capacity and mechanical strength at the same time. Across species, tree diameter had a stronger effect than age on all parameters except for fibres.
5. Given the very substantial within-tree size-related variation in wood traits, tree size is an essential parameter to include in comparative studies on the functional ecology of wood. Analysing ontogenetic changes in wood can advance our understanding of the different ecological strategies of trees.

KEYWORDS
functional traits, ontogenetic trends, tropical tree, wood density, wood radial variation, xylem anatomy

1 | INTRODUCTION

Wood typically serves for mechanical support of the whole tree; it transports water and nutrients from the roots to the leaves in the tree crown via hydraulic conduits and stores essential compounds such as nutrients, carbohydrates and secondary metabolites for defence (Chave et al., 2009; Hacke & Sperry, 2001; Zimmermann, 1983). Because wood performs more than one task, the question arises how plants optimise among these potentially competing functions across a huge range of ecological settings and sizes (Chave et al., 2009). In angiosperms, fibres serve primarily for material strength; vessels transport water axially; and living parenchyma cells, in axial as well as radial alignment, store organic compounds such as carbohydrates, are actively defending against pathogens and are important for refilling embolised conduits (Brodersen & McElrone, 2013; Morris et al., 2016). Both living and dead cells can also store water, although the contribution to wood water capacitance, measured as the change in the water volume fraction per change in tissue water potential, was lower than that of apoplastic water (in fibres, vessels and intercellular spaces) in five temperate species (Jupa, Plavcová, Gloser, & Jansen, 2016). In addition to different cell types, there is substantial variation in the size, form, arrangement and frequency of these (Lachenbruch & McCulloh, 2014; Ziemińska, Westoby, & Wright, 2015). This is associated with different physiological and ecological strategies (Hacke & Sperry, 2001), but the significance of this is only partially understood.

Wood density (WD) is frequently taken as a key functional trait as it is related to the growth: survival trade-off and can be the best predictor of growth (Chave et al., 2009; Wright et al., 2010). The variation in WD among species is strongly controlled by fibre wall thickness and the fraction of fibre walls in the cross-section (Fortunel, Ruelle, Beauchêne, Fine, & Baraloto, 2014; Jacobsen et al., 2007). However, WD is a complex trait and is affected by cell types that perform different functions, resulting in potential trade-offs between these functions and WD (Lachenbruch & McCulloh, 2014; Ziemińska et al., 2015). For instance, a negative correlation between WD and vessel fraction has been interpreted as evidence of a trade-off between hydraulic conductance and strength (Preston, Cornwell, & DeNoyer, 2006). However, others found these correlations to be weak and probably indirect and pointed to the fact that hydraulic efficiency can be achieved by larger vessel diameters without requiring a higher vessel fraction that might compromise strength (Hietz, Rosner, Hietz-Seifert, & Wright, 2017; Zanne et al., 2010).

To understand how wood adapts to different environments and life histories, wood structure and functions are frequently compared among species (e.g. Martínez-Cabrera, Jones, Espino, & Schenk, 2009; Poorter et al., 2010). However, analysing multi-species datasets is complex because wood originating from different sites, species or even individuals may be shaped by phylogenetic constraints, phenotypic adaptations to the site conditions as well as ontogenetic effects related to tree age or size. Alternatively, the functional significance of wood traits can be studied by looking at within-tree variation, where site-related and genetic factors are kept constant.

Within-tree variations in wood traits are rather well-studied in conifers, particularly for traits relevant to timber utilisation. Typically WD, tracheid length, diameter and wall thickness increase from the centre of the stem (the wood produced by small trees) towards the outer stem (wood produced by large trees; Lachenbruch, Moore, & Evans, 2011). However, exploring conifer wood, which is largely composed of tracheids, may tell us little about the adaptive strategies available to angiosperms with more complex wood. In angiosperms the radial changes in WD are less uniform and WD can increase, remain constant or decrease from pith to bark (Hietz, Valencia, & Wright, 2013; Wiemann & Williamson, 1989).

In contrast to the variable trends in WD, studies that looked at the radial variation in vessel size (in a much lower number of species) invariably found an increase with increasing distance from the pith, which translates to higher hydraulic conductivity in outer wood (Sperry, Hacke, & Pittermann, 2006). The few studies of radial variation in parenchyma found a variable pattern. In *Paraserianthes falcataria* the axial parenchyma fraction increases in outer wood while ray parenchyma does not (Ishiguri et al., 2009), in *Hevea brasiliensis* the ray fraction increases while ray density decreases (Naji, Sahri, Nobuchi, & Bakar, 2013), and in *Zelkova serrata* the ray fraction remains constant as individual rays increase in size and decrease in density (Tsuchiya & Furukawa, 2010). Apart from WD, why species differ in their radial (ontogenetic) variation in wood functions is largely unknown.

Three main underlying causes of radial changes in wood structure have been suggested (Lachenbruch et al., 2011): the age of the cambium, the different requirements to transport water in small and large trees, and the optimal mechanical construction of trees of different sizes. The age of the cambium might constrain a young meristem to produce small, short cells with high microfibril angle, whereas mature cambium cells in outerwood can produce adaptive wood for optimisation of the tree. Alternatively, the development of cells is determined by factors external to the cambium such as the mechanical load or the need to supply leaves with water, which are mostly associated with tree size. Lachenbruch et al. (2011) reviewed the typical radial pattern of wood traits and discuss the underlying causes, but acknowledged a possible bias by the focus on parameters of interest for wood processing and by the dominance of studies on conifers, as well as the difficulty in distinguishing size from age effect in trees where both are correlated. The differences in WD is often taken as a criterion to distinguish between juvenile or inner wood and mature or outer wood, though other criteria such as fibre length have also been used (Zobel & Sprague, 1998).

In the present study we analysed radial trends in wood anatomy in five tree species from a Thai seasonal forest differing in light demand, phenology and growth trends. We took advantage of distinct annual growth rings to determine tree ages in four of these species. We quantified radial patterns of cell fractions and sizes, and analysed correlations between these and functional traits associated with mechanical support, water transport, and storage. The aim was to clarify the following questions: (1) What is the best parameter to distinguish juvenile from mature wood? (2) What is the pattern of radial variation of wood anatomical traits related to mechanical support, water conductance and storage? (3) How do trees realise the adjustments in wood density and hydraulic conductivity necessary during ontogeny? (4) Are differences in cell fractions and sizes and the radial trends related to differences in the species' growth strategies? and (5) Are radial variations more controlled by tree diameter or cambial age?

2 | MATERIALS AND METHODS

2.1 | Study site and sample collection

Trees were sampled in a 50-ha forest plot (15°40′ N, 99°10′ E) in the Huai Kha Khaeng Wildlife Sanctuary in west-central Thailand, at an altitude of approximately 600 m a.s.l. (Baker, Bunyavejchewin, Oliver, & Ashton, 2005). Mean annual rainfall is approximately 1,500 mm with a dry season between November and April. Monthly mean temperature is between 19°C in January and 27°C in July.

Five tree species were selected that were reported to form annual growth rings and dominate the forest community by basal area or frequency (Baker et al., 2005): *Afzelia xylocarpa*, *Chukrasia tabularis*, *Melia azedarach*, *Neolitsea obtusifolia* and *Toona ciliata* (Table 1). Trees were cored with a 5-mm-diameter increment borer at approximately 1 m height in April 2007 (Nock et al., 2009). *Afzelia*, *Melia* and *Toona* are deciduous, *Chukrasia* is brevi-deciduous, and *Neolitsea* is evergreen (Williams, Bunyavejchewin, & Baker, 2008). *Afzelia*, *Melia* and *Toona* are categorised as shade intolerant pioneer species, *Chukrasia* from shade intolerance to moderately tolerant, and *Neolitsea* as shade tolerant (Baker et al., 2005). A tree-ring study of four of these species (except *Neolitsea*) found a negative effect of temperature and a positive effect on dry-season rainfall on growth at the study site (Vlam, Baker, Bunyavejchewin, & Zuidema, 2014).

2.2 | Laboratory procedures

The annual tree ring width for the three Meliaceae species has been measured previously (Nock et al., 2011) and was measured likewise in *Afzelia*. We used the change in ring-width for four species to plot changes in annual radius increment during growth. Rings of *Neolitsea* were often indistinct, which would make age unreliable and age was thus not obtained for this species.

For the anatomical analysis, we selected cores from five individuals per species and took 1-cm long samples every 2 cm for the inner 10 cm of the core, and every 5 cm for the outer part of the core. We measured the distance to the pith and determined cambial age for each sample analysed. When the 1-cm sample covered more than one growth ring, this was the age at the centre of the sample. The distance to the pith is the wood (stem without bark) radius, and we use 2× distance to pith as a measure of tree diameter. When the core did not include the pith, we estimated the distance to the pith following the direction of the rays to where they would intersect (Hietz, 2011). We obtained the cambial age of each sample by counting annual rings from the innermost part of the core. For the cores that did not hit the pith, we estimated the age of the innermost sample as the distance to the pith of this sample divided by the average annual growth during the first years for each species. The average initial growth was 5, 5, 25 and 10 mm per year for *Afzelia*, *Chukrasia*, *Melia* and *Toona*, respectively. On average the first 3, 3, 0.2 and 0.4 years were missing, i.e. had to be estimated for the four species.

Wood density had previously been measured in a different set of cores from the same trees by X-ray densitometry (Nock et al., 2009) but since we wanted to compare wood anatomy to the density of the exact sample, we measured WD for each sample by the water displacement method.

Samples were softened with 10% ethylenediamine overnight at 60°C prior to cutting of 20 μm thick transverse sections with a microtome. Sections were stained with methylene blue (Carl Roth, Karlsruhe, Germany), dehydrated with a graded alcohol series and mounted on slides embedded in Euparal (Carl Roth). We also made tangential sections of one sample per species to get familiar with the wood structure of the species studied. These are helpful in distinguishing fibres from parenchyma, but are not suitable for quantifying cell types.

We took images of sections (Figure 1) with a DM5500B transmission light microscope equipped with a DMC2900 microscope camera (Leica, Wetzlar, Germany) using lenses with low magnification (5× or 10×). Adjacent images were merged and fibres, radial parenchyma, axial parenchyma and individual vessels were colour-coded manually with Photoshop CS6 (Adobe Systems, Mountain View, USA). We classified cell types based on cell size and cell wall thickness though for some cells the classification as fibres or parenchyma was difficult. In *Toona*, axial parenchyma is scarce, but could not be distinguished from fibres reliably enough in transverse sections and the two cell types are thus not differentiated in this species. Depending on vessel size and

TABLE 1 Tree species studied and sampled trees. Wood anatomical samples were analysed from five trees per species. Except for *Neolitsea*, were tree rings were too indistinct, *n*, the diameter of breast height (DBH) and age are the number, size and age ranges of trees used for tree ring analysis in Figure 2

Species	Family	Light-demand	Phenology	*n*	DBH (cm)	Age (year)
Afzelia xylocarpa Craib	Fabaceae	Shade intolerant	Deciduous	14	37–166	25–276
Chukrasia tabularis A. Juss.	Meliaceae	Moderately shade tolerance	Brevi-deciduous	36	9–80	25–123
Melia azedarach L.	Meliaceae	Very shade intolerant	Deciduous	23	28–94	6–84
Neolitsea obtusifolia Merrill	Lauraceae	Shade tolerant	Evergreen	5	22–70	na
Toona ciliata M. Roem.	Meliaceae	Shade intolerant	Deciduous	14	16–74	24–121

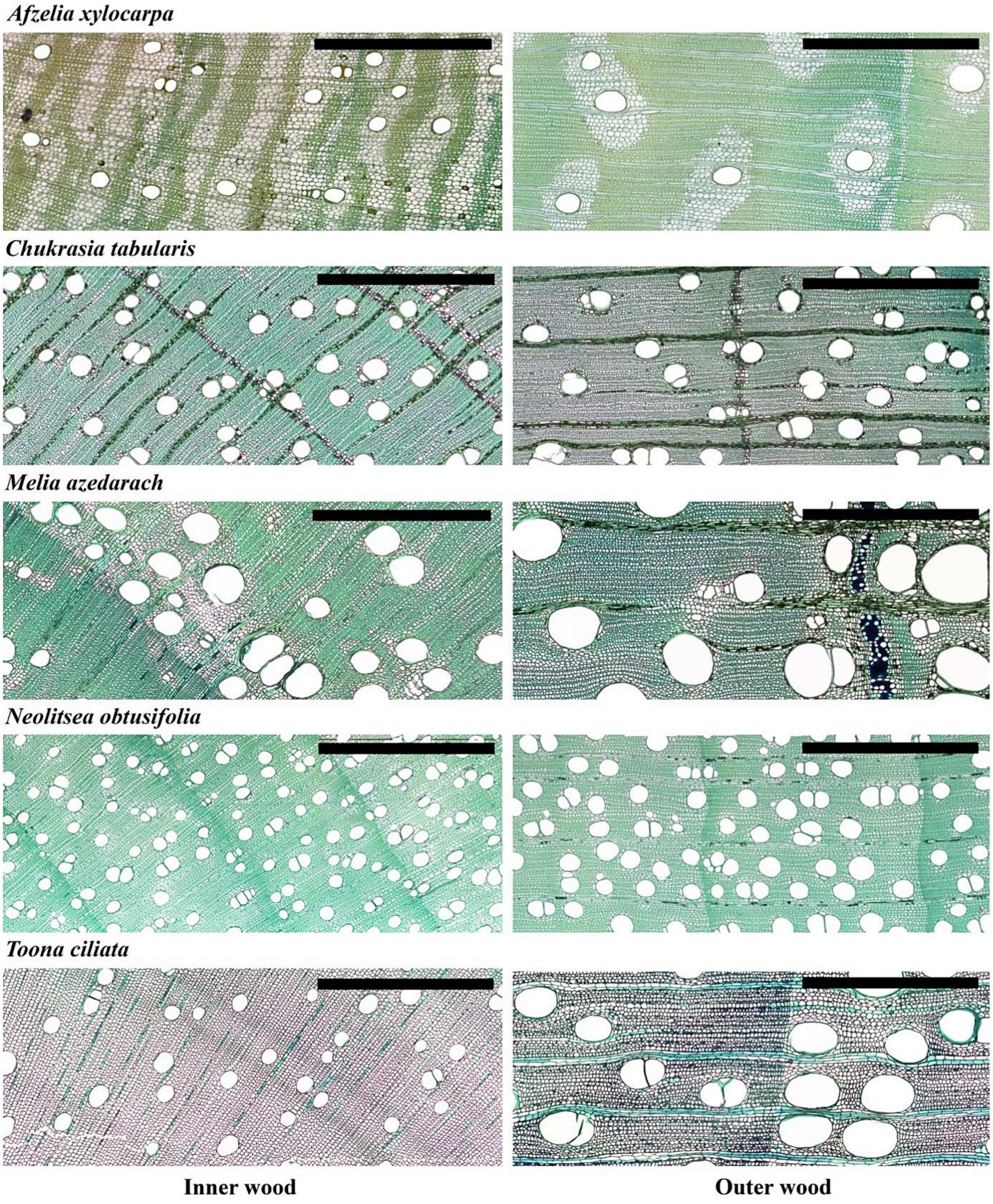

FIGURE 1 Inner wood (left) and outer wood (right) of five Thai canopy tree species. Scale bars are 1 mm

the sizes of groups of the same cell type, images covered 2–20 mm^2 and included on average 73 (11–233) vessels. To measure individual fibres, we used a 63× lens. Fibre size and wall thickness can be quite variable within one radial position, and we therefore randomly selected five points within each section and took images covering 0.01 mm^2 to calculate the average fibre size over these five images.

TABLE 2 Wood traits of five canopy tree species. Values are mean (max/min) of all samples measured for each species. Fractions (P) are the proportion of the cross-sectional area occupied by the cell types or fibre cell walls. K_h, theoretical hydraulic conductivity calculated from vessel size and densities

Wood trait	*Afzelia*	*Chukrasia*	*Melia*	*Neolitsea*	*Toona*
Axial parenchyma (P)	0.25 (0.56/0.16)	0.04 (0.08/0.01)	0.12 (0.24/0.03)	0.04 (0.10/0.01)	na[a]
Vessels (P)	0.04 (0.16/0.02)	0.10 (0.17/0.07)	0.15 (0.22/0.06)	0.19 (0.29/0.08)	0.11 (0.19/0.05)
Ray parenchyma (P)	0.15 (0.21/0.07)	0.15 (0.19/0.12)	0.12 (0.16/0.07)	0.17 (0.22/0.09)	0.15 (0.21/0.05)
Fibres (P)	0.56 (0.65/0.33)	0.71 (0.77/0.60)	0.61 (0.82/0.44)	0.60 (0.60/0.50)	0.75[a] (0.89/0.62)
Fibres wall (P)	0.35 (0.48/0.18)	0.48 (0.56/0.40)	0.38 (0.50/0.30)	0.44 (0.51/0.38)	
Vessel area ($mm^2 \times 10^3$)	14.30 (28.54/3.85)	7.92 (13.51/3.42)	40.03 (68.06/6.69)	7.58 (12.69/2.34)	18.17 (36.06/4.25)
Vessel density (mm^{-2})	3.12 (7.19/1.87)	13.25 (20.57/8.61)	4.27 (8.67/1.80)	25.89 (36.23/19.14)	6.75 (12.89/2.76)
K_h ($kg\ m\ s^{-1}\ MPa^{-1} \times 10^5$)	4.21 (27.70/0.46)	4.56 (11.90/1.20)	35.28 (70.30/2.15)	7.34 (16.80/0.98)	16.26 (46.90/1.75)
Fibre wall thickness (μm)	2.47 (3.57/1.39)	2.73 (3.79/2.18)	2.82 (4.20/1.84)	3.84 (4.77/2.73)	2.18 (2.69/1.74)
Wood density (g/cm^3)	0.63 (0.76/0.42)	0.66 (0.80/0.55)	0.50 (0.63/0.33)	0.60 (0.67/0.50)	0.38 (0.47/0.28)

[a] In *Toona*, a small proportion of axial parenchyma is included in the Fibre fraction.

Tissue fractions and cell sizes on colour-coded images were measured with ImageJ 1.49o (http://imagej.nih.gov/ij).

We calculated the fractions of fibres, radial parenchyma, axial parenchyma, vessels and fibre walls, as the proportion of these tissues in the transverse section. The latter is the proportion of fibre walls in the entire cross section, calculated as the fibre fraction in the low-magnification images times the fraction of fibre walls in the high-magnification fibre images. We calculated the mean area of individual vessels (VA), fibre wall thickness, vessel density per mm^2 (VD) and theoretical hydraulic conductivity (K_h). K_h was calculated according to the Hagen–Poiseuille law (Sterck, Zweifel, Sass-Klaassen, & Chowdhury, 2008):

$$K_h = (\pi \rho_{w/128} \eta) \times VD \times D_h^4,$$

in which K_h is the xylem hydraulic conductivity ($kg\ m\ MPa^{-1}\ s^{-1}$), η is the viscosity of water at 20°C (1.002×10^{-3} Pa s at 20°C), ρ_w is the density of water (998.2 kg/m^3 at 20°C), VD is the vessel density (m^{-2}), and D_h is the hydraulically weighted vessel diameter (m), using the average of major and minor axis of the diameter of individual vessels. Calculated K_h is not the same as true conductivity as it ignores resistance of water flowing through the vessel walls, but walls were found to contribute a relatively constant fraction of total resistance in vessels (Sperry et al., 2006), and therefore K_h serves as a good proxy for true conductivity.

2.3 | Statistical analysis

For each species, we tested the effect of the distance from the pith on wood cell fractions, cell sizes, K_h, WD, and fibre wall thickness with linear mixed effects models, with individual trees as random effects. VA, VD and K_h were log-transformed. Slopes of these regressions represent the radial gradients for each wood trait. Since linear mixed effects models do not provide a coefficient of determination, we used simple linear regressions to calculate r^2 values.

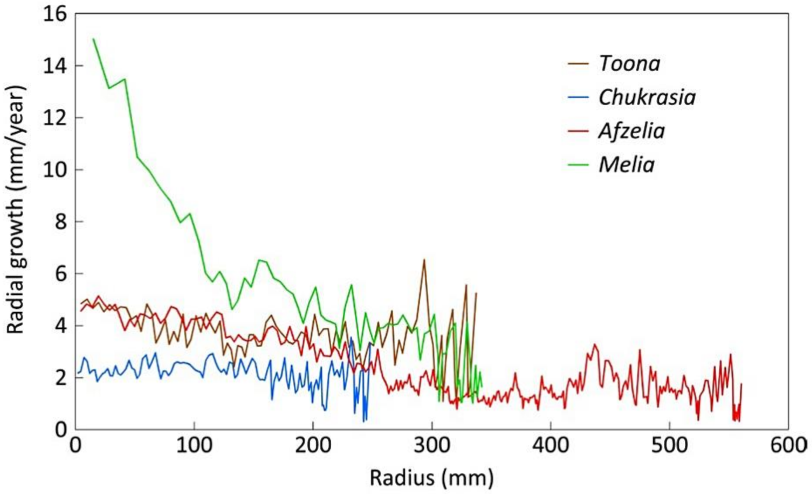

FIGURE 2 Growth trends with tree size in four species with distinct annual growth rings. Lines are the average growth of several individuals (Table 1)

To assess how the intra-specific variation in WD is related to tissue fractions, we calculated multiple linear mixed models that started with the fractions of axial parenchyma, ray parenchyma, vessels and fibre walls and were reduced by stepwise dropping non-significant variables until only significant or marginally significant ($p < .1$) variables remained. We used the wall fraction for fibres but the total fraction for other cell types because in fibres, which have a high wall-to-lumen ratio, the contribution to WD would come from their thick walls, whereas in the other cells with low wall-to-lumen ratios the effect on WD should come from their lumen fraction, which is close to their total fraction.

Testing if age or diameter is the main driver of radial changes is complicated by the fact that tree diameter and age are strongly related ($r^2 = 0.64–0.77$ in our data). Variance inflation factors between age and diameter were moderate (2.8–4.3), which suggests a multiple regression is not too biased by a correlation between these two factors. Since attempts to deal with collinearity by some form of partial regression can also introduce a bias (Quinn & Keough, 2002), we calculated mixed effect models of the form trait ~age + diameter for all species and traits to test which of the two factors has a stronger effect on trait variation, and linear regressions of traits against age and diameter to compare r^2 values.

Statistical analyses were calculated with R 3.2.2 (R Core Team, 2015).

3 | RESULTS

In all species, fibres occupied the largest fraction (56%–71%) of the cross-sectional area, the average ray fraction was relatively constant across species (12%–17%), the vessels fraction ranged between 4% in *Afzelia* and 19% in *Neolitsea*, and axial parenchyma between 4% in *Chukrasia* and *Neolitsea* and 25% in *Afzelia* (Table 2). In *Toona*, fibres and axial parenchyma were sometimes too indistinct to quantify seperately, but tangential sections showed that axial parenchyma is also scarce. Mean individual vessel area ranged from 7,600 μm^2 in *Neolitsea* to 40,000 μm^2 in *Melia* and vessel density between 3 mm^{-2} in *Afzelia* and 26 mm^{-2} in *Neolitsea*. K_h was the highest in *Melia*, followed by *Toona*, *Neolitsea*, *Chukrasia* and *Afzelia* (Table 2).

Mean WD in *Chukrasia*, *Afzelia* and *Neolitsea* was approximately 0.65 g/cm, in *Melia* 0.50 and in *Toona* 0.38. Similarly to WD, fibre wall thickness was relatively uniform ranging between 2.18 μm in *Toona* and 3.84 μm in *Neolitsea*. The average fibre wall fractions in *Chukrasia* and *Neolitsea* (48% and 44%) were higher than those in *Melia* and *Afzelia* (31%–38%).

3.1 | Radial variation in growth and wood traits

Melia had the highest juvenile growth rates with >10 mm per year of radial increment and *Chukrasia* the lowest (*c.* 2 mm). In *Melia*, growth declined fast, in *Afzelia* and *Toona* the decline was more gradual and in *Chukrasia* the annual diameter increment was almost constant (Figure 2).

We found significant radial trends in all wood traits, but which traits showed significant trends and whether the trait measures were increasing or decreasing towards the bark differed substantially among species (Table 3, Figures 3 and 4). The ray fraction increased in all except *Neolitsea*, but the axial parenchyma fraction was significantly related to tree diameter only in *Afzelia*, where it decreased from about 50% close to the pith to *c.* 20% at distances >10 cm. The vessel fraction (Figure 3) and mean VA (Figure S1) increased with tree diameter in all species, VD decreased (significantly only in two species, Table 3, Figure S2) and K_h invariably increased (Figure 4). The fibre fraction decreased in all except *Afzelia* (Figure 3), whereas the fibre wall thickness increased in all (Figure S3) and the fibre wall fraction increased (in *Afzelia*), decreased (in *Neolitsea*) or did not change significantly (Table 3). WD increased with tree diameter in *Afzelia*, *Melia* and *Toona*, but decreased in *Neolitsea* and did not change in *Chukrasia* (Figure 4). While some characteristics of wood anatomy changed strongly over the first 5–10 cm and then were more or less constant (e.g. VA and fibre fraction in *Afzelia* or WD in *Afzelia* and *Melia*), most measures do not offer a clear distinction between juvenile and mature wood.

3.2 | Relationships between wood density, K_h and wood cell features

Within and among species, K_h was strongly related to VA and vessel fraction, but weakly and rather negatively to VD, with a strong negative VD: VA relationship (Figure 5). Within and among species, there was very little to no relationship between WD and K_h or vessel size. The relationship between WD and its underlying anatomical components varied substantially among species (Table 4). In *Afzelia* ,WD was negatively correlated with axial parenchyma, in *Chukrasia* and *Melia* positively related to axial parenchyma and fibre walls, in *Neolitsea* negatively and in *Toona* positively related to the vessel fraction.

3.3 | Diameter vs. age effect

When age and diameter were used to explain the variation in wood anatomy, diameter was mostly the better predictor (Table 5). Age was the better predictor for the fibre fraction and fibre wall thickness in *Afzelia* and *Chukrasia*, for the vessel fraction in *Afzelia* and the parenchyma fraction in *Melia*. Diameter was always the better predictor for vessel traits (including K_h) and WD. K_h (which is largely controlled by VA) and VA were the only traits with significant radial gradients in all species in the Diameter + Age models.

4 | DISCUSSION

We first discuss the concept of juvenile/mature wood and then how wood anatomical traits and the functions served by different cell types vary over a tree's life and how trends in wood functions are linked to differences in radial growth trajectories and shade tolerance of the five species studied. We ask which cell types either control or potentially trade-off against wood density and finally if the radial

TABLE 3 Correlations between wood traits and tree radius (distance from the pith). Regression slopes (*S*, change per m radial distance) and significance levels (*$p < .05$; **$p < .01$; ***$p < .001$) were obtained with linear mixed models, r^2 is adjusted r^2 from simple linear models. Vessel area, vessel density and K_h were log-transformed

	Afzelia		*Chukrasia*		*Melia*		*Neolitsea*		*Toona*	
Wood traits	r^2	*S*	r^2	*S*	r^2	*S*	r^2	*S*	r^2	*S*
Parenchyma	0.21	−0.56**	0.02	−0.06	0.04	0.08	0.02	0.03		
Vessels	0.39	0.18***	0.49	0.35***	0.27	0.25**	0.59	0.50***	0.44	0.38***
Rays	0.34	0.26***	0.27	0.16***	0.09	0.11*	0	0.04	0.12	0.24*
Fibres	0.01	0.14	0.34	−0.45***	0.17	−0.48**	0.49	−0.58***		
Fibres wall	0.35	0.74***	−0.03	−0.04	−0.03	0.1	0.22	−0.24**	−0.03	0.07
Vessel area (mm^2)	0.47	4.30***	0.6	4.55***	0.43	3.68***	0.39	2.83***	0.62	0.07***
Vessel density (mm^{-2})	0.02	−0.89***	0.08	−1.36***	0.23	−2.06***	−0.01	−0.15	0.35	−0.03***
K_h (kg m s^{-1} MPa^{-1})	0.51	7.68***	0.7	8.31***	0.39	6.02***	0.48	5.52***	0.68	11.2***
Fibre wall thickness (μm)	0.21	5.43***	0.06	1.73*	0.3	3.54***	0.1	2.66*	0.29	2.39**
Wood density (g/cm^3)	0.32	0.55***	−0.01	0.1	0.1	0.30*	0.1	−0.27**	0.66	0.68***

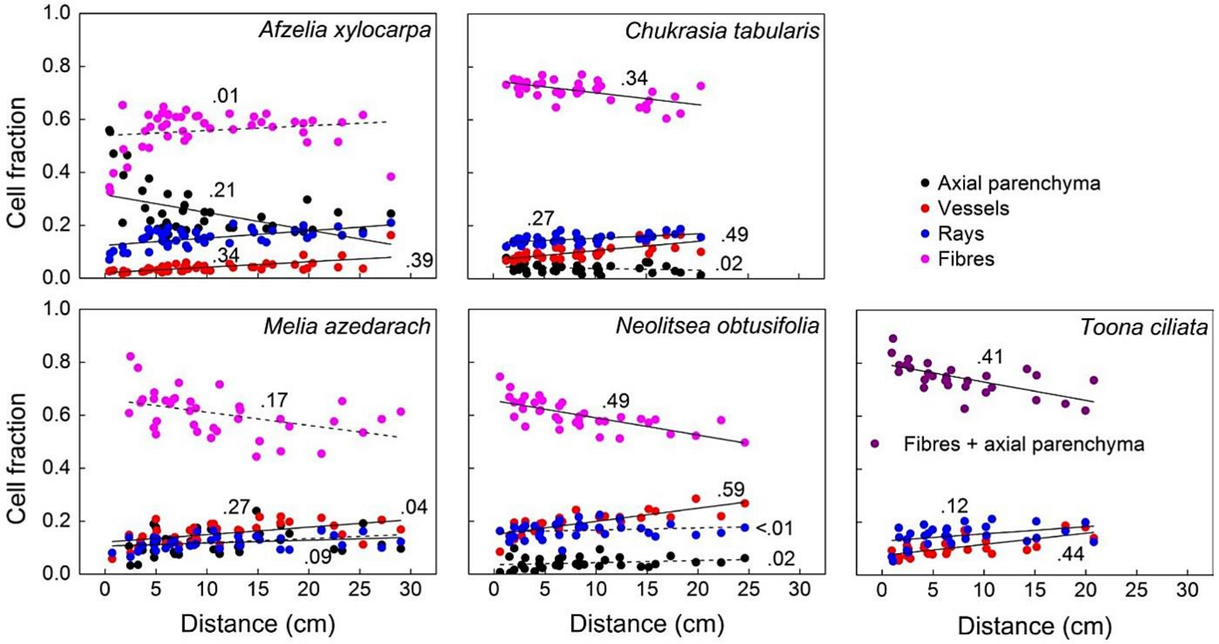

FIGURE 3 Variation in cell fractions with tree size (distance from the pith) in five species from a Thai monsoon forest. The solid lines represent significant ($p < .05$), the dashed lines non-significant correlations. In *Toona*, the small fraction of axial parenchyma could not be distinguished reliably from fibres and the two tissue types are pooled here

variation observed is best explained by cambial age, or by changes in mechanical or hydraulic demands of a growing tree.

4.1 | No universal measure to distinguish juvenile from mature wood

In the species we studied, traits that increase theoretical K_h (vessel size and fraction) and fibre wall thickness were the only ones significantly increasing with diameter in all species (Table 3) and would therefore appear to be a suitable parameter to distinguish juvenile from mature wood. However, all observed changes were gradual, and while vessel and other traits do appear to level off or at least change less after the trees had reached a certain diameter in some species, none of the traits investigated had reached constant values in all five species, which were measured to diameters of 50–60 cm. Thus, none of the measures we took can be used to define the limit between juvenile

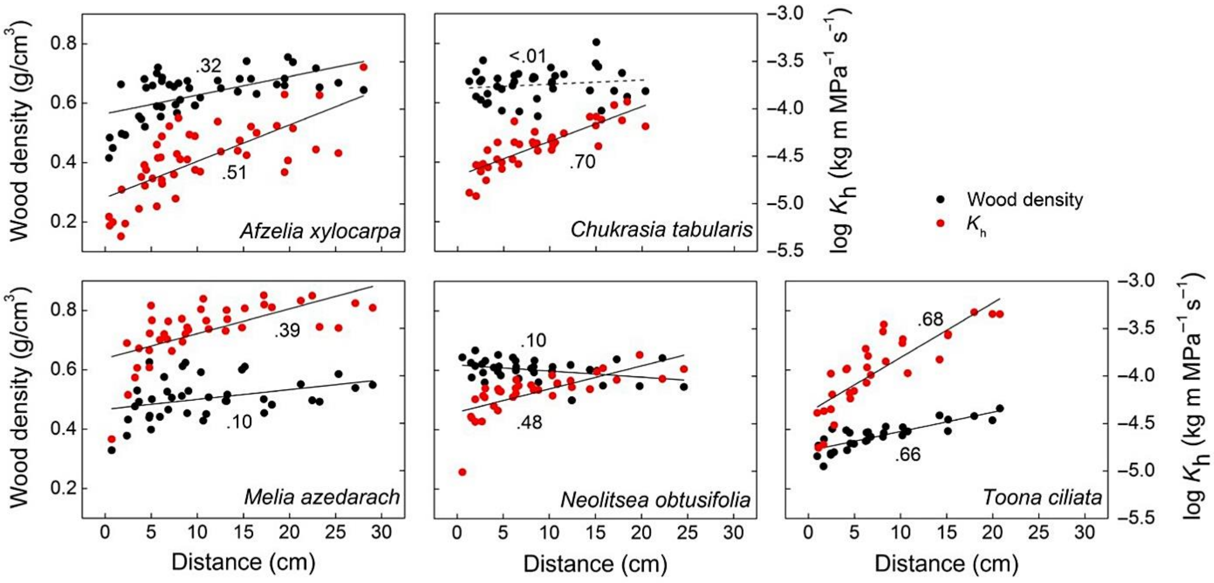

FIGURE 4 Variation in wood density and hydraulic conductivity (K_h, on a log-10 scale) with tree size (distance from the pith) in five species from a Thai monsoon forest. The solid lines represent significant ($p < .05$), the dashed lines non-significant correlations

and mature wood. Distinguishing juvenile from mature wood is important for the wood industry, but is not an ideal measure when comparing different species for ecological questions. Moreover, with radial changes being gradual, the difference between inner wood and outer wood depends on the tree diameter the samples represent, i.e. the radial distance from the pith. Without this information, comparing juvenile vs. mature wood to understand differences among tree species can be misleading. We, therefore, suggest to record tree size for all wood traits and to use the radial gradient, i.e. change over distance, and indicate the size range for an analysis of ontogenetic changes.

4.2 | Radial variations in hydraulic and storage functions

K_h increased about three- to eightfold in the first 20 cm of radial growth (Figure 4). Higher conductance in larger trees is necessary to account for the increased resistance of the longer and more tortuous path (McDowell et al., 2002). Increases in stem hydraulic conductance can be achieved by increasing sapwood area, or by increasing sapwood-specific hydraulic conductivity, which are substantially different ways to keep supplying leaves with water. The leaf area:sapwood area ratio (LA:SA) was found to increase by about 40%–400% as trees grow (McDowell et al., 2002), but this was not observed in all species (Ryan, Phillips, & Bond, 2006). Our results show that K_h can increase by at least as much or more. Increasing sapwood area incurs metabolic costs to either produce more new wood or to keep wood parenchyma cells alive, which is needed to maintain water transport (Brodersen & McElrone, 2013). Producing larger vessels, by contrast, would not incur extra costs, unless these are frequently embolised and require active removal of emboli. Unfortunately, few studies looked at both changes in hydraulic conductivity per sapwood area and LA:SA as related to tree size (Ryan et al., 2006), which would be important to understand why trees differ in the ontogenetic adjustments of their capacity to transport water.

We may also frame the question of why vessel size increases by asking why small trees have smaller vessels. In conifers, inner wood is known to have higher resistance to cavitation (Domec & Gartner, 2001), which is needed because smaller trees have a lower water storage capacity and a less developed root system. Little is known about stem-size related xylem vulnerability in angiosperm trees, but smaller vessels tend to have lower vulnerability to cavitation (Cai & Tyree, 2010; Schuldt et al., 2016). Thus, the increase in vessel size with tree diameter may reflect the need for high hydraulic efficiency in large trees as well as that for hydraulic safety in small trees.

A study of 800 tree species in China found axial parenchyma more related to theoretical hydraulic conductivity, but ray parenchyma scaling with wood density and thus mechanics (Zheng & Martínez-Cabrera, 2013). These different functions of ray and axial parenchyma may explain why the ray fraction is rather constant among species and mostly increases radially, whereas the axial parenchyma fraction is very variable (Table 2, Morris et al., 2016). In addition, many tropical trees have living, septate fibres, which are cells with thick and lignified walls that are long-lived and perform the mechanical function of fibres but can also storage organic compounds (Carlquist, 2015). If these partially take over the function of parenchyma, this may also explain why axial parenchyma is scarce in some species.

Living parenchyma can dynamically release water and thus contribute to wood capacitance in tropical dry forests (Borchert & Pockman, 2005). In addition, parenchyma associated with vessels contributes to hydraulic safety by actively repairing emboli (Brodersen & McElrone, 2013). In *Afzelia*, the species with by far the largest axial parenchyma fraction, hydraulic capacitance and possibly embolism repair may be

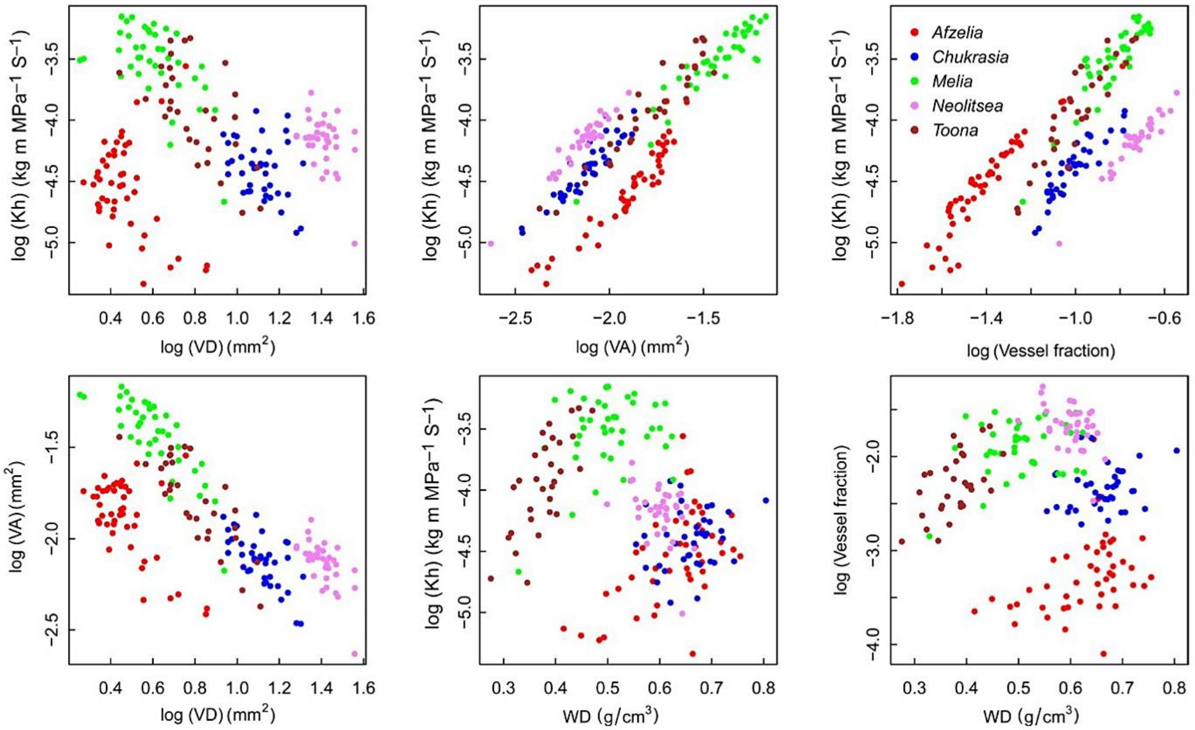

FIGURE 5 Correlations among wood traits for five tree species. K_h, theoretical hydraulic conductivity; VA, vessel area; VD, vessel density; WD, wood density

provided through paratracheal axial parenchyma (Figure 1). When cored, *Afzelia* stems released copious amounts of liquid water, even during the dry season (Figure S4). Positive xylem pressure can be produced via an osmotic pressure gradient between the xylem apoplast and living cells (Yang & Tyree, 1992), which is related to the hydrolysis of starch in living cells (Braun, 1984). Stem water storage delays the effect of drought (Pineda-García, Paz, & Meinzer, 2012) and permits leaf flushing in the dry season (Borchert, 1994). By storing water and carbohydrates, the large parenchyma fraction of *Afzelia* could provide the anatomical basis for its strategy in a seasonal dry forest.

4.3 | Variations in wood mechanics and potential trade-offs

Wood density is strongly related to mechanics and the increase in WD observed in many species reflects the fact that increasing strength on the periphery of a column makes it stronger at a given diameter and biomass investment (Hietz et al., 2013). However, a radial increase in WD is not uniform nor seen in all species (Figure 4). The investment in mechanical support vs. diameter growth should be advantageous at any life stage of a tree, also considering that low wood density and high growth rates tend to trade off against high mortality rates (Wright et al., 2010).

Fibres are cells for mechanical support and occupied the largest fraction of the cross-sectional area (Table 2). Although there might be a trade-off between the area occupied by fibres and other cell types and their respective functions, WD and thus mechanical strength can be increased by increasing fibre wall thickness, which affects the fibre wall fraction but not the cross-sectional area available for other cell types (Ziemińska, Butler, Gleason, Wright, & Westoby, 2014; Ziemińska et al., 2015). The increase fibre wall thickness, which is seen in all species, is necessary to increase WD or to compensate for the reduction of the fibre fraction when the parenchyma fraction increases.

TABLE 4 Results of linear mixed models describing the relationship between wood density and wood tissue or cell wall fractions for five canopy tree species

	Afzelia	*Chukrasia*	*Melia*	*Neolitsea*	*Toona*
Parenchyma	−0.63***	0.98**	0.86***		
Vessels		0.37		−0.41**	0.93***
Rays					
Fibre walls		0.61***	1.10***		
r^2	0.67	0.43	0.56	0.17	0.26

Values are the slope of significant or nearly significant variables and the coefficient of determination (r^2) for the linear model. Significance levels are $p < .1$; *$p < .05$; **$p < .01$; ***$p < .001$.

TABLE 5 Significance (*p*-values) for multivariate mixed-effects models relating wood traits to tree diameter (Diam) and age. Parenchyma to fibre walls are fraction out of the cross-sectional area. $p < .05$ are printed in bold, the factor with the highest significance level in each correlation is shaded grey

	Afzelia		*Chukrasia*		*Melia*		*Toona*	
Wood traits	**Diam**	**Age**	**Diam**	**Age**	**Diam**	**Age**	**Diam**	**Age**
Parenchyma	**.0002**	**.0142**	.7179	.8241	.0592	**.0168**		
Vessels	**.0173**	**.0000**	**.0026**	.6832	.6053	.1958	.3151	.3654
Rays	**.0035**	.9820	.0511	.7861	.8304	.4523	**.0062**	**.0430**
Fibres	**.0002**	**.0001**	.0904	.5728	.3292	**.0223**		
Fibres wall	**.0000**	**.0436**	.5867	.4439	.0819	.1540	.5882	.7456
Vessel area	**.0000**	.5420	**.0378**	.1322	**.0030**	.3834	**.0012**	.2312
Vessel density	**.0001**	**.0002**	.5419	.6950	**.0057**	.1086	**.0011**	**.0318**
K_h	**.0000**	.8444	**.0065**	.1188	**.0264**	.8674	**.0051**	.8090
Fibre wall thickness	.1298	**.0057**	.1686	.5744	.8010	**.0102**	.4098	.3346
Wood density	**.0000**	**.0003**	.4936	.7237	**.0313**	.2563	**.0006**	.4862

Although the variation in WD among species is strongly controlled by fibre wall thickness and the fraction of fibre walls in the cross section (Fortunel et al., 2014; Jacobsen et al., 2007), we found the within-species variation of the fibre wall fraction significantly related to WD only in *Chukrasia* and *Melia* (Table 4). In *Afzelia*, while the fibre wall fraction and WD both increased radially, the very strong variation in axial parenchyma alone explained 67% of the variation in WD. The vessel fraction was negatively correlated with WD only in *Neolitsea*, the species with the highest vessel fraction. In most species, the vessel fraction may be too low to have an effect on WD that could not be compensated or surpassed by the effect of other cell types. Although the negative correlations between parenchyma and vessel fractions and WD suggests trade-offs between mechanical demands and other functions, such trade-offs could be avoided by increasing WD through fibre wall thickness or increasing K_h through larger vessels without the need to increase the fibre or vessel fraction.

4.4 | Associations between wood traits and species performances

Trees in tropical dry forest have been classified along a gradient between two main strategies to cope with drought (Méndez-Alonzo, Paz, Zuluaga, Rosell, & Olson, 2012). Deciduous species typically have low WD, which is related to high stem water capacitance, high hydraulic conductance but low cavitation resistance. Trees that are evergreen or drop their leaves late in the dry season typically have high WD, a conservative water use with low hydraulic conductance and high cavitation resistance. With the exception of *Afzelia*, the average WD and K_h of the trees studied reflects this pattern with deciduous and shade-intolerant *Toona* and *Melia* having low WD and high K_h and shade-tolerant brevi-deciduous *Chukrasia* and evergreen *Neolitsea* having higher WD and low K_h. Deciduous *Afzelia*, however, had the lowest K_h and second-highest WD, again reflecting its unusual water relations strategy.

Wood density is the functional trait that best explains differences in growth rates among species (Wright et al., 2010). We found that WD also explains within-species differences in growth rates during ontogeny with positive radial gradients of WD in *Afzelia*, *Melia* and *Toona* (Figure 4), the three species with declining growth rates (Figure 2), and no trend in either growth or WD in *Chukrasia*. Variation in WD is thus also explained by growth trajectories, where extremely high juvenile growth rates can only be achieved by producing cheap (i.e. low density) wood.

Among species, K_h has been found to be correlated with growth rates (Hoeber, Leuschner, Köhler, Arias-Aguilar, & Schuldt, 2014), possibly because high growth rates require productive leaves and a water transport system that supplies them (Hietz et al., 2017). As individual trees grow, the increase in K_h is needed to compensate for the increasing hydraulic resistance (Zach et al., 2010). While K_h always increased significantly in the species we studied, the radial gradient in K_h was highest in *Toona* and lowest in *Neolitsea* and *Melia* (Table 3). Thus, the relatively high K_h in small *Melia* trees may be due to their very high initial growth rate, with the subsequent increase in K_h is smaller than in other species. In addition, shade-intolerant pioneers such as *Melia* typically regenerate in gaps with higher evaporative damand, where high K_h may be needed to sustain transpiration (Maherali, DeLucia, & Sipe, 1997). In contrast, trees of species typically regenerating in the shade and then growing to the canopy typically have slow initial growth and experience a stronger change of microclimate and evaporative demand, which may explain the continuing increase in K_h in these species.

4.5 | Does age or size control radial variation in wood traits?

Understanding age vs. diameter control of wood structure and functions is important to understand a tree's potential to respond to demand. The correlation between tree diameter and age makes a clear separation between the two difficult and we limit our analysis here to asking whether age or size had the stronger effect in multiple regression (Table 5). A size effect could be explained by the changing demand for mechanic, hydraulic and perhaps storage functions of wood as outlined above. An age effect, by constrast, would point to an intrinsic control of wood development whereby the younger and often shorter cambium cells are limited in the size of cells they can yield. In this case, one would still expect the development and size of cambium cells to be related to the number of cell divisions, and thus tree diameter, and not to the age per se. Alternatively, if higher growth rates result in cells having less time to expand because the cambium is producing new cells at a high rate, then faster growing trees would, at a given diameter, have more juvenile wood such as shorter tracheids or thinner walls and thus lower wood density (Lachenbruch et al., 2011).

Few studies tested the age vs. diameter effect in angiosperms with more complex wood than conifers. In *Populus* hybrids with different growth rates fibre length appears to be controlled by age rather than diameter (DeBell, Gartner, & DeBell, 1998), whereas a study on tropical hardwoods found fibre length to be controlled by diameter in *Acacia* and *Paraserianthes* but by age in *Eucalyptus* (Kojima, Yamamoto, Yoshida, Ojio, & Okumura, 2009). Also wood density, which is strongly controlled by fibres, was more strongly correlated with cambial age than with tree diameter (Clark & Saucier 1989; de Castro, Williamson & Jesus 1993; Nock et al., 2009; Williamson & Wiemann 2010).

For other cell types in angiosperms, the age vs. size effect is largely unexplored. Overall, we found a stronger effect of tree diameter compared to age on all parameters except for fibre wall thickness and the fibre fraction, which were more strongly correlated to age than diameter in *Afzelia* and *Melia* (Table 5). The cambium can produce very different vessel sizes within a short timespan, also in young trees, so that the variation of vessel size within a very small space is often larger than the gradient from pith to bark (Leal, Sousa, & Pereira, 2007). Except for perhaps the first few centimetres of diameter growth, it therefore appears implausible that vessel development is constrained by what cell types and sizes can be derived from cambium cells.

5 | CONCLUSIONS

With the possible exception of fibres, our data thus support a stronger effect of tree size and no or a substantially weaker effect of tree age on wood structure and function. The ontogenetic (radial) variation can be explained by the changing demands for mechanical stability, the efficiency and safety of the water transport system, and in some cases the stem storage capacity. By modulating tissue fractions, cell sizes and cell wall thickness, growing trees can adjust their wood functions, increasing water transport capacity and mechanical strength at the same time, without an apparent trade-off in terms of cross-sectional area. This size-related change in wood structure and function is important to understand difference in ecological strategies among tree species. Given the very substantial radial variation in wood traits, tree size is an essential parameter to include in comparative studies on the functional ecology of wood.

ACKNOWLEDGEMENTS

We thank the Thai Royal Forest Department for permission and help to sample wood. K.R. is supported by a grant from the Austrian Federal Ministry of Science, Research and Economy (BMWFW) within the framework of the ASEA UNINET. We appreciate support from the Austrian Science Fund (P19507-B17) and helpful comments by Sabine Rosner, Daniel Tholen and two anonymous reviewers. The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

P.H. collected samples and conceived the ideas, K.R. made anatomical measurements, P.H. and K.R. contributed equally to data analysis, discussion and writing.

DATA ACCESSIBILITY

Data are deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.k78c0 (Rungwattana & Hietz, 2017).

ORCID

Peter Hietz http://orcid.org/0000-0002-0458-6593

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SUPPORTING INFORMATION

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How to cite this article: Rungwattana K, Hietz P. Radial variation of wood functional traits reflect size-related adaptations of tree mechanics and hydraulics. *Funct Ecol*. 2018;32:260–272. https://doi.org/10.1111/1365-2435.12970

General conclusions

To better understand the responsible mechanisms and variations in wood structure and functions, a tool for quantitative wood anatomy is very important. Here, this thesis in the chapter 2 have presented the new measurement approach by using a laser scanning microscopic (LSM) technique which speeds up sample preparation and still procedure reliable results, by a clear distinction between cell lumina and walls. The LSM has no requirement for sectioning, staining and embedding in relation to conventional thin-section procedure. Additionally, the LSM provided a high-quality image that is suitable for automated image analysis both under low magnification for vessels and high magnification for fibres. I believe that the increased power and versatility of this technique might allow to efficiently creating comprehensive dataset of cell anatomical features for a wide range of novel research applications such as wood structure and function relationship, an evolutionary adaptation to a changing environment in woody plants, dendrochronology etc.

Wood features vary among and within species. This thesis showed that the intraspecific trait variation has important ecological significance and helps to study and understand evolutionary adaptation in tree species (e.g. *Hevea brasiliensis* in chapter 3). The results of a common garden experiment of the rubber trees from multiple provenances showed that genotype explained a substantial proportion of the variation in leaf and wood functional traits and the dry season intensity could be linked to the phylogenetic signal in several traits. *Hevea* has evolved drought-avoidance strategies for adaptive traits instead of drought tolerance traits that researcher have usually looked for. Trees from stressful habitats had a higher growth potential than trees from more humid regions and leaf carbon isotope also indicated reduced drought stress in trees from drier locations. In the search for adaptive traits (and promising genotypes for breeding program), failing to identify drought tolerance should not lead to the conclusion that species are not drought resistant or do not have the genetic diversity to adjust to drought. Trees can survive drought with different strategies such as drought avoidance as the example of *Hevea* shows.

This thesis also showed that the ontogenetic variation of wood structure has evolved to optimize wood functions for the whole plant at all life stages. The results in chapter 4 show that the considerable ontogenetic variations are relatively can be explained by the changing demands for mechanical stability, the efficiency and safety of the water transport system, and in some cases the stem storage capacity. Wood anatomical components such as cell (vessels, fibres, ray and axial parenchyma) fractions, cell sizes and cell wall thickness change during ontogeny. This chapter also shows that the ontogenetic changes are determined plastically by tree size rather than intrinsically by tree age. Thus, this size-related change in wood structure and function is important to understand difference in ecological strategies among tree species. Given the very substantial radial variation in wood traits, tree size is an essential parameter to include in comparative studies on the functional ecology of wood.

To conclude, this thesis shows that wood is very variable with difference occurring among species, among geographic sources within a species, among trees within a geographic source as well as within each individual tree. These variations in xylem structure and function make substantial contributions to the success or failure of the plant through optimizing three main functions relating to mechanical, hydraulic and storage constraints. With whatever underlying cause that shapes adaptive traits through either genetic control or plasticity, the main goal of a plant is to increase its chance of survival and reproduce for the continued existence of the species. Humans can benefit from a better understanding of the causes and consequences of the variation in xylem structure and function in many different applications such as breeding genotypes with increased drought resistance or drought avoidance when specific gene loci have been explored to control a given adaptive trait; or speeding up artificial migration of tree genotypes which fail to adjust to rapid climate change when this species has a high degree of intra-population genetic variability.

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Curriculum Vitae

Personal information

Date of birth	03.09.1987
Place of birth	Bangkok, Thailand
Nationality	Thai

Educational background

2015 – present	**Doctoral Program in Agriculture (Forest and Woodscience)** University of Natural Resources and Life Science Vienna, AUSTRIA Institute of Botany
2009 – 2012	**Master of Science (Botany)** Kasetsart University, BANGKOK, THAILAND Department of Botany, Faculty of Sciences
2011 – 2012	**Master's Program (Horticultural Sciences) as a degree student** University of Natural Resources and Life Science Vienna, AUSTRIA
2005 – 2009	**Bachelor of Science (Agricultural Sciences) with First Class Honours** Kasetsart University, BANGKOK, THAILAND Department of Horticulture, Faculty of Agriculture
2002 – 2005	**High School Certificate (Sciences-Mathematics)** Bodindecha (Sing Singhaseni) School, BANGKOK, THAILAND

Professional Experiences

2017 – 2018	Evolution Adaptation to Drought for *Hevea brasiliensis* University of Natural Resources and Life Science Vienna, AUSTRIA Collecting wood and leaf sample, Preparing wood anatomical section, Processing image analysis, Analyzing data, Producing manuscript
2016 – 2017	New Insight of Hydraulic Conductivity of Temperate Angiosperm Tree Species University of Natural Resources and Life Science Vienna, AUSTRIA Preparing wood anatomical section, Processing image analysis, Analyzing data
2015 – 2017	Radial Variation in Wood Structure and Function for Tropical Tree Species University of Natural Resources and Life Science Vienna, AUSTRIA Preparing wood anatomical section, Processing image analysis, Analyzing data, Producing manuscript

2015 – 2016	Novel Technique to Assessing Quantitative Wood Anatomy University of Natural Resources and Life Science Vienna, AUSTRIA Preparing wood anatomical section, Processing image analysis, Analyzing data, Producing manuscript
2009 – 2012	Water and Light Regimes Reflecting Photosynthesis for *Calathea* spp. Kasetsart University, BANGKOK, THAILAND Measuring gas-exchange, chlorophyll fluorescence and osmotic adjustment, Analyzing data, Producing manuscript

Publications

- Rungwattana K, Hietz P (2018) **Radial variation of wood functional traits reflect size-related adaptations of tree mechanics and hydraulics**. Funct Ecol 32:260-272. http://dx.doi.org/10.1111/1365-2435.12970
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Awards

2015 – 2018	Technologiestipendien Südostasien (Doctoral Study in Vienna, Austria) ASEA-UNINET: the Austrian Federal Ministry of Science, Research and Ecnomy – BMWFW
2011 – 2012	EURASIA 2 Project (Exchange Master student in Vienna, Austria) ERASMUS MUNDUS (European commission)
2007	Phumiphon Scholarship (King's Scholarship Thailand) HM King Bhumibol Adulyadej