



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA

**El potencial ontogenético de las células troncales de la madera en la
evolución morfológica de las plantas vasculares**

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

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CIUDAD UNIVERSITARIA, CD. MX., NOVIEMBRE, 2023.



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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **18 de septiembre de 2023** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **PETRONE MENDOZA EMILIO** con número de cuenta **410066119** con la tesis titulada **“EL POTENCIAL ONTOGENÉTICO DE LAS CÉLULAS TRONCALES DE LA MADERA EN LA EVOLUCIÓN MORFOLÓGICA DE LAS PLANTAS VASCULARES”**, realizada bajo la dirección del **DR. MARK EARL OLSON**, quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
“POR MI RAZA HABLARÁ EL ESPÍRITU”
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COORDINADOR DEL PROGRAMA



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Resumen

La biología evolutiva del desarrollo, o evo-devo, investiga los procesos y mecanismos que generan variación morfológica durante el desarrollo de los organismos y la relación de estos con los procesos evolutivos. En las últimas dos décadas, la evo-devo se ha centrado principalmente en estudiar la evolución de la expresión genética. En esta tesis argumento que, en las plantas, las mismas características de su desarrollo permiten hacer estudios de evo-devo a nivel morfológico que no es posible realizar en muchos animales. La relevancia del desarrollo de las plantas como una fuente de datos para hacer estudios de evo-devo, permite hacer inferencias que van más allá de la asociación de un patrón de expresión genética con la formación de una característica fenotípica. En particular utilicé el sistema vascular secundario de las plantas leñosas, un excelente archivo ontogenético, para identificar desde decenas hasta cientos de diferenciaciones o “decisiones” realizadas por las células troncales del tejido. Información detallada de las series de “decisiones” celulares de las células troncales llamadas cambium vascular no se había documentado previamente. Las “decisiones” del cambium vascular son relevantes porque los tipos celulares de la madera determinan las características del transporte y almacenamiento de agua y nutrientes, así como del soporte mecánico de los tallos. Utilizando teoría del lenguaje, representé a los tipos celulares como letras y las combinaciones de los tipos celulares como palabras. Esto me permitió cuantificar la diversidad de “decisiones” celulares y compararla en un grupo de especies del clado *Pedilanthus* (género *Euphorbia*) que varían morfológica y funcionalmente. Las especies del clado *Pedilanthus* varían, principalmente, de arbustos con tallos suculentos a arbustos y árboles con tallos leñosos. Descubrí que una mayor diversidad ontogenética en las formas con tallos leñosos que en las formas con tallos suculentos, está posiblemente asociada con una mayor diversidad funcional del tejido. Por otra parte, analicé la relación entre el hábito de crecimiento de “no auto-sostén” y la presencia de variantes vasculares en las angiospermas. Las variantes vasculares son una categoría de organización vascular que

agrupa a especies que no desarrollan la configuración típica de un anillo de cambium vascular que resulta en la acumulación concéntrica de células del xilema. A partir de una base de datos pública, demostré que hay una asociación preferencial entre el hábito de crecimiento de “no auto-sostén” y la presencia de variantes vasculares. Propongo que la evolución del hábito de crecimiento de “no auto-sostén” es una característica que favorece la evolución de las variantes vasculares. En conjunto, los estudios propuestos en la presente tesis son una contribución al entendimiento de la evolución morfológica del sistema vascular de las plantas leñosas.

Palabras clave: *Evo-devo, adaptación, morfología, desarrollo secundario, cambium vascular, potencial ontogenético.*

Abstract

The processes and mechanisms generating morphological variation during development and their relationship with evolutionary processes are studied by evolutionary developmental biology or evo-devo. Over the past two decades, evo-devo has mainly focused on studying the evolution of gene expression. In this thesis, I argue that in plants, due to the unique characteristics of their ontogeny, it is possible to conduct evo-devo studies at the morphological level that may not be feasible in many animals. Moreover, the relevance of plant development as a rich source of evo-devo data makes plant evo-devo vastly more than which gene is involved in which transformation. To show the relevance of plant morpho evo-devo, I used the secondary xylem of woody plants, an excellent ontogenetic record, to identify tens to hundreds of differentiations or “decisions” made by the stem cells of the tissue named vascular cambium. Detailed information about the series of “decisions” made by vascular cambium cells had not been documented previously. The series of “decisions” of wood stem cells are relevant because cell types determine strategies of water and nutrient transport and storage, as well as the mechanical support of stems. Using language theory, we represent cell types as letters and combinations of cell types as “words.” This allowed us to measure the diversity of cellular “decisions” and compare it among a group of tropical species, the *Pedilanthus* clade (genus *Euphorbia*). The *Pedilanthus* clade varies from succulent shrubs to woody trees. We found that greater ontogenetic diversity is associated with greater tissue functional diversity in woody trees with respect to succulent shrubs. Furthermore, I analyzed the relationship between the “non self-supporting” growth habit and the presence of vascular variants. I propose that the evolution of the “non self-supporting” growth habit is a feature that promotes the evolution of vascular variants. Overall, the studies proposed in this thesis contribute to the understanding of the morphological evolution of the vascular system in woody plants. Furthermore, I analyzed the relationship between the “non self-supporting” growth habit and the presence of vascular variants in angios-

perms. Vascular variants are a category that encompasses species that do not develop a “typical” vascular organization, consisting of a vascular cambium ring that accumulates xylem cells in concentric layers. Using a public database, I show that there is a preferential association between the “non self-supporting” growth habit and the presence of vascular variants. I propose that the evolution of the “non self-supporting” growth habit is a feature that facilitates the evolution of vascular variants. Collectively, the studies proposed in this thesis contribute to the understanding of the morphological evolution of the vascular system in woody plants.

Key words: *Evo-devo, adaptation, morphology, secondary growth, vascular cambium, developmental potential.*

Introducción general

Características de la biología evolutiva del desarrollo

La biología evolutiva del desarrollo o evo-devo es la disciplina que busca entender la relación entre los procesos que subyacen a la formación de los organismos y los patrones evolutivos derivados de estos procesos. Aunque la disciplina comenzó a gestarse a finales del siglo XIX, producto de los trabajos de embriología comparada, la evo-devo se consolidó durante la década de los años 1980 con el descubrimiento de los genes homeobox ([Arthur, 2002](#); [Gilbert, 2003](#)). Hoy en día la evo-devo se caracteriza por abordar diversos problemas de evolución y desarrollo mediante una diversidad de aproximaciones epistémicas y metodológicas ([Moczek et al., 2015](#); [Nuño de la Rosa, 2017](#); [Nuño de la Rosa and Müller, 2021](#)). La formación y el mantenimiento de estructuras que evolucionan de forma cuasi-independiente ([Bolker, 2000](#); [Wagner, 1996](#)), las bases biológicas de las homologías ([Wagner, 1989, 1996, 2014](#)), o la tendencia de los individuos dentro de poblaciones a formar en mayor proporción ciertos fenotipos que otros ([Uller et al., 2018](#)), son algunos de los temas de investigación de la evo-devo. Una buena parte de la investigación en evo-devo se enfoca en estudiar la acción de los genes durante el desarrollo de los organismos y su evolución ([Müller, 2007](#)).

La genética evolutiva del desarrollo es una de las áreas de la evo-devo que ha tenido mayor atención, aportando conceptos clave para el entendimiento de la evolución de los caracteres biológicos. La manipulación genética de los organismos en un número limitado de especies incluyendo la mutagénesis, la transgénesis o los análisis clonales, ha permitido comparar los patrones espaciales y temporales de expresión genética ([Carroll, 2008](#)). En un número aún más limitado de especies, se ha dilucidado la actividad y regulación de grupos de genes que actúan en conjunto durante el desarrollo; es decir, qué genes activan o inhiben la expresión de otros genes. Los estudios de la regulación

genética durante el desarrollo han culminado en la creación de modelos de redes de regulación genética. Al estudiarse de forma comparativa, las redes de regulación genética se han postulado como participantes en la aparición de caracteres “novedosos” (Abouheif and Wray, 2002; Monteiro, 2012; Monteiro and Gupta, 2016). Por ejemplo, el desarrollo de los cuernos en los escarabajos evolucionó a partir de la reutilización de una red de regulación genética que participa en el desarrollo de las extremidades en los artrópodos (Moczek and Rose, 2009). Además, el desarrollo de técnicas de secuenciación a nivel genómico y transcriptómico han ampliado el número de sistemas modelo, características estudiadas y preguntas evolutivas específicas. Sin embargo, la tendencia a enfocarse en el estudio de los cambios moleculares que subyacen a la generación de la diversidad fenotípica ha creado sesgos que limitan la representación de otros enfoques convenientes y prometedores en la evo-devo.

En los últimos 20 años, la evo-devo ha mostrado una tendencia a estudiar aspectos genético-moleculares del desarrollo. Por ejemplo, palabras relacionadas con aspectos moleculares, como “gen”, estuvieron sobrerrepresentadas en los títulos y resúmenes del Congreso Panamericano de Evo-Devo de 2015 y 2017 (Diogo, 2016, 2018). Ocho de los 10 artículos más citados en evo-devo desde 1997 hasta la fecha se centran en el estudio de aspectos genético-moleculares (Tabla 1 y consultar Anexo 1 para detalles del análisis bibliométrico). Un análisis de co-ocurrencia de las palabras clave más comunes en las publicaciones dentro del campo muestra que el tema conceptual más común explora aspectos genético-evolutivos, con palabras como “evolución,” “expresión,” “genoma”, y “morfogénesis” (Figura 1A, color rojo). El segundo grupo (color azul) muestra una serie de conceptos relacionados con la evolución y adaptación a nivel morfológico, mientras que el tercer grupo (color verde) tiene que ver con la investigación en genética del desarrollo de *Drosophila melanogaster*. En resumen, la figura 1A muestra que los temas de principal interés en evo-devo tienen que ver con aspectos de genética del desarrollo, con énfasis particular en animales. La relativa independencia en la investigación de animales y plantas podría sugerir que los temas de interés en evo-devo de animales es diferente de la de plantas.

Los estudios de evo-devo en plantas siguen la misma tendencia que los de animales, al centrándose principalmente en aspectos de la genética del desarrollo. De los diez artículos más citados en el campo de la evo-devo de plantas, ocho se enfocan principalmente en aspectos de biología molecular (Tabla 2). A partir del análisis de las palabras que co-ocurren con mayor frecuencia en las publicaciones sobre evo-devo en plantas, se

identificaron tres temas conceptuales (Figura 1B y el Anexo 1). El tema más destacado, en términos de número de palabras, se centra en comprender las bases genéticas y la evolución de características en las plantas terrestres (resaltado en azul en la Figura 1B). Este grupo incluye términos como “evolución,” “plantas terrestres,” “*Arabidopsis*” y “expresión genética.” El segundo grupo está relacionado con el uso de *Arabidopsis* como sistema modelo para investigar mecanismos moleculares (también resaltado en azul en la Figura 1B). El tercer grupo conceptual aborda el origen y la diversificación de características morfológicas utilizando herramientas filogenéticas (resaltado en rojo en la Figura 1B). Dentro de los diez artículos más citados en el campo de la evo-devo de plantas, el segundo artículo más citado se enfoca en aspectos de plasticidad fenotípica (Sultan, 2000). Este descubrimiento sugiere un creciente interés en el estudio de características morfológicas dentro del ámbito de la evo-devo de plantas, en comparación con la investigación en evo-devo de animales. Esta inclinación podría estar relacionada con las características únicas del desarrollo en las plantas.

La información y los datos a nivel morfológico desempeñan un papel fundamental en la investigación de la evo-devo en plantas. La mayoría de las plantas vasculares crecen de forma continua, dejando rastros de su desarrollo en forma de características anatómicas específicas. Por ejemplo, las cicatrices foliares a lo largo de los tallos permiten inferir el área foliar producida durante la ontogenia de una planta, o la variación de las flores dentro de inflorescencias facilita la cuantificación y delimitación de módulos. El crecimiento continuo es producto de la proliferación y diferenciación celular en los meristemas (Rudall, 2020). Las células derivadas de los meristemas generalmente pierden su capacidad meristemática, permanecen en su lugar y se diferencian, lo que permite identificar a las células precursoras de diferentes linajes celulares (Mauseth, 2008). Aunque algunos autores han aprovechado estas características de las plantas para, por ejemplo, hacer inferencias de evolución por heterocronía (Hearn, 2009b; Isnard et al., 2012; Olson, 2007), es esencial enfatizar y promover este enfoque para enriquecer las discusiones relevantes en campo de la evo-devo.

La tendencia a utilizar organismos modelos con ciclos de vida corta, así como condiciones de crecimiento óptimas y limitadas han sesgado el planteamiento de preguntas sobre la generación de variación que se produce durante la ontogenia, especialmente en organismos de vida larga que crecen en condiciones naturales. La variación fenotípica se genera por una constante interacción entre los procesos ontogenéticos y el ambiente (West-Eberhard, 2003). Las condiciones de crecimiento en laboratorios pue-

den introducir sesgos en los intervalos de variación fenotípica de los organismos modelos (Rivera-Yoshida *et al.*, 2020). Por estas razones, se requiere un mayor número de mediciones, cuantificaciones y comparaciones detalladas de la diversidad ontogenética en organismos con ciclos de vida largos y que crecen en condiciones naturales. Por ejemplo, comparar el desarrollo de especies con organogénesis continua permitiría dilucidar si hay una relación entre la diversidad ontogenética, ya sea en términos de mecanismos ontogenéticos o diversidad en número de tipos celulares, y la diversidad funcional.

En la presente tesis, utilicé características del desarrollo de las plantas para abordar preguntas de investigación en evo-devo. En específico, estudié el xilema secundario o madera de las plantas como un excelente archivo ontogenético. La amplia diversidad funcional y anatómica hacen del xilema secundario un modelo apropiado para hacer estudios de evo-devo. Dada la importancia del desarrollo y evolución del xilema secundario en el contexto de la evo-devo de características morfológicas, a continuación, presento una breve descripción de su desarrollo.

Desarrollo del xilema secundario

El cambium vascular es uno de los meristemos fundamentales para el engrosamiento radial de los tallos mediante la formación del xilema y floema secundario en las angiospermas y en las gimnospermas (Lucas *et al.*, 2013; Tomescu and Groover, 2019). En la mayoría de las plantas leñosas, el desarrollo del cambium se describe por una serie de pasos, o una “trayectoria ontogenética,” que resultan en la formación de una anatomía del tallo conocida como cambium regular (Carlquist, 2001; Cunha Neto, 2023; Tomescu and Groover, 2019). En la trayectoria ontogenética que da lugar al cambium, las células tienen dos orígenes distintos: de tejido fundamental (en el caso del cambium interfascicular) y de tejido vascular (en el caso del cambium fascicular). Por un lado, las divisiones celulares en el procambium dan lugar a la formación del cambium fascicular, mientras que las divisiones y diferenciación de las células localizadas entre los haces vasculares conducen a la formación del cambium interfascicular (Figura 2) (Fischer *et al.*, 2019). El proceso de división en las células cambiales se puede dividir en dos tipos principales. Las divisiones anticlinales ocurren perpendicularmente a la superficie del órgano y generan dos nuevas células cambiales. Las divisiones periclinales, por otro lado, son paralelas a la superficie del órgano y de ellas se forma el xilema secundario al interior del tallo y floema secundario al exterior del tallo.

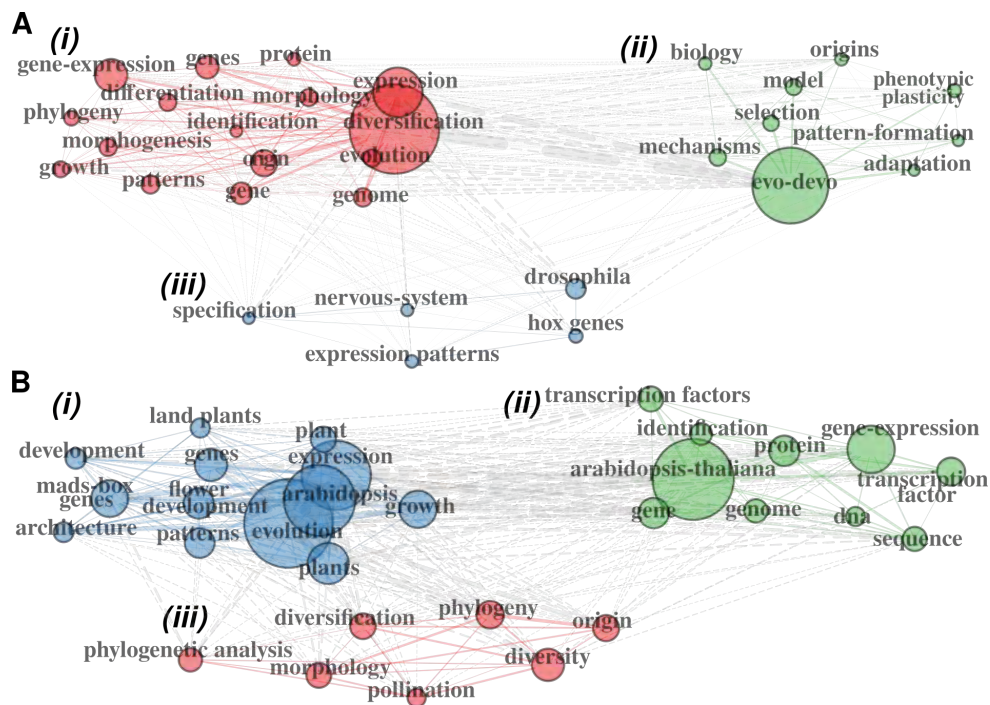


Figura 1: Sesgos en el campo de la evo-devo relacionados con la investigación de aspectos genéticos del desarrollo, revelados por el análisis de co-ocurrencia de palabras clave más comunes en artículos encontrados en la base de datos Web of Science. **(A)** Tres grupos de palabras clave co-ocurren con mayor frecuencia en artículos de evo-devo. El primer grupo (*i*, color rojo) engloba temas relacionados con evolución, morfología, diferenciación y expresión genética. El segundo grupo (*ii*, color verde) engloba temas relacionados con evo-devo, plasticidad fenotípica y adaptación, lo que posiblemente comprenda un grupo de publicaciones no relacionadas con geno-evo-devo. El tercer grupo de palabras (*iii*, color azul) está relacionado con *Drosophila melanogaster*, genes *hox* y expresión de genes, lo que muestra la relevancia de este modelo para la geno-evo-devo. **(B)** Tres grupos de palabras clave que co-ocurren con mayor frecuencia en el campo de evo-devo de plantas. El primer grupo (*i*, color azul) está relacionado con los genes MADS-box, la planta *Arabidopsis*, plantas terrestres, evolución, entre otras. Esto comprende un grupo de publicaciones relacionado con geno-evo-devo comparativo en el contexto de plantas terrestres. El segundo grupo (*ii*, color verde) está relacionado con la planta *Arabidopsis* y factores transcripcionales, lo que sugiere que un grupo de publicaciones relacionado con la genética del desarrollo en *Arabidopsis*. El tercer grupo (*iii*, color rojo) y el más pequeño, está relacionado con morfología y patrones de diversificación, lo que sugiere un grupo pequeño de publicaciones relacionado con morfología y evo-devo.

Investigaciones recientes han confirmado que el cambium vascular es uniseriado (Miyashima et al., 2019; Smetana et al., 2019). Esto significa que una única célula progenitora del cambium es responsable de generar tanto el floema como el xilema secundario. Además, las divisiones de estas células progenitoras se conservan con un

nivel de detalle que permite rastrear la historia de las diferenciaciones celulares en el xilema secundario (Bossinger and Spokevicius, 2018), desde que la planta era joven, al centro del tallo, hasta su edad más reciente en la zona cambial. Por lo tanto, el cambium vascular y sus células derivadas en el xilema secundario representan un archivo ontogenético que permite estudiar si hay una relación entre la diversidad ontogenética y la diversidad funcional en organismos con ciclos de vida largos que crecen en condiciones naturales.

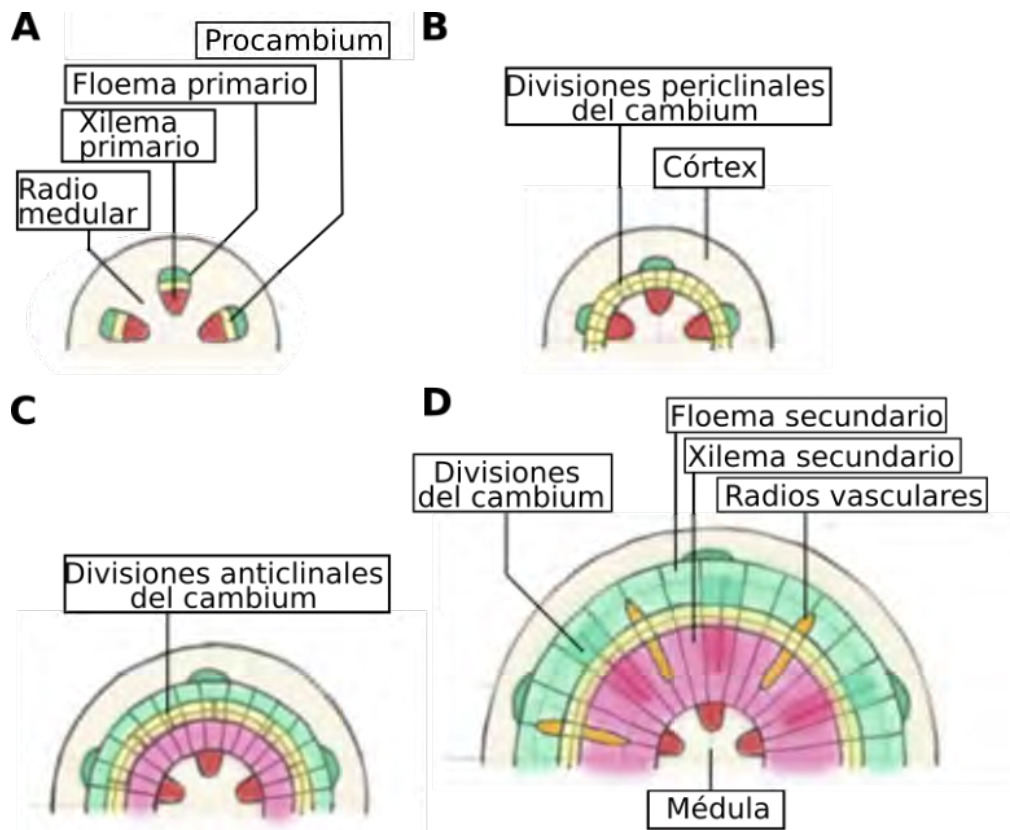


Figura 2: Desarrollo secundario y formación regular del cambium vascular en angiospermas y en las gimnospermas. (A) En el crecimiento primario cada uno de los haces vasculares están formados y contienen procambium y floema y xilema primario. (B) Divisiones del procambium (cambium fascicular) y de células parenquimáticas localizadas entre los haces vasculares (cambium interfascicular) dan lugar al cambium. (C) Las divisiones del cambium dan lugar a la formación de las primeras células del floema y xilema secundario. (D) Más divisiones del cambium y formación de radios vasculares. Modificada de (Tomescu and Groover, 2019).

Las trayectorias ontogenéticas involucradas en la formación del xilema secundario, junto con las variaciones ontogenéticas que resultan de estos procesos y que dejan un registro en la morfología de los tallos, constituyen una valiosa fuente de información para

comprender la evolución de las plantas leñosas. El orden de los cambios que resultan en la evolución de un determinado fenotipo a partir de otro fenotipo se conoce como “trayectoria evolutiva” (Edwards, 2019). Por ejemplo, en algunas especies de la tribu Bignoniae (Bignoniaceae), se forma un cambium vascular continuo parecido al de un cambium regular. Sin embargo, la trayectoria evolutiva de los tallos de la familia se desvía del cambium regular. En cuatro puntos equidistantes del tallo, la tasa de división de las células meristemáticas del xilema se reduce mientras que la del floema aumenta, creando “arcos del floema” (Pace et al., 2009). En una de las especies, los arcos del floema son el punto final de la trayectoria ontogenética, pero en la mayoría de las especies de la tribu, el cambium localizado en la zona de los arcos deja de dividirse anticlinalmente, lo que tiene como consecuencia la disrupción del cambium y dando lugar a la formación de las denominadas “cuencas del floema” (Pace et al., 2009). Este ejemplo subraya la importancia de trazar tanto las trayectorias ontogenéticas como las evolutivas para comprender el origen de la diversidad anatómica. En este contexto, mi tesis contribuye en dos aspectos del desarrollo del cambium vascular que hasta ahora han recibido poca atención.

El primer aspecto tiene que ver con documentar las diferenciaciones de las células progenitoras del xilema. El cambium consta de dos tipos de células progenitoras: las células iniciales fusiformes y las células iniciales radiales. Las células iniciales fusiformes forman el sistema axial del xilema y principalmente se diferencian en tres tipos celulares en angiospermas, que son elementos de vaso, parénquima axial y fibras (Carlquist, 2001; Mauseth, 2008; Montes-Cartas et al., 2017). Las células iniciales radiales, por otro lado, contribuyen al sistema radial y se diferencian principalmente en células parenquimáticas radiales. Las distintas tendencias de las células iniciales cambiales para producir diferentes tipos celulares tienen como resultado plantas funcionalmente diferenciadas (Bouda et al., 2019; Hearn, 2009a; Isnard et al., 2012; Lachenbruch and McCulloh, 2014; Lahaye et al., 2005; Martínez-Vilalta et al., 2012). Sin embargo, hasta ahora no se ha documentado con detalle cómo las series de diferenciaciones de las células iniciales ni cómo la variación en estas diferenciaciones repercute en la diversificación funcional y anatómica. La documentación precisa de estas diferenciaciones ayudaría a mejorar nuestra comprensión del papel de las hormonas y de las redes de regulación que influyen en la tasa de división celular en el cambium y en la determinación celular durante la formación de la madera Groover (2023).

El segundo aspecto de mi investigación se centra en la identificación de trayectorias

ontogenéticas que favorecen la evolución de fenotipos convergentes. Las configuraciones o formas ontogenéticas que facilitan o sesgan la evolución de características convergentes se conocen como “facilitadores ontogenéticos” (Edwards, 2019). Por ejemplo, la presencia de abundante parénquima axial o de radios parenquimáticos anchos, como una característica ancestral en plantas con hábito lianoide, actúa como un “facilitador ontogenético” de la evolución de árboles paquicaules, como en *Dendrosicyos socotrana* de la familia Cucurbitaceae (Olson, 2003) o en especies del género *Adenia* de la familia Passifloraceae (Hearn, 2009b). En el contexto del desarrollo de las variantes cambiales, la documentación de “facilitadores ontogenéticos” no se ha abordado explícitamente. Identificar tales “facilitadores” sería fundamental para comprender mejor los múltiples orígenes independientes de las variantes vasculares en plantas vasculares.

Basándome en los dos aspectos previamente expuestos relacionados con la evolución del desarrollo del cambium vascular y del xilema secundario, establezco los objetivos de la presente tesis. El objetivo general consistió en utilizar las características morfológicas y anatómicas de las plantas y en particular el xilema secundario, para profundizar sobre la evolución del desarrollo en el contexto de la diferenciación y función de los tejidos. En particular, realicé una revisión y una propuesta de características morfológicas que, por las particularidades del desarrollo de las plantas, pueden utilizarse para abordar cuestiones significativas para la evo-devo. Además, comparé los linajes celulares de las células progenitoras del xilema secundario en un clado de plantas tropicales morfológicamente diversas. Por último, exploré la relación ontogenética y evolutiva del desarrollo de variantes cambiales y del hábito de crecimiento lianoide.

Este trabajo consiste en una tesis compuesta por artículos que han sido publicados o están en proceso de revisión, donde cada capítulo corresponde a un artículo. La tesis consta de tres capítulos. El primer capítulo consiste en un artículo de revisión y opinión, en el que, junto con mis coautores, presento ejemplos de cómo se pueden aprovechar las características del desarrollo de las plantas para abordar temas de evo-devo, prescindiendo del uso de tecnologías moleculares. Esta aproximación busca que la evo-devo sea más accesible para botánicos con diversos perfiles, presupuestos y niveles de infraestructura. La propuesta no excluye el papel o la relevancia de los genes; más bien, pone énfasis en la promoción del estudio de la evo-devo a nivel morfológico.

En el segundo capítulo, junto con mis coautores, comparo los linajes celulares provenientes de las células progenitoras del cambium vascular y que dan lugar a la formación

del xilema secundario en el clado *Pedilanthus* del género *Euphorbia*. El clado *Pedilanthus* es un grupo de plantas tropicales con amplia diversidad de formas de crecimiento y de hábito. Para comparar los linajes celulares utilicé técnicas de la teoría del lenguaje. Representé los tipos celulares del xilema como “letras” y las combinaciones de tipos celulares como “palabras.” La codificación de “letras” y el conjunto de “palabras” me permitió medir la similitud de los linajes celulares utilizando matrices con las frecuencias de palabras. Identifique una mayor diversidad de “palabras” y, en consecuencia una mayor diversidad ontogenética en una forma de crecimiento particular dentro del clado *Pedilanthus*. La aproximación es novedosa para estudiar la evolución del desarrollo en organismos con ciclos de vida largos.

Finalmente, en el tercer capítulo analicé la relación entre el desarrollo de variantes vasculares en los tallos de plantas leñosas y la evolución del hábito de crecimiento “lianoide” o de “no auto-sostén.” Planteo la hipótesis de que el hábito de “no auto-sostén” es una característica que favorece la evolución de las variantes vasculares. Para someter a prueba esta hipótesis, recopilé datos de “Inside Wood” ([Wheeler, 2011](#); [Wheeler et al., 2020](#)) y construí una base de datos codificando la presencia o ausencia de variantes vasculares y el hábito de crecimiento (plantas de auto sostén o no-auto sostén). Sometí a prueba la asociación de las dos variables construyendo una tabla de contingencia. La tabla mostró que hay una asociación preferencial entre el hábito de crecimiento de “no auto-sostén” y la presencia de variantes vasculares. Sugiero que esta asociación preferencial puede explicarse a través de las modificaciones que ocurren durante el crecimiento primario de las plantas.

Cuadro 1: La lista de artículos con más citas, según de Web of Science, en el campo de la evo-devo muestra un claro interés en el área de la genética evolutiva del desarrollo.

Título del artículo	Autores	Año	Citas
Evo-Devo and an expanding evolutionary a synthesis: genetic theory of morphological evolution	Carrol S.B.	2008	1279
The genomic basis of adaptive evolution in threespine sticklebacks	Jones FC. et al.	2012	1197
Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a <i>Pitx1</i> enhancer	Chan YF. et al.	2010	718
Proteinortho: detection of (co-)orthologs in large-scale analysis	Lechner M. et al.	2011	716
The locus of evolution: evo devo and the genetics of adaptation	Hoekstra HE. y Coyne JA.	2007	690
Cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence	Wittkopp P.J. y Kalay G.	2012	633
Divergence of duplicate genes in exon–intron structure	Xu G. et al.	2012	529
The extended evolutionary synthesis: its structure, assumptions and predictions	Laland KN. et al.	2015	493
The African coelacanth genome provides insights into tetrapod evolution	Amemiya CT. et al.	2013	485
The road to modularity	Wagner et al.	2007	482

Cuadro 2: Lista de artículos con más citas, según de Web of Science, en el campo de evo-devo de plantas.

Título del artículo	Autores	Año	Citas
Significance of inducible defense-related proteins in infected plants	van Loon L.C. et al.	2006	2183
Phenotypic plasticity for plant development, function and life history	Sultan SE.	2000	1024
Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids	Chen ZJ.	2007	692
A short history of MADS-box genes in plants	Theissen G. et al.	2000	655
Heat shock protein 101 plays a crucial role in thermotolerance in <i>Arabidopsis</i>	Queitsh C. et al.	2000	491
The plant vascular system: evolution, development and functions	Lucas WJ. et al.	2013	429
Genome-wide classification and evolutionary analysis of the bHLH family of transcription factors in <i>Arabidopsis</i> , <i>Poplar</i> , Rice, Moss, and Algae	Carretero-Paulet L. et al.	2010	385
Integrative plant biology: role of phloem long-distance macromolecular trafficking	Lough TJ. et al.	2006	358
Inheritance and natural selection on functional traits	Geber MA. y Griffin LR.	2003	342
Strigolactone signaling and evolution	Waters MT. et al.	2017	336

Capítulo 1. Plant morpho evo-devo

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Feature Review

Plant morpho evo-devo

Emilio Petrone-Mendoza,^{1,2} Francisco Vergara-Silva,³ and Mark E. Olson ^{1,*}

Evo-devo is often thought of as being the study of which genes underlie which phenotypes. However, evo-devo is much more than this, especially in plant science. In leaf scars along stems, cell changes across wood growth rings, or flowers along inflorescences, plants trace a record of their own development. Plant morpho evo-devo provides data that genes could never furnish on themes such as heterochrony, the evolution of temporal phenotypes, modularity, and phenotype-first evolution. As plant science surges into increasingly -omic realms, it is essential to keep plant morpho evo-devo in full view as an honored member of the evo-devo canon, ensuring that plant scientists can, wherever they are, generate fundamental insights at the appropriate level of biological organization.

Plant evo-devo: genes and more

The effort to understand phenomena, such as the developmental changes underlying important evolutionary transitions in morphology, how development **constrains** (see [Glossary](#)) or facilitates evolution, the biological causes of homology, and other aspects of the evolution–development relationship, are often regarded as involving studies of gene expression patterns throughout development [1–8]. As important and insightful as this gene-based agenda is, it reflects only a part of plant evo-devo.

Crucial plant evo-devo data abound in plant morphology. Unlike most animals, plants grow continually and, in doing so, record the progress of their meristems through space. Thus, plants represent archives of their own ontogenetic decisions, sketched out in the tracery of their stems and roots ([Figure 1](#)). Leaf scars along stems allow for reconstruction of the ages of twigs ([Figure 1B,C](#)) [9,10]. In plants that branch after flowering, the history of reproductive events can be reconstructed ([Figure 1D](#)) [11]. Changes in leaf shape along stems provide some of nature's most easily seen sequences of developmental transformation [12–16]. Within their trunks, trees lay down concentric layers of secondary xylem cells that retain their characteristics and relative positions for the life of a tree, leaving ontogenetic records that can span centuries or millennia ([Figure 1E](#) and [Box 1](#)) [17–19]. Reiteration of parts along the plant body is readily modeled, such as how plants fill stem or inflorescence morphospace [20–22], or for the characterization of functional divergence among similar parts, such as flowers, leaves, or wood cells ([Figure 1A](#)) [23–27]. Given that morphological features can be observed even in fossil plants [28–30], morpho evo-devo is the essential bridge that connects paleontology to evo-devo. With plants bearing so many signatures of development, botanists are accustomed to thinking of plants in ontogenetic terms (see [31] for a historical perspective).

The access that these properties of plant development give to central themes in evo-devo means that plant evo-devo is vastly more than which gene is involved in which morphological transformation. As evo-devo surges into important but costly -omic realms, it is essential to maintain morphological approaches in view as legitimate members of the evo-devo toolbox. For one, no matter how detailed genetic data are, the significance of countless key evo-devo phenomena is cashed out at the morphological level. For example, biological **modularity** is the phenomenon of one 'part' of an

Highlights

Evo-devo studies usually examine which genes are expressed in which tissue, but the ways in which plants develop allow for a wider range of evo-devo approaches.

Plant morpho evo-devo bases evolutionary inferences on the archive of development in plant morphology in features such as changes in leaf shape along stems, flowers along inflorescences, biomechanical properties along stems, or cell characteristics across rings of wood.

These features and others provide detailed information regarding the ways in which development is involved in evolutionary diversification.

Given this richness of biological information, the morpho evo-devo level is the appropriate one for key evo-devo questions from modularity and heterochrony to 'genes-as-followers' evolution. Moreover, in contrast to costly -omic approaches, it is accessible not only to scientists in higher income countries.

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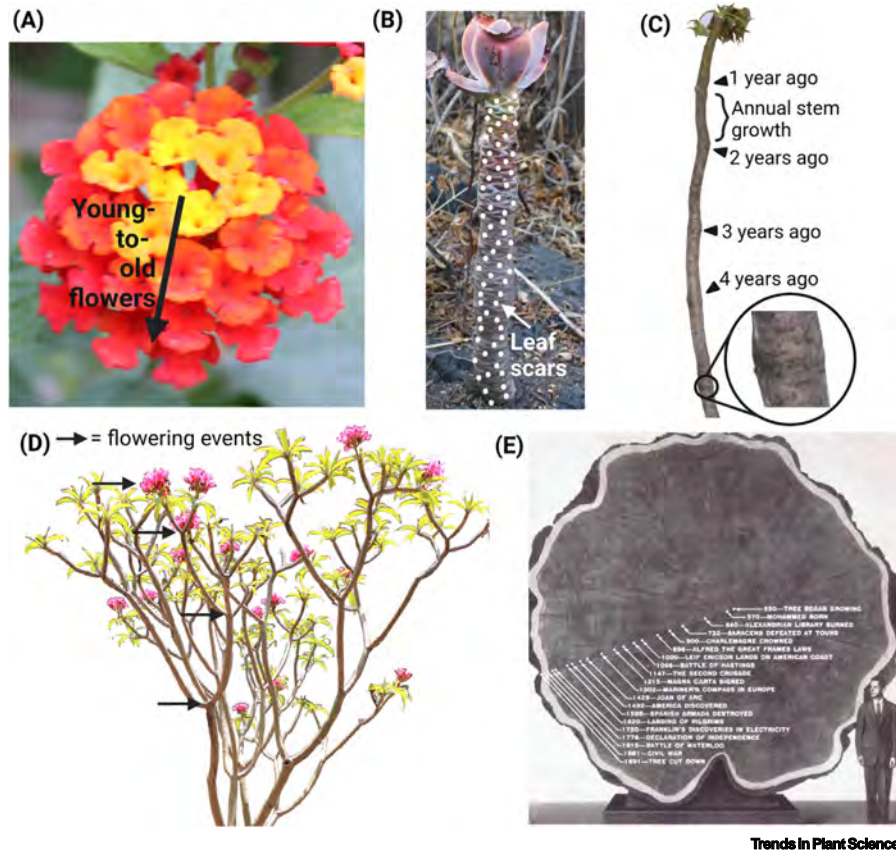


Figure 1. Plant morpho evo-devo. (A) Organs often differ in their characteristics along plant axes, meaning that developmental sequences can be readily reconstructed. For example, in the inflorescences of *Lantana camara*, corollas change from yellow to red as flowers mature from the center to the outside of the inflorescence. (B) Leaves leave conspicuous scars (highlighted by white dots) on the stems of *Echeveria gibbiflora*. Given that petiole cross-sectional area predicts leaf area [128], total leaf area produced during the lifetime of a plant can be estimated. (C) In the water-storing asteraceous *Pittocaulon praecox*, dense packing of leaves at the end of the growing season leaves an annual annular scar (inset), allowing reconstruction of stem growth rates over the life of a stem [66]. (D) Many plants branch after flowering, exemplified by the frangipani tree (*Plumeria rubra*), which allows estimation of the total flowering events. Arrows highlight four flowering events along the same branch. (E) Section of the famous ‘Mark Twain Tree’ (*Sequoiadendron giganteum*) in the New York Natural History Museum, relating historical events to growth rings. Wood cells are produced in concentric layers and remain in place for the life of a tree, providing the longest ontogenetic series available anywhere in the living world (see Box 1 in the main text).

organism developing and evolving (quasi-) independently of the rest of the organism [32–34]. Whatever genes might be doing is of course of interest, but developmental autonomy can only be observed at the level of an organismal part and its relationship to other parts, that is, the morphological level (Box 2). Moreover, this-gene-is-involved-in-that-part style evo-devo is costly and inaccessible to most scientists in the world. As a result, morphological studies crucially enrich evolutionary **explanations** by including researchers of all budget levels. To ensure that morphological evo-devo takes its rightful place in the canon of plant evo-devo, in the following sections we give examples from major themes relevant not only to botanists, but also to a broad evo-devo audience, including molecular evo-devo. We conclude by emphasizing the broad reach that plant morpho evo-devo provides as an affordable and inclusive approach to evo-devo.

Glossary

Constraint/constrain: delinquent terms that scientists use to mean everything from factors that oppose selection to selection itself [74,112,125,126]; potentially useful to designate factors that impede the production of variants that would be favored by selection if they could be produced, but, given the vagueness of the term, it is best avoided or at least defined.

Developmental bias: tendency for a developing individual to produce functional phenotypes rather than the panoply of less-functional ones that it is capable of producing. Whereas constraint (usefully construed) opposes selection, developmental bias is a product of selection; indeed, some would argue (as included) that it is the main outcome of selection itself [11].

Developmental potential or evolvability: range of phenotypes an individual, species, or clade can produce developmentally, from the commonly produced to the rare but natural, to the rare and teratological, to variation induced experimentally [111]; key for discovering developmental bias, constraint (as we understand it here), and the action of selection.

Explanation: accounts of the cause of organismal form (broadly construed). Explanations comprise complementary layers of direct evidence, historical assumptions, the pattern to be explained, and the mutual confirmation among them (see Box 4 in the main text). The explanation or explanations with the best mutual fits among evidence, pattern to be explained, and assumptions are the preferred ones.

Externalist–internalist (adaptation–constraint) artificial dichotomy: caricature of efforts to explain organismal form, in which biologists naïvely assume that any variant is developmentally possible, with selection determining which are observed in any given population (externalism) versus the notion that (usually mysterious) ‘constraints’ limit possible forms, with selection having little Lebensraum (internalism). In reality, any good explanation of form involves both of these aspects [112].

Genetic assimilation: given intrapopulation variation in the propensity to produce a given plastic response given a certain stimulus, natural selection favoring phenotypes

Heterochrony: morpho evo-devo par excellence

Heterochrony is a key evo-devo process, the evolution of functional diversity via alterations in the timing of developmental events [35,36], one which plant morpho evo-devo is uniquely well suited to study [18,37]. By mapping the sequence of developmental steps for different species across a phylogeny, ancestral and descendant-type ontogenies can be inferred. Pedomorphic descendants resemble the juveniles of the ancestral type, whereas peramorphic descendants recapitulate the developmental steps of the ancestral type before going beyond it with additional steps [38]. Given that plants add new structures sequentially, developmental sequences are readily characterized in rich detail.

A compelling example of heterochronic evolution comes from the flowers of a clade of orchids [39–41]. Flowers are particularly amenable to comparative developmental studies because the position and order of primordium appearance, usually sepals->petals->anthers->gynoecium, are relatively constant [42,43]. This constancy makes it easy to detect alterations in timing or rates of development. In *Gymnadenia* s.s. in the subtribe Orchidinae (Orchidaceae) [44], the plausible ancestral type is represented by flowers with a deeply trilobed labellum (the most ornate petal in an orchid flower), from which a long nectar spur develops (Figure 2). Moreover, in ancestral-type flowers, the ovary twists, rotating the flower 180° in a process called ‘resupination’ (Figure 2) [39,45]. The descendant-type morphology is represented by flowers with scarcely lobed labellums, short spurs, and without resupination [46]. Spur development in all species starts at the same moment, immediately after the gynostegium (the central male–female column) starts elongating and before lateral labellum lobes start growing. While spurs of the ancestral-type species continue growing after anthesis, in the descendant type, spurs stop growing before anthesis, remaining short (Figure 2B) [46]. In addition, growth in the lateral labellum lobes of ancestral-type species result in deeply trilobed labellums, while scant growth in the lateral lobes of the descendant type results in scarcely lobed labellums. Immediately before anthesis, the flowers in the ancestral types resupinate, while those of the descendant type remain in place. Thus, pedomorphic descendant types end up with short spurs and small lateral labellum lobes. Even the ovary can be considered pedomorphic, because it does not twist as it does in the resupinating ancestral type. *Gymnadenia*

that more readily produce a given plastic response [84,127].

Heterochrony: evolution of diversity, as in morphology, via evolutionary alterations in the timing of developmental events. Pedomorphic evolution results in descendants that resemble the juveniles of ancestors, whereas peramorphic evolution results in descendants that recapitulate the developmental stages of ancestors and ‘go beyond’ them.

Modularity: property of organisms to comprise subsets or ‘parts’ that develop and evolve to some extent autonomously from the rest of the organism. Lewontin [3] termed this ‘quasi-autonomy.’ Given that organismal parts are found as parts of whole organisms, covariation between parts is expected. Thus, the conspicuous phenomenon to be explained is not covariation but how and why some organismal subsets can be so developmentally and, therefore, evolutionarily decoupled.

Phenotypic plasticity: property of a single genotype to produce the phenotype that maximizes performance/fitness in the environment it finds itself in, relative to the other phenotypes that it could produce in that environment. Plasticity is an adaptive property favored by natural selection. Notions of ‘non-adaptive’ plasticity make it impossible to distinguish plasticity from pathology (heart attack in the environmental stimulus of high fat), poisoning (death in the environmental stimulus of strychnine), and so on.

Box 1. The archive of ontogeny in the wood (secondary xylem)

A largely untapped morpho evo-devo treasure trove is secondary xylem (wood) [27], which provides the richest ontogenetic record available in a single tissue anywhere in the living world. Wood is produced by a lateral meristem called the vascular cambium (Figure 1). The vascular cambium lies just beneath the bark, producing wood cells to the inside and bark to the outside. Wood cells are produced in concentric layers, which remain permanently in place for the life of the plant. The oldest cells, produced when the plant was young, are found in the center of the trunk. Each layer toward the outside comprises successively younger cells, produced as the tree ages. By following files of cells from the inside to the outside, every developmental ‘decision’ that a vascular cambium cell made, from the types of wood cell produced to their shapes, sizes, cell wall characteristics, and the spatial relations between cell types, can be traced. What is more, these ontogenetic records routinely span thousands of such decisions and even thousands of years. All of these reasons make wood an unparalleled resource for evo-devo at large.

Much of the diversity across woody plants, from tundra shrubs to rainforest trees, can be traced to differences in form and function in their wood, which in turn is due to different characteristics and proportions of wood cell types [27], differences resulting from different ‘decisions’ made by the vascular cambium cells. Vessel elements join together to form long tubes that transport water. Imperforate tracheary elements (ITEs) are long, slender cells that participate in mechanical support, water conduction, and water and starch storage. Fibers are one type of ITE. Parenchyma cells (of two types, ray and axial) are key in storage and movement of photosynthates in the xylem. Different ontogenetic trajectories, for example, those that produce more of one cell type than another, can lead to drastically different functional morphologies. Some studies have examined heterochronic evolution by comparing xylem ontogenetic trajectories across species in a phylogenetic context [129,130]. Other studies have characterized changes in ITE morphology across wood rings to evaluate changes in hydraulic properties or ecological strategies [131,132]. For the most part, however, this rich archive of ontogeny remains unexploited, and wood remains a vast unexplored territory for morpho evo-devo.

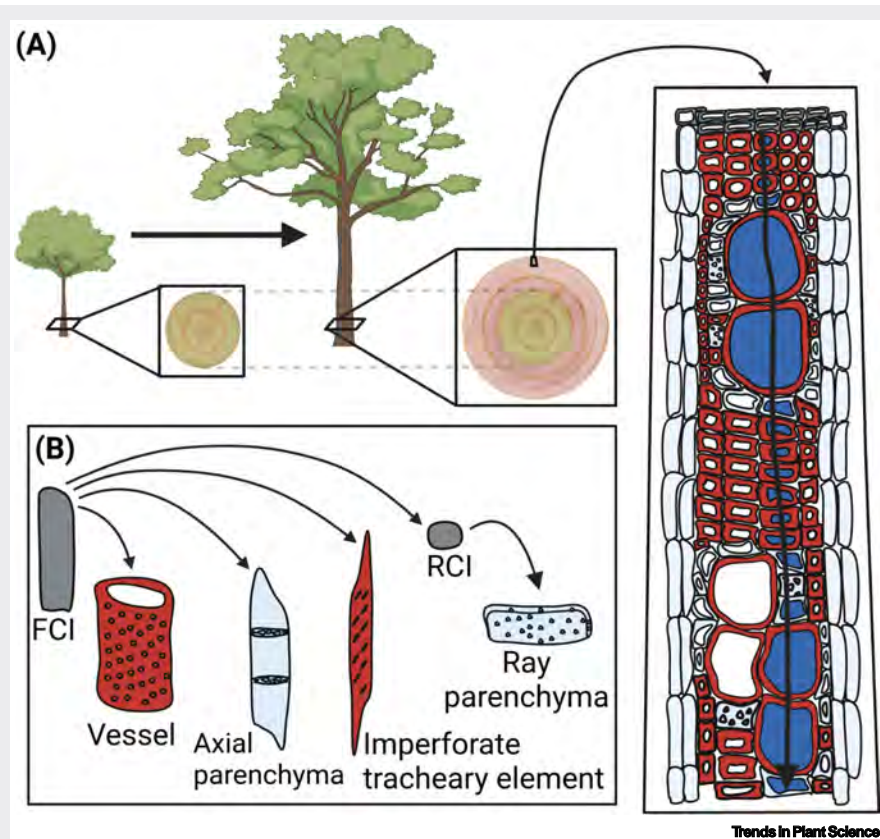


Figure 1. Wood as an archive of ontogeny. (A) Woody plants lay down concentric layers of cells to the outside of the woody cylinder. Here, green layers denote wood cells produced when the tree was small, red layers when the tree was larger. These cells are produced by a meristem called the vascular cambium. Each cell of the cambium produces radial files of cells, which can be dozens or thousands of cells long. It is possible to follow these files to trace the developmental 'decisions' that a given cambial cell took throughout decades or centuries. The inset on the right zooms in on the outermost xylem to illustrate this traceability. In blue, the cells derived from a single fusiform cambial initial (FCI) cell. The arrow highlights the file, with the most recent cells, produced when the tree was older, toward the top, and the older cells, produced when the tree was younger, toward the bottom. (B) Vascular cambium cell types and their derivatives. Fusiform cambial initials (FCI) give rise to more FCI, such that the population of nondifferentiated cells grows as the trunk girth increases. FCI differentiate into three cell types: vessel elements, imperforate tracheary elements (ITEs), and axial parenchyma. Fusiform cambial initials can also form ray cambial initials (RCI), which divide to form the ray system, which comprises entirely of ray parenchyma cells.

highlights two morpho evo-devo insights. One is that evolutionary modification in the onset and rates of development of flower parts can give rise to remarkable floral diversity. The second is that unraveling the origin of floral diversity by heterochronic evolution can require nothing more than forceps, a microscope, and the patience to dissect buds. Such insights are not limited to flowers, and we turn now to a whole-plant example.

Using size and age to explore ecological diversification

Key plant morpho evo-devo data are also stored along the lengths of stems, providing inroads to study habit diversification. More-apical parts of stems were produced more recently; more basal

Box 2. The morpho evo-devo level as the appropriate one for key questions

The concepts of emergence, downward causation, and the sorting/selection distinction are helpful in understanding why the morphological level is an essential one for studying key evo-devo issues.

'Emergent' properties are attributes that 'emerge' (i.e., are observed) at a higher level of organization, and depend on the ways that the lower level components interact [133]. The weight of a group of cells is the same whether they comprise a leaf or are in a pile; thus, 'weight of cells' is not an emergent property. However, leaf function, as reflected in photosynthetic efficiency or transpiration rate, very much depends on how the lower-level elements are arranged and interact and is in no way inferable by reference to disarticulated or isolated cells. Downward causation refers to the effect that emergent properties have on the lower level elements of a system. If morphological traits are associated with allelic differences, then changes in the frequency of bearers of the trait across the members of a population of a species will lead to changes in allele frequencies.

This leads to the sorting/selection distinction [134]. Sorting is any intergenerational change in the frequency of an attribute in a population. Selection is one cause of sorting. Sorting of lower level components always occurs where there is selection on higher level emergent properties. Take a situation in which differences in alleles and post-translational modification of proteins are associated with heritable differences in flower shape within a population. Differences in flower shape are associated with differences in seed set; thus, one variant comes to predominate. The alleles and protein variants involved in the production of the favored variant also come to predominate. Sorting has occurred in floral morphological variants, alleles, and protein variants. The proteins and alleles, as part of the lower level factors involved in the production of the morphological variant, merely sorted, dragged along as a consequence of selection involving properties emergent at the morphological level.

In this example, no amount of protein or genetic data could reveal the causes of sorting at those levels. Lower level processes and entities are involved in generating the higher-level properties, but because morphological properties are not reducible or inferable with reference the lower-level components, the morphological level is an appropriate and necessary level at which to study key evo-devo phenomena, from morphological adaptation to genes-as-followers evolution, homology, and modularity.

parts are older, meaning that the direction of developmental change can be reconstructed unambiguously (Figure 1C,D). Developmental variation in stem length–diameter relations results in dramatically different stem biomechanical behavior, as illustrated in a morpho evo-devo study of the ecologically and morphologically diverse *simarubia* clade in the tropical tree genus *Bursera* [47]. Some species are slender rainforest trees; others fat, water-storing trees of dry forests; *Bursera instabilis* has lianescent branches, and *Bursera standleyana* is a hemiepiphyte with lianescent roots (Figure 3). In plants in general, stems the girth of which keeps pace with their length growth are able to support their own weight. In water-storing species of *Bursera*, the wood is soft and flexible. In these species, a given increase in stem length growth is met with a large increase in girth, keeping the stem from flopping over. In species with stiffer wood, the stems remain thinner for a given length and still support themselves. The lianescent stems of *B. instabilis*, shown by molecular phylogeny to have evolved from self-supporting ancestors, are achieved by ontogenetic responses that barely thicken the stem for a given length increase. Long, skinny stems inevitably fall over, resting on other plants. The *simaruba* clade shows the way in which small adjustments in the ontogenetic relationship between tissue mechanical properties and length–diameter proportions along the lengths of stems are involved in plant functional and morphological diversification, illustrating the power of plant morpho evo-devo. Functional diversity not only manifests across species, as in the *simaruba* clade example, but, as we illustrate in our next example, can also be expressed across contrasting selective conditions in the ontogeny of single individuals.

Temporal phenotypes

The inferential richness of plant morpho evo-devo is admirably showcased by the study of what have been referred to as 'temporal phenotypes'. The term describes the situation in which selection favors one function at one ontogenetic stage, and then another at a later stage in the same structure [48,49]. If selection favors a given morphology at one stage, it can preclude other morphologies being favored at a different stage. As a result, examining only a single developmental

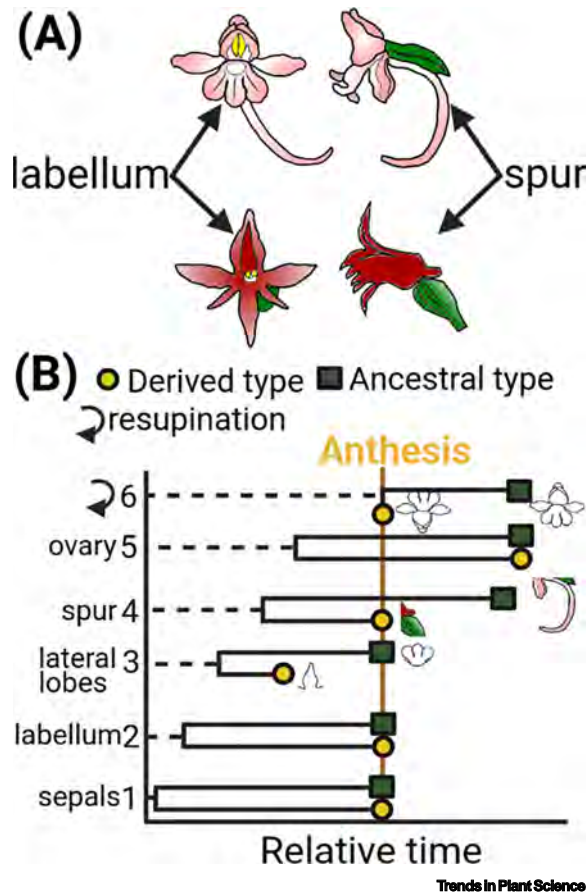


Figure 2. Heterochronic flower evolution in *Gymnadenia* orchids. (A) (Top) Ancestral-type flower morphology with deeply-trilobed labellums, long spurs, and floral resupination. (Bottom) Derived-type flower morphology with barely trilobed labellums, small spurs, and flowers that do not resupinate. (B) Reconstruction of floral developmental stages showing that spurs and lateral lobes stop growing earlier in the derived type ontogeny, while, in the ancestral type, they continue growing. In addition, the ovary does not rotate in the derived type, whereas it does in the ancestral type, yielding resupinate flowers. Dashed lines represent the time between the beginning of floral development and the onset of development of the part in question. Solid lines indicate the relative timing of development between the ancestral and derived types. (B) Modified from [46].

stage can make it impossible to understand why one species has the particular morphology that it does at that stage.

Plant morpho evo-devo provides precisely the tools for examining temporal phenotypes. For example, the familiar female cones of the conifer family Pinaceae pass through two very different developmental stages. The first stage involves protecting and nourishing the maturing seeds within closed compact cones. The second stage involves liberating the seeds. After cones dry, seeds can be dispersed by scale flexion or scale shedding (Figure 4A) [50,51]. In 'flexing' species, the compact closed cones open flower-like, during bract and scale dehydration, liberating the seeds. In 'shedding' species, cones fall apart as they dehydrate, releasing the seeds. In attempting to understand why some species have flexing dispersal and others shedding, focusing only on the final morphology would result in a vain search for a traditional adaptive explanation of the sort where the dispersal type is explained exclusively by factors such as the environment.

A morpho evo-devo approach shows that selection acting at the protection phase influences which morphologies can be produced at the liberating phase. There are two conspicuously different protective-phase morphologies, one with a large amount of seed tissue per cone and the other with only a small amount of seed tissue per cone (Figure 4B). Massive cones with small

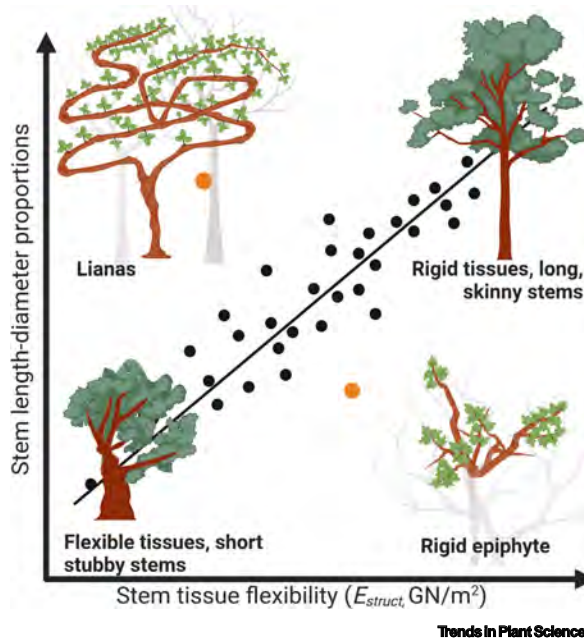
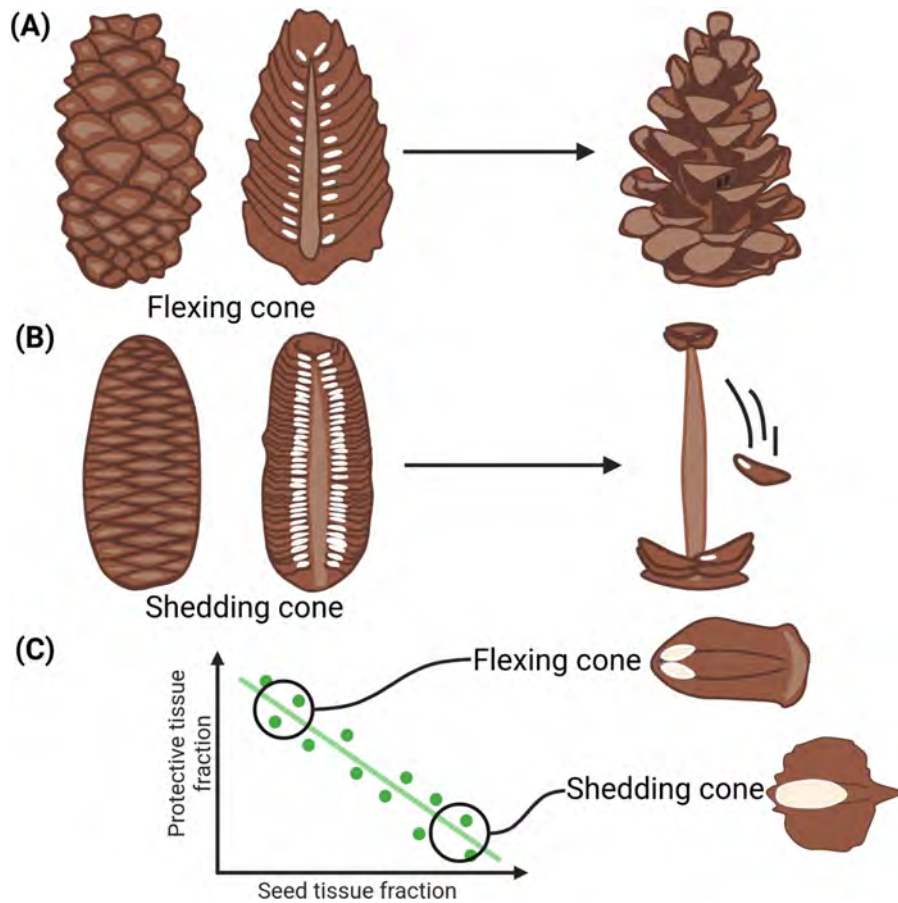


Figure 3. Developmental modulation of branch dimensions and the evolution of biomechanical diversity. Small changes in development to the relationship between stem length and stem diameter, together with tissue mechanical stiffness, leads to marked differentiation in habit across the members of the *simuarba* clade in the genus *Bursera* (Burseraceae). On the x-axis is the stiffness, as measured by Young's modulus of elasticity E , of the tissues (wood, bark, and pith together) that comprise the whole 'structure' of the stem, hence E_{struct} . Lower E_{struct} = more flexible, higher E_{struct} = more stiff. On the y-axis is stem length–diameter proportionality. Most species, of more or less conventional tree habit, fall along a scaling line, with species with flexible stem tissues having short, stubby branches. Trees with stiffer stem tissues have longer, more slender stems for a given diameter. This spectrum of covariation spans small water-storing trees of tropical dry forests to tall rainforest canopy emergents. Modulations of development that result in displacement from the scaling

line is also associated with habit diversity. Above the line (very flexible tissues, stems that are long for their diameter) is the odd *Bursera instabilis*, a dry forest tree with lianescent branches. Below the line (stiff tissues, stems that are short for their diameter) is the even odder *Bursera standleyana*, the stiff, stubby branches of which jut from its epiphytic perch high in Costa Rican rainforests.

amounts of seed tissue per cone are thought to be favored by selection in situations of heavy predation or fire [50–53]. For example, the lipid-rich seeds of pines are heavily subject to predation, favoring their protection in cones with massive, thick scales [54,55]. This protective strategy produces few, expensive cones and seeds with greater seed survivorship. Species with lightweight cone scales and abundant but smaller seeds, as in cedars or firs, exemplify a different strategy, producing many more seeds at the expense of individual mass and survivorship. This trade-off between seed protective tissue and seed tissue holds when controlling for phylogenetic relationships across the family [56].

It appears that cone flexion can only be produced when there is sufficient cone scale mass that can achieve the necessary mechanical stresses. As a result, selection cannot favor a 'flexing' morphology on the substrate of a lightweight, high seed mass per cone mass morphology. In these lightweight cones, the modality favored is shedding. Along the same lines, a shedding morphology in massive-scaled cones would require the production of metabolically costly abscission zones at areas with large investments in tissues, such as xylem, connecting scales to axes [55,56]. Therefore, flexion is apparently favored in massive cones. In this way, information regarding the protective phase is essential to understanding why a species has a given dispersal phase morphology, illustrating the essential insights that only a morpho evo-devo approach can provide in explaining organismal form and function. Temporal phenotypes also illustrate the ways in which different parts can vary to an extent independently of others; such ontogenetic 'quasi-independence' is an entire area of evo-devo study in itself, and we turn to it now.



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Figure 4. Evolution of temporal phenotypes exemplified by Pinaceae cone morphologies. The term 'temporal phenotypes' describes situations in which selection favors one function at one ontogenetic stage and then another at a different stage of the same structure. Pinaceae cones have two ontogenetic stages, a protective phase, which involves seed development, and a dispersal phase, which involves liberation of the seeds. (A) Morphology of cones in 'flexing species' have bracts and scales that open flower-like, liberating seeds. (B) By contrast, shedding species liberate seeds when bracts and scales break apart from the central axis. (C) Which seed dispersal mechanism a given species has is determined, at least in part, by which protective strategy it has. During the seed protective phase, flexing species have a higher amount of protective tissue per seed tissue, favored in high predation conditions, while shedding species have less protective tissue per seed tissue fraction, favored in low predation conditions. A morpho evo-devo approach shows that, given this protection volume-seed volume trade-off, high tissue investment in the protective phase will be associated with flexing dispersal, and low tissue investment in the protective phase will be associated with shedding dispersal. Without taking into account the protective phase, why a given species has a given dispersal syndrome is opaque, illustrating the need for a morpho evo-devo perspective.

Modularity

'Modularity' has different meanings in traditional botany versus evo-devo. Botanists traditionally call any parts that are repeated (leaves, petals, even combinations, such as a leaf plus its node) 'modules'. These are usually delimited simply by eye or intuition [57,58]. However, in evolutionary biology, modularity is the property of organisms that comprise subsets or 'parts' that develop and evolve to some extent autonomously from the rest of the organism [33,59,60]. Modules from this point of view need to be discovered empirically rather than intuited, and how and why modularity

evolves is a central evo-devo theme, one that plant morpho evo-devo is perfectly positioned to examine.

Current evo-devo can be argued to suffer from inadequate engagement with adaptationist thinking [61–64], and plant morpho evo-devo studies of modularity offer ideal bridges. The favored hypotheses for why modularity evolves are adaptationist: because variation in one part of the body does not derange other parts of the body, intrapopulational variants with modular structure, and moreover in which modules are aligned with functional domains, have a greater propensity for adaptive form–function fit and, therefore, persist [60,65–68].

Thus, modularity unites both evo-devo and adaptation, as showcased by the tribe Merianeae of Melastomataceae, in which the evolution of different pollination syndromes allows testing the hypothesis that modularity is shaped by selection [69]. Dellinger *et al.* found different patterns of modularity under contrasting selective contexts [69]. In species with buzz pollination, the ancestral syndrome in the tribe, one module was found to comprise stamen appendages, which covary strongly among one another but little with the rest of the flower. Stamen appendages are structures at the apices of stamen filaments that covary among one another in shape, size, and color. Strong covariation between stamen appendage traits is favored because bees are attracted to flowers with color contrasts between the petals and the stamen appendages. Variants with stamen appendages clustered together have high color contrast and, thus, have higher chances of being detected by bees compared with conspecifics with stamen appendages that are not clustered. By contrast, different modularity patterns were identified in species pollinated by hummingbirds and bats. In vertebrate-pollinated species, stamen appendages covary with other stamen traits, having the same inconspicuous coloration as the rest of the stamens [70]. In vertebrate-pollinated species, close covariation between the corolla, stamens, and the gynoecium is favored because campanulate corollas with stamens with pores close to the stigma have higher probabilities of pollen transfer when vertebrate tongues or beaks are inserted in search of nectar. That different patterns of trait covariation are possible across species and, moreover, that they make sense in the context of their pollination syndrome, is strongly consistent with modularity being shaped by adaptation.

Plant morpho evo-devo studies also provide compelling examples of ways in which the dynamics of development limit the possibilities for the evolution of developmental independence and, therefore, modularity forged by adaptation. Wood or secondary xylem offers a tractable system because it has only three main cell types, which, in angiosperms, are vessel elements, fibers, and parenchyma (Box 1). While each participates in different functions, vessels are traditionally regarded as the main sites of water transport, fibers as the main support cells, and parenchyma for the transport and storing of photosynthates [71,72]. From the modularity-as-adaptation view, selection should favor functional domains that are as developmentally decoupled as possible.

If selection favors developmental decoupling between morphological domains that differ functionally, then we would expect to see high levels of developmental independence of xylem cell types from one another. A study across the *simaruba* clade of *Bursera* revealed features of development that apparently impede developmental independence even if selection were to favor it [66]. This study found strong covariation (and low phylogenetic signal) between the lengths of vessel elements and those of fibers. There is nothing in the traditional formulation that ‘vessels conduct and fibers support’ that leads to an expectation of covariation; instead, their very different functions lead to an expectation of developmental independence ([73], see p. 193). However, in the secondary xylem, both vessel elements and fibers are produced from the same mother cells. Therefore, minimum cell size is set by the initial size of the mother cell.

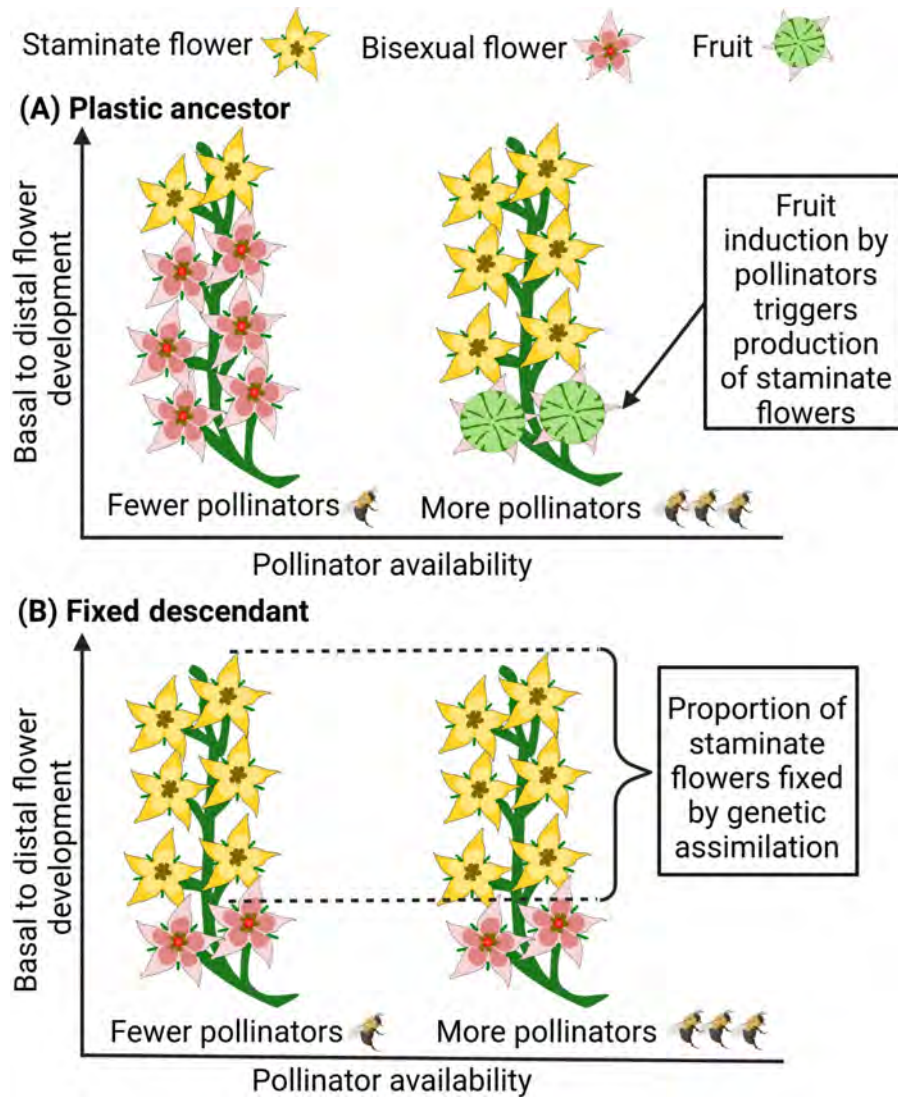
This shared ontogenetic origin imposes a powerful limit on the morphological variation that can be produced. Even if selection were to favor longer or shorter minimum cell sizes between fibers and vessel elements, such a condition would never be observed because it is developmentally impossible. Thus, rather than selection favoring certain combinations from among a wide set of developmental possibilities, the strong covariation between vessel element length and fiber length tells a story of limited **developmental potential** imposed by shared developmental pathways. Explaining the tight covariation between fiber and vessel element length cannot appeal only to selection and must include their developmental dependence. Thus, morpho evo-devo provides convincing examples of ways in which modularity is shaped by adaptation as well as limited by development, key aspects of phenotypic evolution [74,75]. How quickly this evolution might occur is the focus of another key morpho evo-devo theme, phenotype-first evolution, which we now examine.

Phenotype-first evolution: development driving adaptive evolution

Rather than waiting around for just the right mutation to arise in a single individual and spread through a population, morphological novelty can evolve quickly in novel environments via a scenario called 'phenotype-first' evolution, and studying this scenario demands a morpho evo-devo perspective. Organisms adjust their phenotypes depending on the conditions they find themselves in, a property known as **phenotypic plasticity** [76]. If a novel phenotype triggered by changing conditions has better performance than other phenotypes possible in that environment, the variants that most readily produce it will be favored, leading to it rapidly coming to prevalence under the new conditions [77]. Natural selection on standing variation in how easily a given plastic response is produced is known as **genetic assimilation** [78,79]. While there are reasons to suspect that phenotype-first adaptation is common, flagship examples are still few. Plant morpho evo-devo has a key role here.

One potential example comes from the genus *Solanum* in studies by Diggle and Miller. In the subgenus *Leptostemonum*, flowers are produced along long inflorescences, opening from the base toward the tip [80,81]. Some flowers are the usual hermaphrodite and some are only male, and the proportions of hermaphrodite versus male flowers has important implications for pollen export and fruit set. *Leptostemonum* provides a likely example of phenotype-first evolution, with the ancestral inflorescence type in the clade being one of marked plasticity [82]. In these 'plastic' species, when many early-flowering hermaphrodite flowers are pollinated at the base of the inflorescence, and sufficient fruit-set insured, the plant then shifts to producing male flowers, ensuring pollen export. Instead, when few hermaphrodite flowers are pollinated at the base of the inflorescence, the plant continues to produce hermaphrodites, hedging its bets for sufficient fruit set. 'Fixed' species of *Leptostemonum* always have basal hermaphroditic flowers and distal male flowers (Figure 5). In producing high proportions of staminate flowers regardless of whether their early flowers were pollinated, these species display only a subset of the plastic response of their ancestors.

The authors give evidence to suspect that evolution of 'fixed' inflorescences was via genetic assimilation from plastic ancestors because the cue of pollinated basal flowers is no longer needed to induce staminate flowers. 'Fixed' species are found in situations in which pollination of hermaphrodite flowers is almost assured. As a result, individuals maintaining costly phenotypically plastic responses direct fewer resources to growth and reproduction [83]. Therefore, individuals that produce the 'fixed' hermaphrodite-to-male proportion without requiring the costly plastic machinery would be favored. In this way, selection plausibly acted on standing variation in the thresholds of plastic induction of the favored phenotype, favoring individuals with the lowest thresholds of induction, in other words, via genetic assimilation.



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Figure 5. Potential plasticity-first evolution in flowers along inflorescences. The ancestral type in the *Leptostemonum* group of *Solanum* is plastic, with plastic species producing different proportions of staminate flowers depending on the proportion of flowers pollinated. (A) When pollinator availability is low, the first-produced flowers do not form fruits, triggering prolonged production of bisexual flowers, ensuring fruit formation. When pollinator availability is high and the early flowers are pollinated, the inflorescence shifts to exporting pollen via male flowers. (B) Fixed descendant-type species no longer require high pollinator availability and fruit induction to produce higher amounts of staminate flowers; this production in the absence of a cue suggests evolution by genetic assimilation in high-pollinator environments.

Phenotype-first evolution might be the most frequent mode of adaptive evolution [76,84,85], in contrast to the view in standard evolutionary theory (Box 3). Given that phenotype-first evolution crucially involves developmental and morphological driving mechanisms, it inherently must be studied at the morpho evo-devo level. Phenotype-first evolution could underlie the tendency in the fossil record for new forms to appear suddenly, with few intermediates with respect to

Box 3. Plant morpho evo-devo and the Extended Evolutionary Synthesis

Traditional evolutionary biology is enshrined in textbook versions of the Modern Synthesis, the extrapolation of population genetic theory from the 1930s–1950s that remains in place in much of the field. Some biologists regard some of the tenets of such ‘standard’ evolutionary theory to be unfaithful depictions of biological reality, such as the notion that phenotypes are encoded in the genome [135]. The latter view implies that development is simply a reading-out of a computer software-like blueprint; as a result, nothing of relevance to the evolutionary process can occur in development (**externalist–internalist** dichotomy). Evo-devo is an emphatic reaction to this position [111, 135, 136]. A vivacious group of researchers argue that the Modern Synthesis is so confining that it is worth recognizing a wider canon of processes. Their perspective is often identified as an ‘Extended Evolutionary Synthesis’ (EES) [137].

Plant morpho evo-devo is a key source of information regarding central EES processes, such as niche construction. From the traditional perspective, organisms are shaped by their environments. Niche construction highlights that organisms exert choice in where to live, and in the process actively modify the conditions of selection that they are exposed to [138]. So-called ‘ecosystem engineers’ adapt to the habitats that they themselves construct. Trees are consummate ecosystem engineers, and their study has proven crucial in raising organism–environment evolution to an object of study in its own right [139, 140]. The process by which organisms shape the conditions of their own ontogeny is inherently a morpho evo-devo phenomenon.

Another EES theme ripe for plant morpho evo-devo study is developmental potential (evolvability), the range of variants a species can produce. Some developmental variants are common and even favored. At the same time, certain variants that would seem plausibly favored by selection are apparently impossible to produce [39]. This is the notion of developmental constraint [74, 112]. A related EES concern is **developmental bias**, the tendency for a developing individual to generate functional phenotypes rather than the panoply of less-functional ones that it is capable of producing. No amount of study of genes can reveal the range of phenotypes that a given species can produce; only the morpho evo-devo level can map developmental potential. As a result, by bringing to light essential data on key EES processes, plant morpho evo-devo promises to remain a leading front in expanding the horizons of evolutionary biology.

previous ones [86,87]. Nothing puts ‘evo’ in ‘evo-devo’ better than reference to fossils, and we turn now to the greatest merit of morpho evo-devo of all, the fact that it unlocks the paleontological treasure trove.

The only way to put the ‘paleo’ in paleo-evo-devo

Morphological approaches are the only bridge connecting extant plant evo-devo to the vastness of the fossil record. In extant species, similar morphological outcomes are often produced by different developmental pathways [88–90]. For example, while toadflax (*Linaria*) species with longer spurs result from higher rates of cell division [91], columbine (*Aquilegia*) species with longer spurs result from higher rates of cell elongation [92]. Therefore, a persistent puzzle facing plant evolutionary biologists is whether, in any given case, similarity of structure across species denotes similarity of developmental mechanism.

In extant species, developmental mechanisms can be investigated directly. It goes without saying that no such luxury exists for fossils. As a result, evo-devo attempts to bridge extant and fossil species involve detecting morphological diagnostics of particular developmental mechanisms in extant species, and then searching for these diagnostics in extinct plants [30,93]. Such searches require realistic expectations regarding how definitive or not the results of a single study will be regarding the inference of developmental processes in extinct plants. Demands for ‘definitive proof’ and the like misunderstand not only paleo evo-devo, but also evolutionary inference in general. To calibrate their expectations regarding the epistemic power of any single study, evo-devo practitioners need to head into the lab armed with an understanding of the structure of a well-supported evolutionary **explanation** (Box 4). This need is particularly vivid in the case of paleo evo-devo, and we illustrate this point with an example from plant vascular systems.

Vascular plants (those that transport water in tracheids or vessels) deploy conduit diameters throughout their bodies in a strikingly universal pattern. Across all vascular plant lineages, from club mosses to spike mosses (for, that matter, even true mosses, which transport water in

hydroids [94]), in ferns, and across all orders of seed plants, from lianas to cacti to the world's tallest trees, conduits widen predictably from the tip of a plant to the base [95]. The cause of tip-to-base conduit widening is unambiguously adaptation by natural selection [96]. Selection favors narrow conduits in leaves, where water leaves the xylem and enters the mesophyll. Narrow conduits maximize surface area per water volume, ensuring efficient transpiration [97]. However, as a plant gets taller, the distance between the leaves transpiring water and the roots taking up the water increases, often by tens of meters. If conduit diameter remained unchanged as transport distance and, thus, conduit length increased, hydraulic resistance would increase and water supply would decrease. While hydraulic resistance increases only linearly with increases in conduit length, it decreases as the (whopping) fourth power of conduit diameter [98]. Thus, small increases in diameter are sufficient to offset even very large increases in length. This happy balance has not gone unnoticed by natural selection, with the result that, across vascular plants, conduits not only widen tip-to-base but do so at almost the same rate no matter the species, habit, or habitat [95]. The pattern of tip-to-base conduit widening across the vascular plants is so conspicuous that developmental biologists wish to understand its mechanistic basis.

This interest in understanding the ontogenetic mechanisms underlying tip-to-base conduit widening focuses, as is usually the case, mostly on extant plants, but also extends to extinct ones, providing a paradigm paleo evo-devo case study. In all vascular plants that have been studied, including all angiosperms, lycophytes, spike mosses, and mosses [99–101], the development of vascular tissue involves polar auxin transport. Polar auxin transport from shoot tips and leaves toward the roots [102] creates a concentration gradient [103]. Water-transporting conduits develop along these gradients, with conduit expansion time and, thus, conduit diameter apparently negatively proportional to auxin concentration [104]. Extinct lepidodendrolean lycopsids evolved secondary growth independently from other vascular plants [105,106], and, thus, whether their vascular development mechanism arose *de novo* or recruited similar pathways as extant vascular plants is a question of persistent interest. Careful study of fossils reveals that their vascular system

Box 4. Smoking guns and 'definitive proof': the structure of evolutionary explanation

Evolutionary inferences, no matter how apparently simple, always involve manifold assumptions, which are accepted given how well they would explain the observed data if they were true [141]. It is universally accepted that globular succulent *Euphorbia*, cacti, and Apocynaceae are derived via convergent evolution from nonglobular ancestors [142,143]. However, even such an ironclad explanation involves accepting crucial and often untestable assumptions. Biologists assume that in past, unobserved and unobservable ancestral populations, there was some degree of variation, with some variants being more and some less globular. Individuals that were slightly more globular tended to have progeny that were also slightly more globular; that is, biologists assume that this variation was heritable. They also assume and accept that this heritable variation was associated with fitness differences, such that slightly more globular variants tended to survive and reproduce better compared with less globular conspecifics. These ancient populations will never be observed: we accept their existence by virtue of how well this assumption would explain the observed data if they were true. Assumptions about unobserved populations are part of all evolutionary explanations.

In constructing their explanations of organismal form, the task of evo-devo (indeed all) biologists is to reduce the relative importance of these assumptions. They do so by adducing as many layers of direct evidence as possible. 'Direct evidence' for a given hypothesis is any result that does not contradict the hypothesis. There is a vast array of sources of such evidence, including across-species patterns of convergence, studies of development showing what variants are and are not possible developmentally, selective breeding to test the notion that the common variants have the highest fitness, and even surgical manipulation to create variants not observed in wild populations, to pit their fitness against natural variants [74,144]. Evolutionary explanation comprises patiently piling layer upon layer of direct evidence from as many sources as possible. Finding that patterns of vascular development in Lepidodendrales is consistent with polar auxin transport is one such layer of direct evidence. It also illustrates that no single study and no single source of direct evidence ever provides 'smoking gun' evidence on its own of any evolutionary process (Figure 1). Evo-devo biologists, paleo and otherwise, must always live with, and take pains to make explicit, the assumptions that comprise integral parts of their explanations. They must also reach across disciplinary aisles to include complementary sources of evidence, with developmental biologists, comparative biologists, and population biologists all working together.

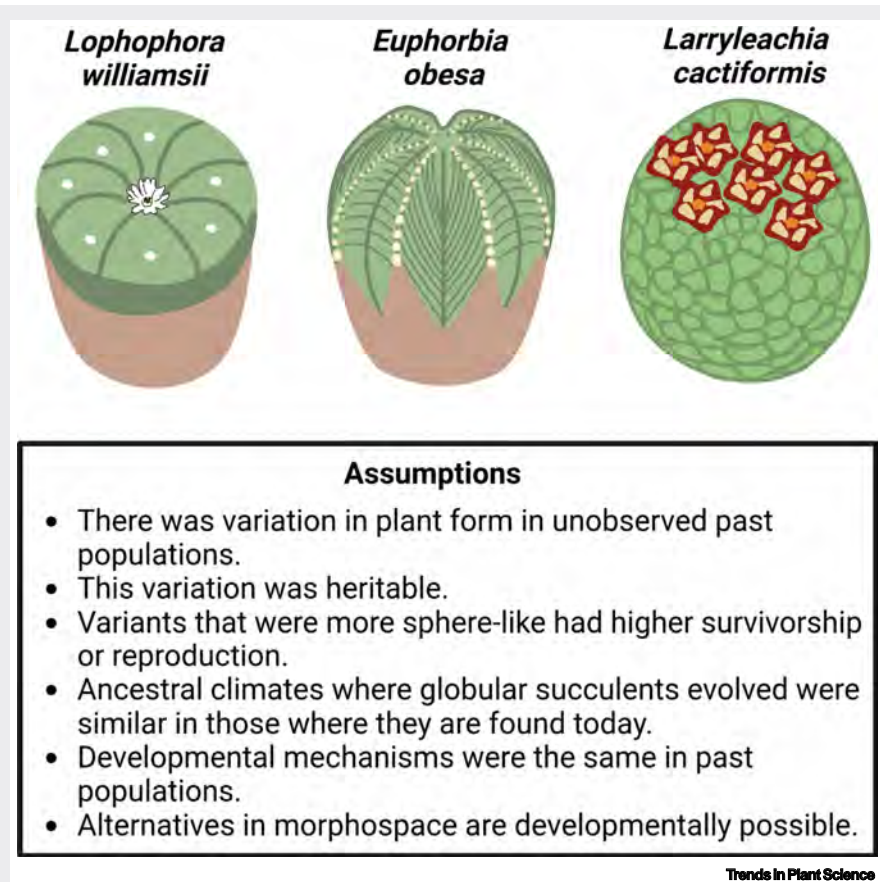


Figure 1. No 'smoking guns' in evolutionary studies. A 'smoking gun' is a single piece of evidence that is so definitive that practically no other information is necessary. Instead of smoking guns, evolutionary studies involve painstakingly bringing together as many complementary pieces of evidence as possible to see which explanation appears the most likely. One reason why so much evidence is needed is that evolutionary biologists make assumptions about past populations that will never be observed. Even the most obvious and well-accepted evolutionary inferences are replete with such assumptions. For example, the world's globular succulents have clearly evolved similar morphologies under similar selective regimes from very morphologically different ancestors. Even such a clear-cut case involves profound assumptions, which biologists accept because they would explain the observed globular succulent-dryland association so well if they were true. Testing these assumptions requires bringing to bear many different types of data, precluding a single definitive study. Moreover, given that the ancestral populations can never be observed directly, the best biologists can hope for is to adduce sufficient data consistent with a given explanation that they can consider it the most well supported from among the alternatives.

shows signatures that some morphogen was, as in extant plants, transported tip-to-base, and that vascular tissue was formed along these gradients [105–107]. While it is no smoking gun (Box 4), this result is consistent with the possibility that polar auxin transport was recruited in Lepidodendrales independently from its evolution in extant vascular plants. In doing so, it illustrates the vital role of morpho evo-devo in linking extant evo-devo to the evolutionary past. Morpho evo-devo not only provides the crucial bridge to the fossil record, but is also ideally paired with traditional molecular evo-devo, and we conclude by highlighting this latter point.

Seamless evo-devo: molecules to morphology

The morphological level is the appropriate level for studying many important questions (Box 2); it is most powerful of all when paired with traditional molecular-focused evo-devo. In celebration of this ideal pairing, we close with a paragon case of molecular and morphological evo-devo complementarity in the study of adaptive evolution. Natural selection is the only process that reliably leads to a fit between organismal form and function and, thus, is of central interest to biologists [108]. However, all biologists interested in adaptation must also be developmental biologists, because adaptation and development are inextricable [74,109,110]. One reason is that selection itself is the biasing of development such that the favored outcomes tend to be produced [111]. As a result, the very biases inherent in development are shaped by selection [112,113]. Another reason is that selection can only favor the variants that are developmentally possible [75]. There are always mechanisms of development at work that make some variants possible or impossible. The range of the possible is known as **developmental potential**. Selection is the favoring of only certain variants from among the possible. Therefore, developmental potential must be coequal with selection in any explanation of organismal form.

Wheeler and Smith [114] provide a masterful example of the inextricability of selection and developmental potential. Flower color is unquestionably a functionally salient attribute because of its role in pollinator attraction. The wide range of flower colors across the nightshade family Solanaceae, which span the rainbow (Figure 6), give no reason to suspect that one color would be any more difficult to produce than any other. 'Difficult' in this context means that some outcomes are more metabolically costly than others and, thus, all else being equal, less likely than more economical ones. Wheeler and Smith modeled the enzyme kinetics of the anthocyanin floral pigment pathway. From the same starting point, 4-coumaroyl-CoA, the pathway branches toward multiple outcomes. Like all developmental pathways, taking one path precludes others.

As a result, there are trade-offs involved in the anthocyanin pathway, such that producing one pigment necessarily decreases the amounts of others that can be produced. Assuming that all enzymes have the same enzyme–substrate binding affinities and the same catalytic rates, and given the same protein concentrations, Wheeler and Smith found that the steady-state concentration of the pigment pelargonidin, which is red, was 20% more likely compared with cyanidin (purple) and delphinidin (blue). Their model dramatically shows that Solanaceae corollas should overwhelmingly be red or pink (Figure 6).

Comparison with empirical reality across the family reveals a stark contrast. Of the nearly 3000 species, only 34 (1%) have red flowers. Of this 1%, two-thirds achieve red flowers without pelargonidin, via carotenoids or modifying pH [115–117]. Wheeler and Smith argue that selection in nearly all species goes against the inherent enzymatic bias leading to pelargonidin. Molecular genetic footprints of this selection bias pathways away from pelargonidin abound. For example, transcriptional regulation of the enzyme flavonoid-3'-5' hydroxylase (F3'5'H), which hydroxylates dihydrokaempferol, diverts pigment synthesis away from pelargonidin [118]. Upregulation of F3'5'H, mediated by MYB transcription factors, forces the pathway toward the production of blue and purple pigments, while downregulation of F3'5'H correlates with the production of red pigments [119,120].

Explaining why Solanaceae species have the flower colors they do demands integration from molecules to morphology. It requires the map of developmental potential and the biases inherent in it. To see what subset of the possible is in fact produced in nature, and why, the selection processes experienced by plants in their interactions with pollinators is also required. Wheeler and Smith illustrate selection shaping development, often against inherently less metabolically costly routes at the molecular level, such that development itself is biased toward the production of

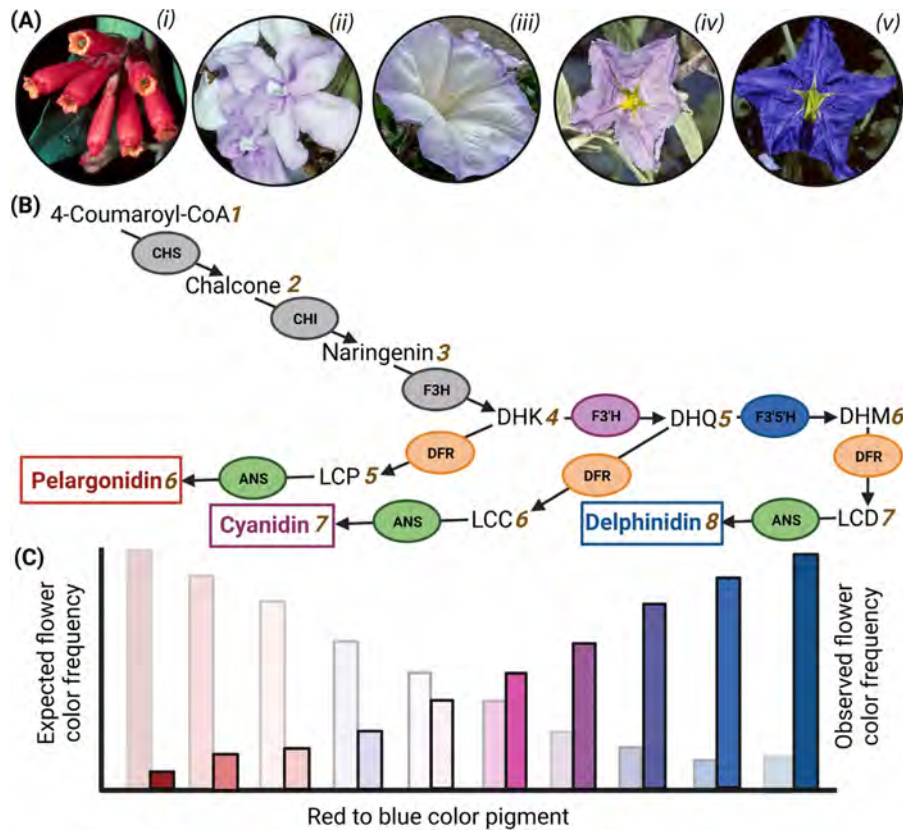


Figure 6. Evo-devo at its finest seamlessly merges morpho- and molecular approaches, and the study of adaptive evolution of floral color in Solanaceae provides a brilliant showcase. (A) Flower color varies strikingly across the family, including red, white, purple, and shades in between. These colors show tight fits with pollinator preferences, from hummingbird-visited red flowers to hawkmoth-visited white ones, the signature of selection honing the form–function fit. (B) Selection can only favor traits that can actually be produced in development. In the anthocyanin enzymatic pathway, getting from one color to another requires passing through a strict series of intermediates, as illustrated in this diagram of the substrates leading to the three main anthocyanin colors. Numbers to the sides of the substrates give the numbers of reactions on the different pathways, leading to red pelargonidin (six), purple cyanidin (seven) or blue delphinidin (blue). (C) Moreover, expected (open bars) versus approximately observed (solid bars) proportions of flower corollas are strikingly at odds with one another, a sure sign of selection. In (B), for simplicity, the pathways leading to flavonols were omitted. Ovals depict enzymes involved in the enzymatic reactions. Abbreviations: ANS, anthocyanidin synthase; CHS, chalcone synthase; CHI, chalcone isomerase; DFR, dihydroavonol-4-reductase; F3H, flavonone-3-hydroxylase; F3'H, flavonol-3' hydroxylase; F3'5'H, avonoid-3'5' hydroxylase. (A) Flowers from different Solanaceae species showing the red–blue color gradient: (i) *Cestrum fasciculatum*, (ii) *Brunfelsia mire*, (iii) *Datura wrightii*, (iv) *Solanum hindsianum*, and (v) *Solanum tridynamum*.

the favored color. Thus, understanding how and why some variants emergent at the morphological level are favored over others demands the seamless integration of molecular and morpho evo-devo perspectives.

Concluding remarks

Having an expansive view of plant evo-devo that embraces everything from molecules to morphology is essential for advances in the field (see [Outstanding questions](#)) [121]. For one, -omic approaches are expensive and available only to the relatively few scientists worldwide

Outstanding questions

To what degree do conclusions regarding evo-devo phenomena, such as modularity, homology, and heterochrony, differ when the same study system is examined from molecular genetic versus morpho evo-devo perspectives? All evidence to date suggests that phenomena observed at one level do not necessarily predict phenomena at another (e.g., non-orthologous genes are routinely involved in the development of indisputably homologous morphological features), underscoring the need for a morpho evo-devo perspective.

To what extent do botanical modules as traditionally defined agree with evolutionarily 'quasi-autonomous' units?

Where might the best hunting ground be for testing phenotype-first evolution? Particularly lacking are vegetative cases. Different morphologies in the sequences of leaves produced along branches (heteroblasty) or different types of shoot (short versus long) are potential study systems for identifying phenotype-first evolution.

Do temporal phenotypes limit or enhance the morphological diversity that can evolve in a lineage? If adaptation at one stage directs the variation that can be presented to selection at another, temporal phenotypes might limit morphological diversification.

Does absolute age ever represent a suitable variable for developmental comparisons? Time is often used as an x-axis for comparison of development between animals, but, in plants, age is often decoupled from development [18,122].

What is the best notion of 'homology' for evo-devo? We advocate the taxic sense as being the solution to the tricky vagueness of this term in evo-devo [123,124].

What should we call the modern study of form? A faithful explanation of any instance of organismal form involves both natural selection and factors that oppose selection ('constraint'). Calling such an explanation adaptationist, or evo-devo, or internalist, or any single side of the artificial selection-

having access to the logistics, facilities, and infrastructure to perform molecular experiments. Even more importantly, and as we exemplify throughout, morpho evo-devo provides data at the appropriate levels of biological organization for studying some of the most consequential of all evolutionary phenomena (Box 2). For example, morphological adaptation emerges via the interaction between whole organisms and their selective contexts. Of course, molecular processes are important players, but anything like a complete story demands focus on the organism–environment dyad and, thus, a morpho evo-devo approach. Phenotype-first evolution suggests in its very name the need to include the richness of morphological study and a morpho evo-devo perspective. Organismal modularity by definition bears on ‘parts’ of organisms and their relationships to the whole, inherently demanding delving into the morpho evo-devo toolkit. All practitioners of evo-devo recognize themes such as modularity, phenotype-first evolution, heterochrony, and developmental processes that limit or direct phenotypic evolution as central to not only evo-devo, but also evolutionary biology at large. Therefore, studying these fundamental phenomena requires a field of plant evo-devo that wholeheartedly encompasses, from the fossil past to molecular techniques, morpho evo-devo in its fullest expression.

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Declaration of interests

None declared by authors.

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constraint dichotomy, sells it short [112]. What to call this true science of form? We like ‘adaptationist evo-devo.’

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Capítulo 2. The evolution of ontogenetic “decision-making” in the wood of a clade of tropical plants

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The evolution of ontogenetic “decision-making” in the wood of a clade of tropical plants

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EPM, conceived the experiments, collected the data, developed the code, and wrote the original draft. MO, designed the study, conceived the experiments, collected the samples, collected the data, and wrote the original draft. EPM, MB, and MEL designed the analyses. All authors reviewed and edited the writing at all stages of revision.

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Data Availability

All data analyzed in the manuscript, and R and Python code code for analyses will be deposited in github and figshare upon acceptance.

Conflict of Interest

This manuscript has been viewed and approved by all co-authors, and we are unaware of any conflicts of interest.

Abstract

Greater diversity in functional morphology should be associated with the evolution of greater ontogenetic diversity, an expectation difficult to test in most long-lived wild organisms. In the cells derived from the wood meristem (vascular cambium), plants provide extraordinary systems for reconstructing ontogenies in often long-lived organisms. The vascular cambium produces files of cells from the stem center to the periphery, with each cambial derivative “deciding” which of four cell types it differentiates into. Wood cell files remain in place, allowing tracing of the ontogenetic “decisions” taken throughout the life of a stem. We compared cell files from 14 species of the Pedilanthus clade (genus *Euphorbia*), which span a range of growth forms from small trees and shrubs of tropical habitats to desert succulents. Using language theory, we represented wood cell types as “letters” and combinations of cell types in cell files as “words,” allowing us to measure diversity of “decisions” based on word frequency matrices. We also used information content metrics to compare levels of predictability in “decision-making.” Our analyses identified a wider array of developmental “decisions” in woody trees as compared to succulent shrubs, illustrating ways that woody plants provide unparalleled systems for studying the evolution of ontogeny in long-lived, non-model species.

Keywords:

adaptation, ontogeny, cell lineage, morphological diversity, xylem, evo-devo

Introduction

Organismal parts and tissues vary in the numbers of functions and roles that they participate in. Some, are involved in a panoply of functions, such as respiration, osmoregulation, thermoregulation, defense, water absorption, and communication in the skin of amphibians (Akat Çömden et al., 2023). Others carry out fewer functions, e.g. not including respiration or water absorption in mammal skin (Liu et al., 2013). This variation in organismal form-function relation implies that natural selection shapes the diversity of ontogenetic processes within and across species. Morphological and functional diversity is produced in development, the processes in which manifold components, including genes, proteins, membranes, and even environmental resources, interact as a system in the construction of new individuals or new organismal parts (Blumberg, 2009; Jablonka & Lamb, 2005; Oyama et al., 2001; Sultan, 2015). In many, perhaps most, cases, greater tissue-level functional diversity requires greater ontogenetic diversity. This reasoning suggests that selection can alter the breadth of ontogenetic processes, even in the same structure, across species.

Testing expectations regarding the ways that selection should favor differing degrees of ontogenetic diversity in the context of variation in functional diversity is difficult because detailed developmental information, as at the cellular level, is often unavailable for non-model species. Such information is difficult to come by either because of the large sizes of non-model animals or difficulty of culture in the lab and, often, challenges for the identification of cell lineages. A cell lineage is the group of cells derived from the same progenitor cell (Baron & van Oudenaarden, 2019). In most animals, cell migration, cell intercalation, and cell rearrangements, crucial factors for animal morphogenesis (Fulton et al., 2022; Solnica-Krezel & Sepich, 2012; Uriu et al., 2014), occur during the brief period of embryogenesis, requiring the use of sophisticated techniques to identify cell lineages, thus limiting their study to specific developmental stages in short-lived model species (McDole et al., 2018; Mongera et al., 2018; Schauer et al., 2020). Moreover, model organisms are usually raised under very specific conditions, biasing the potential range of morpho-anatomical variation (Rivera-Yoshida et al., 2020) and excluding organism-natural environment interaction. These factors have delayed studies addressing the ways that variation in progenitor cell division rates and final differentiation states leads to variation in cell lineage composition, which in turn gives rise to morpho-anatomical variation in long-lived organisms in wild conditions.

To identify ontogenetic changes in a comparative context for long-lived and often very large organisms, woody plants are an extraordinary and virtually untapped system (Petronne-Mendoza et al., 2023). Unlike most animal tissues with short embryonic cell proliferation-differentiation periods, the stems of woody plants grow throughout their lives (Groover, 2023; Onyenedum & Pace, 2021; Sillett et al., 2010) via the vascular cambium (henceforth “cambium”), producing secondary xylem (wood) internally and secondary phloem (\approx bark) externally (Groover, 2023;

Schmitt et al., 2016). While it is common for secondary phloem cells to collapse and be pulled apart as trees grow, wood cells remain intact and maintain their relative positions and for the life of a tree. Cells are cut off from the cambium, and then almost immediately, based on their position relative to other cell types, longitudinal position in the trunk, growing season, environmental conditions, and associated hormone levels, cambial derivatives differentiate into a given cell type and enlarge to a certain size (Buttò et al., 2019; Fischer et al., 2019; Johnson et al., 2018; Johnsson et al., 2019; Noyer et al., 2023). This process of each cambial derivative maturing into a given cell type based on the local conditions it experiences is what we refer to as, using the evocative metaphor of previous authors (Bhalerao & Fischer, 2017; Johnsson & Fischer, 2016; Mäkilä et al., 2023; Olson et al., 2019), “decision-making.”

The differentiated products of a single cambial initial are typically arranged in clear radial files (Figure 1A, Figure A1). As a result, the entire history of “decisions” made by the derivatives of a single cambium cell can be reconstructed from the plant’s early stage, at the center of the stem, to its current state in the outermost layers (Bossinger & Spokevicius, 2018; Miodek et al., 2021a,b; Olson, 2007; Olson & Rosell, 2006). These properties of xylem development allow the reconstruction of cell lineage composition giving rise to morphological and anatomical variation in long-lived organisms under wild conditions (Cunha Neto et al., 2022; Groover, 2023; Onyenedum & Pace, 2021; Petrone-Mendoza et al., 2023).

Wood offers a tractable study system for testing the expectation that tissue-level functional diversity should be associated with greater ontogenetic diversity, because wood consists of just four cell types, each of which has well-documented functions. The cambium has two types of progenitor cells, fusiform initials and ray initials. Fusiform initials in most flowering plants are tall, slender cells that, in the xylem, differentiate into three main cell types (Carlquist, 2001b; Mauseth, 2008; Montes-Cartas et al., 2017), vessel elements, axial parenchyma cells, and fibers (Figure 1B). Vessel elements are tube-like cylindrical cells, dead at maturity, with lysed end walls. Vessel elements are arranged end-to-end forming continuous tubes called vessels, through which water flows. Fibers are long, tapered, slender, often thick-walled cells giving mechanical support to stems (see Olson (2023) on the heterogeneity of the “fiber” category), while axial parenchyma cells are living, often with thin cell walls in which nutrients and water are stored (Carlquist, 2001a, 2015a,b, 2018; Plavcová et al., 2023). Fusiform cambial initials occasionally subdivide into ray initial cells, which are considerably shorter than fusiform initials. Ray initial cells give rise to ray parenchyma cells in sheets of cells that stretch from the periphery of the stem toward the inside. Different tendencies for cambial initials to produce differing proportions of wood cell types result in often vastly different whole-plant function (Bouda et al., 2019; Cunha Neto, 2023; Hearn, 2009; Isnard et al., 2012; Lachenbruch & McCulloh, 2014; Lahaye et al., 2005; Martínez-Vilalta et al., 2012; Onyenedum & Pace, 2021; Quintanar-Castillo & Pace, 2022).

Here, we infer the differences in ontogenetic “decision-making” that lead to functionally differing wood types across the members of a clade of tropical plants. We use the *Pedilanthus* clade

of the genus *Euphorbia* because it contains species that should vary in the functional diversity of their wood. The ancestral-type morphology (9 out of 14 species, Figure 2A) are succulent shrubs of deserts and tropical drylands (Cacho et al., 2010). These species have thick, stubby, cane-like stems that quickly drop their leaves, remaining leafless for most of the life of a stem (Figure 2B, Figure S1). They have massive, fleshy barks and piths (the central, water-filled tissue in the center of a stem) with limited accumulation of wood. Woody trees, in contrast, grow in various tropical forest types, and as their name implies, they are of more conventional tree and shrub habit, with leaves borne on thin twigs and trunks with substantial accumulation of wood. With just 14 extant species, we were able to study all of the species in the clade. We used language theory, in which information can be represented as strings of symbols (Hopcroft et al., 2001) to code “decisions,” representing cells with letters. Our cell-to-letter coding allowed us to build word frequency matrices, use information theory metrics such as Shannon entropy (Shannon, 1951) and the Lempel-Ziv algorithm (Ziv & Lempel, 1977), and to build a morphospace to describe the ontogenetic “decision-making” underlying functional diversity across species. We show that the two main growth forms of the *Pedilanthus* clade differ in wood cell “decision-making” in ways consistent with the expected effects of selection on ontogenetic diversity in the context of differing functional contexts. Our data also suggest that this greater ontogenetic complexity in the woody species evolved via the modification of a less-complex ancestral ontogenetic type in the context of likely functional trade-offs in wood function (Figure 2A).

Methods

Study clade

The *Pedilanthus* clade consists of 14 extant species (Olson et al., 2005). Growth form variation goes from clump-forming shrubs with slender stems less than one meter tall in *E. cymbifera* (Dressler, 1957), to succulent stems varying from one to three meters tall in *E. tithymaloides*, *E. personata*, *E. bracteata*, *E. cyri*, and *E. lomelii*, to evergreen treelet species in *E. colligata*, *E. finkii*, *E. konzattii*, and *E. peritropoides*, to deciduous trees reaching up to five meters in *E. calcarata* and *E. coalcomanensis* (Cacho et al., 2010; Olson et al., 2005). Because all of the species are derived from the same common ancestor, all of the morphological, anatomical, and thus developmental, diversity has accrued since divergence from the same ancestral starting point (Figure 2). Also, species from the *Pedilanthus* clade span a wide array of habitats, and thus from the same ancestral starting point, they have adapted to different environmental conditions.

Growth form classification and other measured variables.

Following Cacho et al. (2010), we classified species as succulent shrubs or woody treelets and trees. Succulent shrub species (*E. bracteata*, *E. cymbifera*, *E. cyri*, *E. diazlanana*, *E. lomelii*, *E. personata*, *E. tehuacana*, and *E. tithymaloides*), hereafter succulents, grow mostly in drylands such

as deserts, tropical deciduous forests, thorn scrubs, and even rocky clearings on the fringes of mangrove swamps. Woody treelets and tree species (*E. calcarata*, *E. coalcomanensis*, *E. colligata*, *E. conzattii*, *E. finkii*, and *E. peritropoides*), hereafter woody trees, grow mostly in environments such as tropical deciduous and subdeciduous forests to very wet tropical forests. We estimated ancestral character states based on the maximum likelihood tree from Cacho et al. (2010) (see Appendix A for additional methods). We test the notion that succulents and woody trees differ in their patterns of wood cell “decision-making.” To relate different patterns of decision-making with growth form characteristics known to be functionally important in addition to the succulent/woody tree distinction (Larios Mendieta et al., 2021; McDowell et al., 2002; Niklas et al., 2006; Olson & Rosell, 2013; Vertessy et al., 1995), we also measured stem length, total stem diameter, and diameter of different components of the stem, such as the pith-to-cambium thickness of the xylem and pith diameter (Table S1, Figure S1, Figure S4).

Plant material and cell coding

We coded a total of 27 individuals, with at least one individual from the 14 extant species (*E. dressleri* is apparently extinct (Olson et al., 2005)) (see Table S1 for sample information). We gathered secondary xylem data from the main stem (near the base) from each individual and used standard anatomical techniques to prepare sections for light microscopy (Olson & Carlquist, 2001). Viewing a stem in cross-section, lineages derived from cambial initials form radial files, from the outside of the stem to the inside (Figure 1A). We coded all cells derived from one cambial initial (one radial file) until it was not possible to determine the membership of a cell in a given file (See Appendix A for more details on cell coding). To record “decisions,” we represented the different xylem cell types as members of an alphabet, assigning the following letters to each cell type:

$$\Sigma = \{FI, RI, V, F, P, R\}, \quad (1)$$

where *FI* are the fusiform initials, *RI* are the ray initials, *V* are vessels, *F* are fibers, *P* are axial parenchyma cells, and *R* are ray parenchyma cells. The summation notation represents the alphabet of the cambial initials and their derivatives. A string or “word” consists of a sequence of letters, which can be of length one up to infinite, drawn from the alphabet set (Hopcroft et al., 2001). So, if a cambial fusiform initial gave rise to three fibers, followed by an axial parenchyma cell, then two vessels, followed by five fibers, we would represent this word from left to right as *FFFFVVPFFF*. To recover plausible functional differences and to cover a significant cross-sectional area of the secondary xylem and to ensure wide sampling of cell differentiation patterns spanning growth rings when present, we coded at least 120 cambial files per individual. In the first column of our dataset, we assigned a numeric identifier for each lineage, and in the second column we saved the sequence of letters or wood cells in the file produced by the cambial initial.

Data are available at [pending]. Given that ray initials only form ray cells, in the following analyses we focus on the fusiform initial-derived cells, which vary among F , V , and P .

Cell lineage comparisons

To detect differences in “decision-making” of cambial derivative cells across *Pedilanthus*, we performed analyses that can be divided in two categories. In the first category, each lineage was broken into sub-strings or words of different $k - mer$ lengths ($k =$ number of letters in a word) smaller than the total file length (in the following we refer to file length as the number of letters coded for each file). Further explanation of how we broke words in different $k - mer$ lengths in the Maximum number of words section. By breaking files into smaller words we can compare the short term “decision-making” processes of developing wood cells between individuals. Short-term decision-making is biologically relevant because a developing cell is likely to mature into a given cell type not based on the presence of very distant, often dead, cells, but ones in its immediate neighborhood. For instance, consider a species with non-conductive fibers. In this case selection favors the grouping of vessel elements (Johnson et al., 2023). This means that when a cambium derivative differentiates into a vessel element, it is highly probable that nearby cells will also become vessel elements. Distant cells near the pith are often dead or metabolically inactive, and so are less likely than closer cells to influence the “decisions” taken by a distant cambial derivative. In the second category, we analyzed the complete rows using information theory metrics. By analyzing the complete rows we compared the long-term “decision-making” of cambial derivative cells. Cells in the interior of the wood often become non-functional, and so do not participate in functions such as water transport or starch storage. However, aspects such as stem mechanical performance emerge from the joint contributions of all of the cells present. In this way, natural selection could favor certain long-term patterns of “decision-making” to the extent that they can affect some stem functions, such as mechanical support, to which even dead interior cells can contribute. We performed two analyses on the files broken into word categories. The first consisted of counting the maximum number of words found at different word lengths and the second estimated similarities among individuals based on word frequencies, and we detail these in the following paragraphs.

Maximum number of words.

To test the notion that woody species exhibit greater ontogenetic diversity than succulent species, we counted the total number of different words per individual at different word lengths. To perform this analysis we divided the files into different $k - mer$ lengths. For example, for the file *FFFFVVPFFF* and $k - mer$ length of 3, we would have a set of six different words (*FFF*, *FFV*, *FVV*, *VVP*, *VPE*, *FFF*). To estimate the maximum number of words in each individual, we counted the set of different words, from two to 35 $k - mer$ lengths. Words counted at a $k - mer$ length that only appear once reflect rare events of wood cell ordering, and thus rare developmental deci-

sions. Words counted more than once reflect the tendency of a given species to produce, with greater or lesser degrees of consistency, a particular ordering of wood cells. To account for the tendency to produce wood cells with particular orderings, we only counted words appearing more than once, from two to 35 k – mer length. For each sample, we plotted the total number of different words at different k – mer lengths. The range of k – mer lengths we used allowed us to estimate the maximum numbers of words in each individual. To test the expectation that the maximum numbers of words should differ between succulent and woody species, we fit two linear regression models. The first model predicted the maximum number of words appearing more than once based on mean file length plus growth form and the interaction between the predictors. The second model predicted the maximum number of words based on the total number of coded cells per individual plus growth form and the interaction between the predictors. We regressed maximum number of words against mean file length and total number of coded cells to control for the scaling of number of words with number of cells.

Similarity of “decision-making” based on word frequencies.

We tested the idea that greater similarity in the frequencies of certain words should be expected in species with the same growth form. To compare word frequencies across individuals, we counted words appearing more than once, focusing on typically-produced words rather than atypical ones that might not characterize the common cell combinations favored by selection in the context of a given plant growth form. In an analogous situation, to identify the optimum k – mer length to compare between genome sequences, Sims et al. (2009) argued that the optimum value is based on the k – mer having the maximum number of words because it maximizes overlap in word sets. In our sampling, the k – mer length with the maximum number of words across individuals varied from seven to 30. This range in maximum number of words at different k – mer lengths has an effect on word counting. At higher k – mer values, for the individuals having maximum numbers of words of lower k – mer values, more words with zero counts will be introduced to the matrix, because of few words at higher k – mer values. To minimize the appearance of words with zero counts in the matrix, we built a matrix at the minimum k – mer length value where the maximum number of words was observed for our sampling (7 – mer). The mean k – mer value of the maximum number of words from our individuals was 19 – mer. To maximize the set of possible words to compare across our sampling, we also built a frequency matrix of 19 – mer word counts. To account for differences in the total of cells coded per individual, we built the matrices using a scaling factor for each word:

$$S_{i,j} = W_{i,j} \left(\frac{T_j}{W^*} \right), \quad (2)$$

where $S_{i,j}$ are the scaled counts of word i in individual j , T_j is the total number of words counted in individual j , W^* is the total number of words counted in the individual with the most words, and $W_{i,j}$ is the count of word i in an individual j . We estimated the level of similarity between

individuals using the Bray-Curtis dissimilarity measure using the *vegdist* function from the R *vegan* package (Oksanen et al., 2022). Bray-Curtis dissimilarity is useful for comparing word counts because it returns values between 0 and 1, where 0 means completely similar and 1 means completely dissimilar. To visualize the level of similarity, in terms of word abundances, we generated heatmaps from the Bray-Curtis dissimilarity values, as well as non-metric multidimensional scaling (NMDS) plots for the WCS at seven and 19 *k-mer* lengths, with the default settings of the *metaMDS* function (Oksanen et al., 2022). To test which WCS counts had more influence on the seven and 19 *k-mer* NMDS ordination plots, we ran the *envfit* function. We fitted the WCS on the NMDS plots and assessed the significance of the R^2 fitted values using 9999 permutation tests. We plotted words with a significant influence on the NMDS ordination plot ($p \leq 0.0001$).

Information theory-based methods.

To compare the long-term impact of ontogenetic “decisions,” we used information theory metrics that analyze the letter compositions of complete cell files. The letter composition of a file refers to the order of the cells in a given file, reflecting the overall “decision-making” made by the cells in a file from the origin of the file to the present. For example, two files could have the same cell frequency of fibers and vessels but with drastically different orders of ontogenetic “decisions” (e.g. *FFFFVVFFFF* vs *FFVFVFVFFFF*). Information theory metrics estimate differences in letter composition across files. We used the Lempel-Ziv compression algorithm and the Shannon entropy measure to compare VC files across samples and across species. The Lempel-Ziv complexity algorithm calculates the number of sub-sequences encountered in a file from beginning to end Ziv & Lempel (1977). For example, the previously mentioned file, *FFFFVVFFFF*, has a total of 6 different sub-sequences (*F*, *FF*, *FV*, *V*, *VF*, *FFF*), whereas the file *FFVFVFVFFFF* has 3 sub-sequences (*F*, *FV*, *FFFF*) (explained in Zielezinski et al. (2017). More complicated letter compositions within files have more sub-sequences, therefore more ontogenetic diversity. The formula for calculating Shannon entropy (1951) is:

$$H_x = \sum p(i) \log_2 \frac{1}{p(i)}, \quad (3)$$

where (*i*) denotes the cell type, and *p* is the probability of a cell type based on the frequency within the file H_x . If the heterogeneity or “randomness” in letter composition of a file increases, the Shannon entropy value will increase.

Homogeneity indexes and morphospace evaluation.

We expected that succulents versus woody trees should differ in their decision-making patterns, and that these differences should have implications for whole-plant function. To test this expectation, we evaluated the distribution of putative conductive and support functions along cell files. To do so, based on functional characterization of xylem cell functions, we assigned wood cells to conductive or non-conductive categories, and as support or non-support, and transformed the

letters from cell files into binary sequences. We evaluated the binary sequences using two homogeneity indexes modified from Miramontes et al. (1995). One homogeneity index evaluated conduction (HI_{CO}) and the other mechanical support (HI_S). For HI_{CO} , we categorized vessels as conductive (1). Fibers and parenchyma produced after or before vessels (i.e FV or VP) were also categorized as conductive because they are always implicated in conductive functions (Aritsara et al., 2021; Janssen et al., 2020; Morris et al., 2018; Plavcová et al., 2023). Fibers and parenchyma not produced after or before vessels were categorized as non-conductive (0). For the HI_S index, we categorized vessels and parenchyma cells as non-support (0), as well as fibers (0) produced after or before vessels because of their role in conductive functions. Fibers not produced immediately before or after vessels were categorized as supporting (1), because they are the main cell type giving mechanical support to stems (Pratt & Jacobsen, 2017). Both homogeneity indexes evaluate the distribution of 0s and 1s in the sequence. The formulas for the two indexes are as follows:

$$HI_{CO} = \frac{(D_{00} * D_{11}) - (D_{01} * D_{10})}{D_0 * D_1} \quad (4) \quad HI_S = \frac{(D_{00} * D_{11}) - (D_{01} * D_{10})}{D_0 * D_1}, \quad (5)$$

where D_{ij} is the number of ij cell functions, conductive/non-conductive or support/non-support ($ij= 00, 01, 10, 11$), and D_0 and D_1 are the number of 0s and 1s in a cell lineage. Sequences made of alternating 0s and 1s have index values of -1, while sequences made up only of 1s or 0s have index values of 1 (Miramontes et al., 1995). For each cell row we plotted the value of HI_{CO} and HI_S .

Comparing Lindenmayer systems to Pedilanthus cell files

If natural selection favors a particular ordering of wood cells in a file, these orderings of wood cells should be more common, biasing the universe of wood cell orderings and reflecting non-stochastic “decision- making.” To compare non-stochastic “decision-making” with a stochastic system, we designed two different types of Lindenmayer systems or L-systems (Lindenmayer, 1968; Prusinkiewicz & Lindenmayer, 2012). L-systems consist of an alphabet and a set of rewriting rules, which are instructions to replace letters in a word for other letters. For example, for the word AB , after applying one rewriting rule that replaces A for AB , and another rewriting rule that replaces B for A , we will have the word ABA . By repeating the process of replacing letters with the rewriting rules, longer words are produced. This simulates plant cell division, and has been used to map inflorescence morphologies or stem branching patterns (Prusinkiewicz et al., 2018, 2007; Prusinkiewicz & Runions, 2012). Here we implemented stochastic L-systems to simulate the production of wood cells from the vascular cambium. Stochastic L-systems are those in which cambial derivatives can differentiate into any fusiform-derived cell with an equal probability (See Supplementary material for details of the rewriting rules). The two models of

stochastic L-systems we used differed in cell file length distribution. In one stochastic L-system model, the probL-system, we determined the file length of each of the simulated cell files by selecting a random number from the interquartile range of the cell file lengths of the individuals we sampled. In the other stochastic β L-system, the prob β L-system, for each cell file, we randomly selected a number from a β distribution ($\alpha = 2$, $\beta = 5$, scaled to 500 in length) to approximate the file length distributions of our empirical data. For each L-system, a total of 300 cell files were simulated and saved as “virtual woods.” Code was implemented in python3 (Van Rossum & Drake, 2009) and is available at (will be made available upon acceptance). These then served to detect whether or not our empirical data deviate from stochastic expectations, suggesting that certain specific combinations of cells are favored by selection.

Results

General description of the cell lineages

Our coding of cell lineages from the Pedilanthus clade included a total of 430,727 cells and 4,840 cell files from 27 samples from 14 species (Table S2). Of the 430,727 cells coded, 184,774 (42.9%) were from the succulent species and 245,953 (57.1%) were from the woody species. 1734 (35.8%) files had at least one ray cell, and of these 847 were made up exclusively of ray cells. The other 887 cell files containing ray cells had fusiform-derived cells, meaning that at one point a fusiform initial subdivided into a ray initial. Fibers were the most common cell type for all species (66.5% of the coded cells), followed by ray parenchyma cells (20.5%), and axial parenchyma (11.7%); the least common cell type was vessels, with 1.3%. Woody and succulent species mainly differed in the percentage of fibers and axial parenchyma. In woody species, 62.3% of cells were fibers, and 15.2% were axial parenchyma cells. In succulent species, 72% of cells were fibers, and 7% were axial parenchyma cells.

Word based methods

We recovered differences in the total numbers of words of different lengths. When comparing total number of words and total number of words appearing more than once, woody tree species tended to have more words than succulent species, in agreement with our expectation of higher ontogenetic diversity in woody trees (Figure 3). The maximum number of words across our samples was between seven and 30 *k-mer* length. Both models of L-systems had their maximum number of words at eight and nine *k-mer* lengths. L-systems had higher maximum numbers of words than Pedilanthus clade species, even at lower *k-mer* lengths than most individuals sampled, showing that “decision-making” in the Pedilanthus clade deviates from stochastic production of wood cells. The maximum number of words appearing more than once, for all samples, scaled

with mean cell file length ($R^2 = 0.88, slope = 1.3, p < 0.001$) and with total number of coded cells ($R^2 = 0.94, slope = 1.8, p < 0.001$), justifying the scaling factor $WCS_{i,j}$. Woody species had higher intercepts than succulent species for both models (Table 1), showing that for a given set of cells coded or for a given mean cell file length, woody species had higher maximum numbers of words (Figure 3e,f).

Table 1: Linear models predicting differences in numbers of words between growth forms of the *Pedilanthus* clade, standardizing by mean cell file length and by total number of coded cells.

Model	N	R_2	Model ANOVA	Slope Equality test	Intercept Equality Test	slope (C.I.)	intercept (C.I.)
maximum number of words \sim mean cell file length + growth form	24	0.88	$F_{2,24} = 104.2$	-	$p < 0.001$	1.31 (0.99,1.64)	succulent = 0.18 (-0.32,0.38) woody trees = 0.53 (0.4, 1.74)
maximum number of words \sim total cells coded + growth form	24	0.94	$F_{2,24} = 225.9$	-	$p < 0.001$	1.46 (0.99,1.64)	succulent = -3.21 (-3.71,-3.41) woody trees = -2.93 (-3.06,-1.73)

Our analysis of word frequencies highlighted that the two main growth forms are more similar within themselves than with one another. L-systems were not included in word count matrices because they were very different from the *Pedilanthus* clade. When comparing WCS of k -mer length seven, which was the lowest k -mer length value of maximum number of words from our sampled individuals, the majority of samples from succulent species showed greater similarity to other succulent species than to woody species. By the same token, most samples from woody tree species were more similar to other woody species than to succulent species (Figure 4a). Only one of the woody samples (*E. peritropoides*-2) was more similar to a succulent, specifically, *E. cymbifera*-1. The same pattern of similarity was observed at 19 k -mer, which we used because it was the mean k -mer length value of maximum number of words across the sampled individuals (Fig S2). In the ordination plot, words made up mostly of fibers, such as *FFFFFFF*, tended to be from succulent species, while words having a mix of fibers and parenchyma, such as *PPFPFFF*, tended to be from woody tree species (Fig4b and FigS2b). These results highlight that woody tree species produce more and different words than succulent species, reflecting greater ontogenetic diversity at the cellular level associated with the expectation of higher functional diversity.

Information theory based methods.

We observed differences in the Lempel-Ziv compression algorithm and the Shannon entropy values calculated from complete cell files across species (Figure 4) and growth forms (Figure S3) of the Pedilanthus clade. The corresponding ANOVA for the Lempel-Ziv compression algorithm and Kruskal-Wallis test for the Shannon entropy values showed that at least one species differed from the rest ($p < 0.001$ for both tests). Post-hoc comparisons for the Lempel-Ziv compression algorithm showed that the succulent *E. cymbifera* had the lowest values, while the woody tree *E. coalcomanensis* had the highest values. Similarly, post-hoc comparisons for the Shannon entropy values showed that the succulent *E. diaz-lunana* had the lowest values while the woody tree *E. coalcomanensis* had the highest values. The Lempel-Ziv compression algorithm tended to be similar between succulent species, having lower values than woody species (succulent mean Lempel-Ziv value = 13.11, woody tree mean Lempel-Ziv value = 24.1, $p < 0.001$, Figure S2). Only the succulents *E. tehuacana* and *E. bracteata* showed Lempel-Ziv values similar to the woody species. In the Shannon entropy values, succulent species had lower values than woody species (succulent median Shannon entropy value = 0.44, woody tree median Shannon entropy value = 0.75, $p < 0.001$, Figure S2). Higher Shannon entropy values reflect more heterogeneous ordering of wood cells along files, thus more diverse developmental decisions in the woody than in succulent species. L-systems had higher values of both Lempel-Ziv and Shannon entropy values than species from the Pedilanthus clade. Similar to the maximum number of words results, information theory methods point to a wider array of developmental “decision-making” in woody trees than in succulent species, but not in a stochastic way as in L-systems.

Homogeneity indices.

Conductive (HI_{CO}) and support (HI_S) homogeneity indexes were different between growth forms of the Pedilanthus clade, revealing differences in developmental “decisions” that result in plausibly different functionalities. For the HI_{CO} value, 95% of the files were positive; positive values indicate that cell files are made up predominantly of 00s or 11s. 68% of the cell files had HI_{CO} values of 1, with 71% of these files made up only of 00s, meaning that a high percentage of cell files were made up of fibers or parenchyma cells without any vessels. Only 1.6% of the cell files were negative for the HI_{CO} , and of these files most of them were from succulent species (93%) (Figure 5). For the HI_S value, 65.5% of the cell files were positive, while 34.3% of cell files had negative values (lineages made up predominantly of 01s and 10s). None of the cell files had values of -1 for the HI_{CO} , nor the HI_S (files made up exclusively of 01s or 10s).

The HI_{CO} - HI_S biplot of the Pedilanthus cell files showed different distributions between succulent and woody tree species (Figure 6). 68% of the cell files with values between 0.5 and 0.85 for HI_{CO} were from woody species. More than half of woody tree cell files between 0.5 and 0.85 for HI_{CO} (51%) had a negative value for HI_S (Figure 6). The combination of positive values around 0.5 to 0.85 for HI_{CO} and negative HI_S values, reflects cell files tending to form axial parenchyma alternating with fibers and the sporadic production of vessels in woody species, which are not

observed in succulent cell files. Most of the succulent cell files with values between 0.5 and 1 for HI_{CO} showed positive values for the HI_S (71%), reflecting “decisions” resulting in the formation of more fibers without producing axial parenchyma. Most of the cell files having 0 or negative values for the HI_{CO} were from succulent species and tended to have positive values of HI_S (98% of the cell files) (Figure 6). The combination of negative HI_{CO} and positive HI_S reflects decision-making patterns that tend to form series of fibers alternating with vessels and axial parenchyma, which are not observed in succulent cell files. L-systems showed values in between succulent and woody tree species. Most L-system cell files had HI_{CO} values around 0.1 and 0.7 (82%) and HI_S values around 0.0 and 0.5 (86%), suggesting that the wider distribution of values in cell files from the *Pedilanthus* clade produce functionally significant orderings of cell files that deviate from stochastic “decisions.”

Discussion

The remarkable property of wood to be an archive of its own ontogeny allows tracing the “decisions” made by developing cells over hundreds of cell divisions. We used this archive-of-ontogeny property to infer the relationship between ontogenetic diversity and functional diversity between tree and succulent species of the *Pedilanthus* clade. We found that, even though all species produce the same cell types, tree and shrub species have greater ontogenetic diversity, in terms of “decisions,” compared to succulent species. Here, we comment on the ways that different patterns of developmental “decision-making” likely evolve under different selective scenarios, giving rise to morphological, anatomical, and plausibly functional variation. We comment on likely reasons for trees having more diverse patterns of developmental “decisions” than succulents, and we highlight that estimating the maximum number of words can be a measure of the developmental potential of a species. We conclude with comments on the importance of tracing wood ontogeny as a way of identifying differences in the “decision-making” process of progenitor cell lineages in long lived organisms growing in natural conditions.

Wood cell “decision-making” and natural selection.

Our results are consistent with the notion that in the *Pedilanthus* clade, natural selection favors different tendencies of developmental “decision-making” between succulent and woody tree and shrub growth forms. Given a set of hydraulic, storage, and mechanical demands, variants tending to have certain orderings of cells, and therefore a particular sequence of “decisions,” are favored. If the sequence of “decision-making” of cambium-derived cells tends to be heritable, a favored word or sequence of letters will increase in representation within a species. Our Bray-Curtis dissimilarity estimates (Figure 4a) and the NMDS (Figure 4b), using standardized word counts for total counted cells supported the distinction between succulent and woody species. In succulent species, word counts made up only of fibers were more common than in the woody species, which had word counts with more parenchyma-fiber (PF or FP) orderings (Figure 4b).

Over-represented words in each group reflected the tendency to produce different orderings of wood cells, resulting in woods that potentially vary in hydraulic, storage, and mechanical functions. The great deal of variation in cell sequences illustrates the “decision-making” potential of cambium-derived cells, while the differences in overrepresented cell sequences between growth forms suggests ways in which natural selection favors particular cell “decision-making.”

A functional trade-off in the amount of water and nutrient tissue likely drives differences in the patterns of sequential cell differentiation across the *Pedilanthus* clade. Succulent species have wide piths (the central tissue in the stem), whereas woody ones have narrow piths (Figure S3). Pith is made up of water storage tissue (Olson, 2005). Woody species are of larger stature than the succulent species, and accumulate more layers of wood (Figure S3). As a result, the cambium continually displaces itself farther and farther from the pith. Given that metabolic activity in the wood tends to be highest near the cambium (Chapotin et al., 2006; Pruyn et al., 2002; Spicer & Holbrook, 2007), this means that the living cells that potentially draw upon the pith become more and more distant from it. As a result, selection would seem necessarily to favor water storage within the wood itself, consistent with our finding that tree species “decide” to produce greater numbers of water- and starch -storing axial parenchyma cells (Figure 4 and 6). In contrast, in the small stems of the succulent species, water-storage occurs mostly in succulent bark and the pith, the water-storing tissue interior to the wood. In these species, the wood largely functions in mechanical support of the heavy, succulent stem, and in water transport. As in other water-storing “broomstick” plants, water storage is “outsourced” to the bark and pith, outside the wood (Rosell & Olson, 2007). In this way, habit is plausibly connected to differences in ontogenetic “decision-making” across the species of the *Pedilanthus* clade.

Higher diversity in “decision-making” associated with higher functional diversity.

Our results show that woody species of the *Pedilanthus* clade have a wider array of “decision-making” than succulent species. For a given file length and for a given number of cells, woody species had higher maximum numbers of words than succulent species (Figure 3E and 3F). Also, woody species had higher Lempel-Ziv and Shannon entropy values, both of which reflect less predictable patterns of “decision-making” (Figure 5). In general, leafless succulents have much lower transpirational demand as compared to conventional leafy trees and shrubs (Nilsen et al., 1990; Nobel & Jordan, 1983; Ogburn & Edwards, 2010). The much higher frequency of cells involved in conduction in tree *Pedilanthus* species is congruent with the greater conductive demand in these species. Greater water demand by more leaf area should favor individuals with “decisions” transitioning between vessel and parenchyma cells, thereby increasing the maximum numbers of words for a given length. Trees and shrubs such as *E. calcarata*, *E. peritropoides*, and *E. coalcomanensis*, growing in tropical dry forest, produce leaves during the wet season, and treelets growing in mesic environments such as *E. conzattii* and *E. finkii*, are evergreen. Woody trees had cell files producing words such as *PPFPFP* or *PVVVFP*, which were not observed in succulent

species. Succulent species, growing in deserts and tropical dry forests (Figure S4), are mostly early deciduous, and their wood with sparse vessels is reflected in the lower maximum numbers of words and lower Lempel-Ziv and Shannon entropy values, and thus more homogeneous “decisions.” Thus, greater water demands should favor individuals having sequential differentiations transitioning between vessel and parenchyma cells in comparison with individuals with lower water demands and less total leaf area. Overall, the xylem of woody trees suggest higher functional diversity, including water storage without massive pith and bark, higher water demands due to more total leaf area, and maintaining the mechanical support of taller stems, while the xylem of succulent species exhibits much lower ontogenetic diversity, given low storage and water conduction, with its main function being stem mechanical support.

Maximum number of words, morphospace, and developmental potential.

Developmental potential is the entire range of morphologies that can be produced during ontogeny by the individuals of a particular species (Olson, 2012; Vecchi & Santos, 2023). Natural selection can only favor morphologies that can be produced by development, so mapping developmental potential is crucial for understanding why some variants are observed and not others (Blumberg, 2009; Budd, 2021; Frankino et al., 2009; McGhee, 2006; Pavličev & Wagner, 2012). In our empirical dataset, cambium derivative cells of succulent species tended to produce, for a given $k - mer$ length, fewer words than woody species. Our comparative data showed that succulent species had a smaller set of words, and therefore a lower diversity of ontogenetic “decisions” than woody species. Restricted patterns of trait variation can reflect ontogenetic impossibilities (Galis et al., 2022; Hautier et al., 2010; Minelli, 2019). However, the fact that the woody tree species readily produce wider arrays of decisions, and that even the succulent species do produce at least some of all three axial cell types, suggests that the lower diversity of ontogenetic “decision-making” in the succulent species is probably a result of natural selection and not an ontogenetic impossibility. Similarly, if cambium derivative cells “decided” to form wood cells stochastically, as in the probabilistic L-systems, cell files would have values limited to around -0.1 to 0.6 for the HI_{CO} and from -0.1 to 0.5 for HI_S . Instead, cell files from the *Pedilanthus* clade varied from -0.2 to 1 for HI_{CO} and from -0.6 to 1 for HI_S (Figure 6). Thus, the biplot showed that cambium derivative cells from the *Pedilanthus* clade “decide” to form cells in a non-stochastic manner, pointing to “decisions” being biased by natural selection. This apparent biasing provides a window onto the ways that development evolves in the wood of the *Pedilanthus* clade. Our approach recovered thousands of developmental “decisions,” revealing not just tendencies to produce certain cell sequences, but also a large amount of variation about these modal tendencies (Figure 3, 4). This variation illustrates that xylem development in the *Pedilanthus* clade is nothing like the straightforward unfolding of a genetic “blueprint,” understood as the faithful production of one or a few xylem patterns precisely specified in the genome (Krimsky & Gruber, 2013; Laland et al., 2015; Olson, 2019a,b; Sultan, 2015). Instead, our results are more consistent

with selection shaping plastic responses, perhaps in the context of stochastic patterns of cell fate determination. An expression of plasticity is phenotypic accommodation, in which challenges in development are buffered developmentally in ways that produce maximally functional, even normal, morphologies (Blumberg, 2009; West-Eberhard, 2003). As a stem grows over the years, its architecture changes as shaded branches are sacrificed and new ones invade sunny gaps, as branches are subject to bending or breakage under mechanical loads, and as an individual grows taller and transport distances roots-to-leaves increase. The exact sequence of these events and the magnitudes of their effects are largely unpredictable. In the face of this unpredictability, plants have fine-tuned detection-and-response systems that allow them to buffer these challenges through their growth responses (Brenner et al., 2006; Guerra et al., 2019; Karban, 2015). The broad variation in word frequencies in our data, rather than very low variation within succulent versus wood species, speaks to the continual adjustment of xylem cell type composition in response to the continual fluctuation in the demands imposed on stems. Selection in *Pedilanthus* clade xylem thus seems likely to involve biasing developmental detection-response processes, rather than the rigid production of this or that cell sequence. By the same token, our results are consistent with notions of cell fate determination involving stochastic gene expression (Eldar & Elowitz, 2010; Elowitz et al., 2002; Richard et al., 2016), in which the genes that participate in cell differentiation have certain probabilities of being expressed at any given time. Selection on development is thus the favoring of certain stochastic tendencies of gene expression over others. Such a scenario predicts that there should be variability in developmental “decisions,” with broad modal tendencies, exactly the case in our data.

Conclusion

We provide a cell-to-letter coding of cell “decisions” made by wood progenitor cells that allowed us to reconstruct ontogenetic differences in long-lived organisms. We show that differences in developing wood cell “decision-making” have evolved within species of the *Pedilanthus* clade of *Euphorbia* in its diversification spanning succulents to small trees across a wide array of habitats, from rainforest to desert. We found that woody species of the clade had wider arrays of “decision-making,” reflected in higher maximum numbers of words and larger values of Shannon-Entropy and Lempel-Ziv algorithm. By counting the frequencies of words, standardizing for total number of coded cells, we identified different tendencies to produce particular orderings of cells between woody and succulent species. Thus, we document how differences in “decision-making” of progenitor cells from long-lived organisms have implications in morphological, and thus functional diversification across species and habits.

Figures

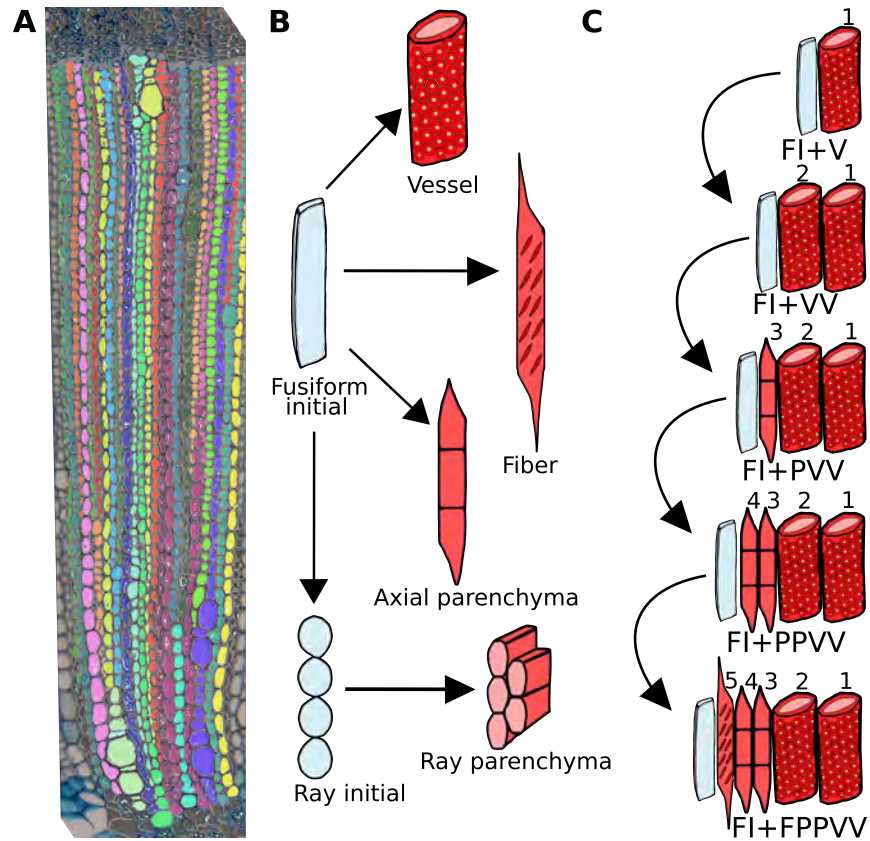


Figure 1: Wood as an archive of developmental “decisions.” **A**, Cross-section of wood showing the cambial zone at top (cells shaded in gray; cells above this are bark) and files of cells derived from the same cambial initial highlighted with different colors. **B**, Developmental cascade of xylem cells. Fusiform cambial initials give rise to the axial system in the wood, made of up fibers, vessel elements, and axial parenchyma. Sometimes, fusiform cambial initials give rise to ray initials, which form the ray system of the wood, made up of ray parenchyma cells. **C**, Representation of a series of developmental “decisions” producing the cell ordering *FPPVV*. The differentiated cells remain in the same place, while the fusiform initial moves outwards. Numbers denote the order of wood cells produced. Abbreviations: *FI* =fusiform cambial initial, *F* =fiber, *P* =axial parenchyma, *V* =vessel.

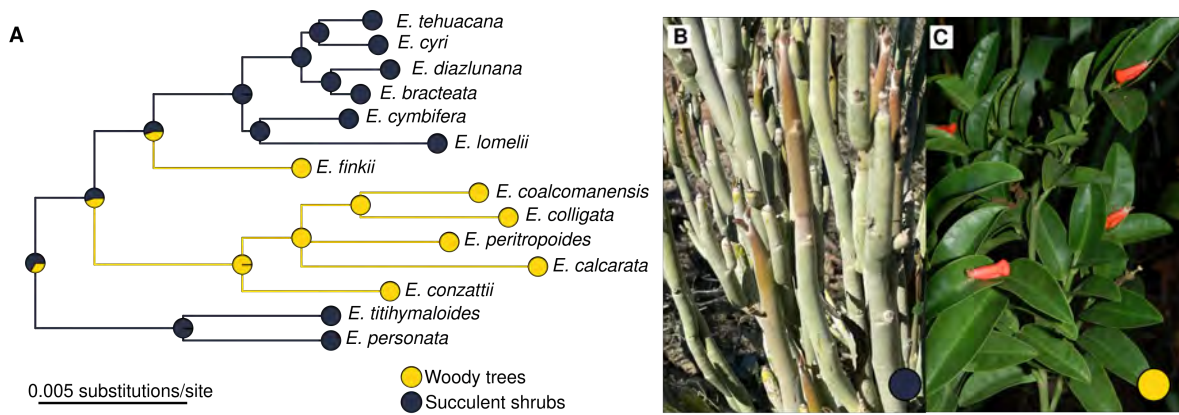


Figure 2: Growth form evolution across the Pedilanthus clade. **A**, Based on the ancestral character state estimation, succulent shrubs was the ancestral growth form of the Pedilanthus clade and woody trees evolved two times. Pie charts represent posterior probabilities of ancestral character states. Phylogeny based on the maximum likelihood tree from (Cacho et al. 2010). Gray color represents succulent shrub growth form while yellow represents woody tree growth form. **B**, Growth form of a succulent plant illustrated by *E. lomelii*. **C**, Growth form of woody trees illustrated by *E. conzattii*.

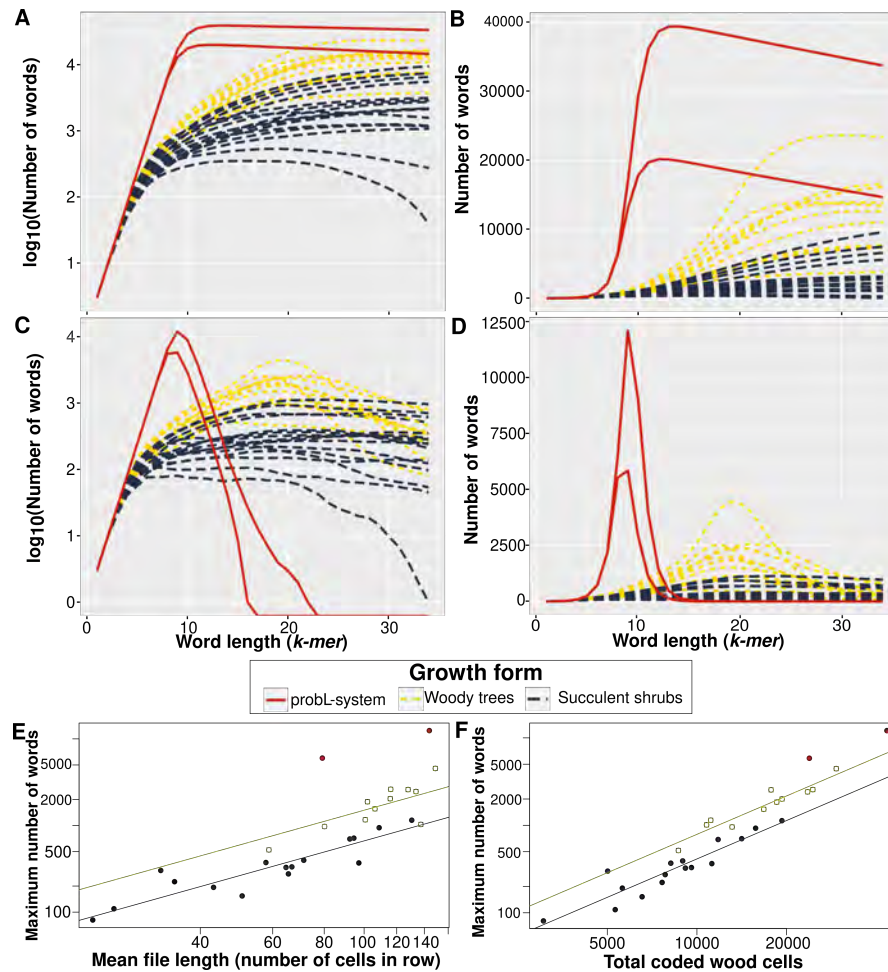


Figure 3: Different patterns of developmental “decision-making” between growth forms from 27 individuals of the 14 extant species of the *Pedilanthus* clade reflected in total and maximum numbers of words at different k – mer lengths. **A-B**, Change in total number of words, from one to 35 k – mer length, logarithmic scale (A) and arithmetic scale (B), showing the highest number of words in L-systems, followed by woody trees, and then succulent species. **C-D**, Change in total numbers of words appearing more than once, from one to 35 k – mer length, logarithmic (C) and arithmetic scale (D), to show that VC initials from woody species produce more words than succulent species with greater degrees of consistency. In **A-D**, each line represent one sampled individual. **E-F**, Linear models showing higher maximum numbers of words produced in the “decision-making” processes of woody trees than in succulent shrubs. Scaling of maximum numbers of words by mean file length (E) and with total number of coded wood cells (F), showing that, controlling for cell file length or total number of coded cells, woody species had higher intercepts, and therefore maximum numbers of words than succulent species. In E and F, blue filled points represent woody species, yellow non-filled points represent succulent species and red points represent L-systems.

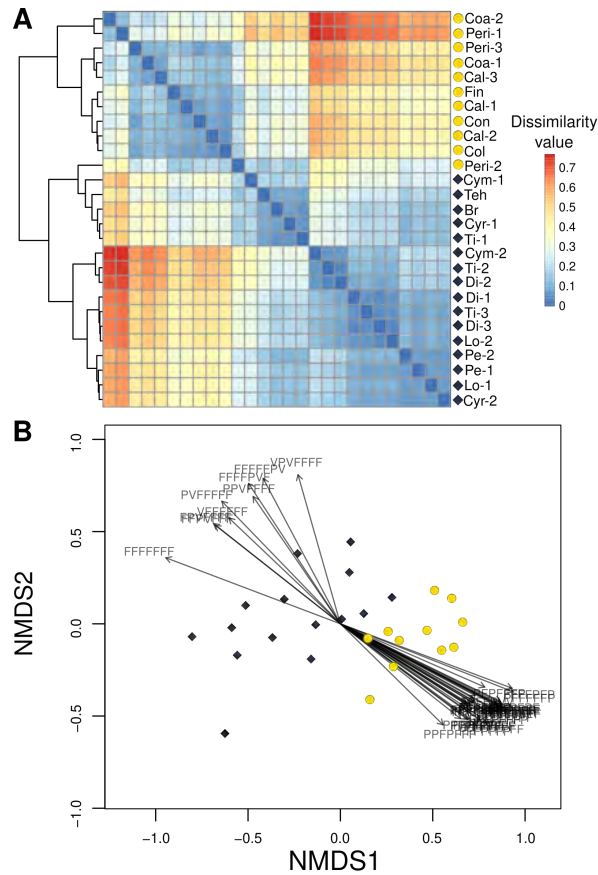


Figure 4: *Pedilanthus* samples tend to resemble other members of the same growth form as compared to the other growth form. **A**, Heatmap showing the pairwise Bray-Curtis dissimilarity values among 27 individuals which were estimated from word counts of k-mer length 7. This length was chosen because it represents the lowest value at which the maximum number of distinct words was observed in our samples, and is thus a useful point of comparison across species. Values closer to zero (blue colors) indicate similarity and values closer to one (red colors) indicate dissimilarity. The same values are shown above and below the diagonal. **B**, Non-metric multidimensional scaling plot (NMDS) based on Bray-Curtis dissimilarity values and the word counts at k-mer length 7, to visualize the similarity among individuals based on word counts. In the plot, individuals are represented by points, with points closer to each other being more similar. Words with a significant influence on the NMDS ordination plot ($p < 0.0001$) are shown. Br = *E. bracteata*, Lo = *E. lomelli*, Ti = *E. tithymaloides*, Cyr = *E. cyri*, Cym = *E. cymbifera*, Teh = *E. tehuacana*, Di = *E. diazlanana*, Pe = *E. personata*, Col = *E. colligata*, Coa = *E. coalcomanensis*, Cal = *E. calcarata*, Fin = *E. finkii*, Con = *E. conzattii*, Peri = *E. peritropoides*. Yellow circles denote woody trees and treelets while blue diamonds denote succulent shrubs.

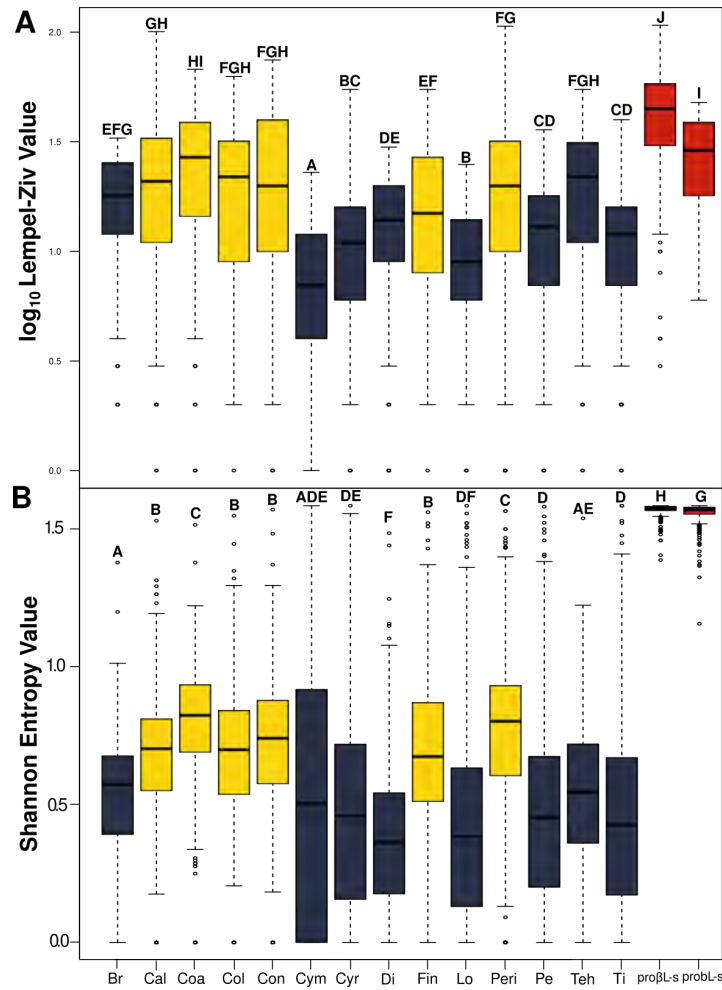


Figure 5: Differences in information content metrics between species and growth forms of the *Pedilanthus* clade suggest more diverse “decision-making” in woody trees than in succulent shrubs. **A**, Boxplots showing the species mean and interquartile ranges of the log-Lempel-Ziv values from cell files, showing that succulent species tended to have lower values than woody trees. Letters denote different groups estimated from Tukey post-hoc tests. **B**, Boxplot showing the species mean and interquartile range of the Shannon entropy values from cell files, illustrating that succulent species had lower values than woody trees. Letters denote different groups estimated from pairwise Wilcoxon rank sum tests. Br = *E. bracteata*, Lo = *E. lomelli*, Ti = *E. tithymaloides*, Cyr = *E. cyri*, Cym = *E. cymbifera*, Teh = *E. tehuacana*, Di = *E. diazlanana*, Pe = *E. personata*, Col = *E. colligata*, Coa = *E. coalcomanensis*, Cal = *E. calcarata*, Fin = *E. finkii*, Con = *E. conzattii*, Peri = *E. peritropoides* Gray = succulent shrubs. Yellow = woody trees. Red = L-systems.

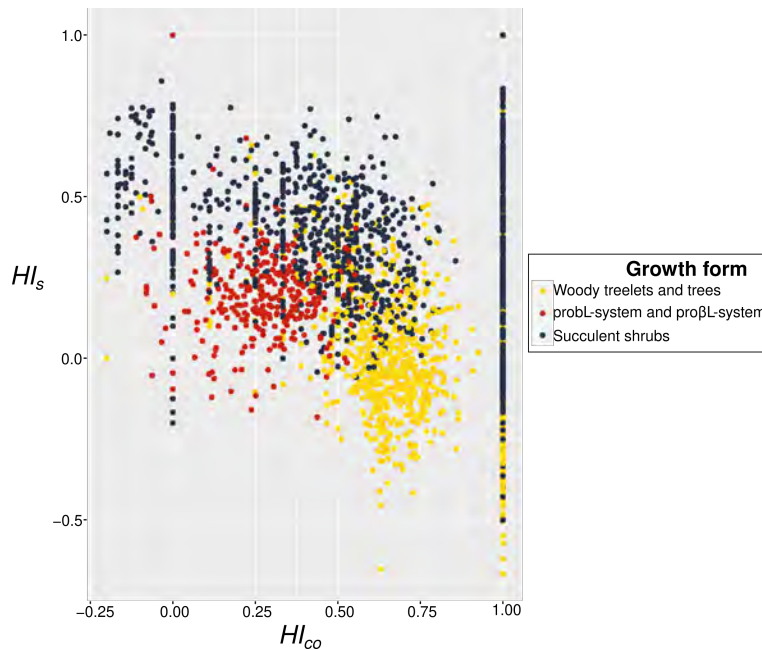


Figure 6: Differences in ontogenetic “decisions” across the *Pedilanthus* clade reflect different wood functionalities. Biplot showing the conduction homogeneity index (HI_{CO}) on the horizontal axis versus the support homogeneity index (HI_S) on the vertical axis. Each point represents the values of HI_{CO} and HI_S from one cell file. Cell files from succulent species had higher values of HI_S for a given value of HI_{CO} , whereas woody species had more files with negative value of HI_S [0,-0.5] for a given positive value in HI_{CO} [0.5,1], reflecting more frequent “decisions” alternating between supporting and non-supporting functions in the woody species as compared to the succulent ones. Woody species had more files with values of HI_{CO} between 0.6 and 0.9 than succulent species, highlighting the sporadic production of vessels. A higher percentage of cell files from succulent species, for a positive value in HI_S had a negative value of HI_{CO} [-0.25, 0], reflecting more frequent “decisions” alternating between conductive and non-conductive functions than are non observed in woody species. In L-systems, cell files had less variation for both indexes (HI_S range= [0, 0.5] and HI_{CO} range=[-0.1, 0.4]). This shows that wood cell production in the *Pedilanthus* clade deviates from stochasticity. For example, L-systems did not produce cell files with values of 1 for HI_{CO} or values lower than -0.25 for HI_S , while species from the *Pedilanthus* clade did.

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Appendix A: Additional methods

Criteria for cell lineage coding.

To reconstruct the ontogenetic decisions made by cambium progenitor cells, we used criteria for two different aspects of cell coding: assigning cells to a particular cell file and identification of cell types. First, we describe the criteria we followed for determining whether a group of cells belongs to the same fusiform or ray initial lineage. Then, we describe some of the anatomical characteristics we used to identify different cell types.

Cell lineage assignment. As mentioned in the introduction, cell lineages can be easily followed because they form radial files of cells that grow from the center of the stem toward the outside. Some factors can potentially lead to incorrect cell lineage assignment, such as intrusive growth of fibers, slight changes in cell position, of a series of cells within a radial cell file, and not identifying when two cell files become one cell file, in other words when one fusiform initial gave rise to two fusiform initials (Figure A1). Fibers are characterized by having intrusive growth. This means that when maturing, the tips of the cells grow in the axial plane, entering into the planes of other cell files, as shown in Figure A1. When assigning cells to a particular cell lineage, anatomical criteria allowed us to identify intrusive growth in fibers not belonging to the coded cell lineage. One anatomical criterion was smaller fiber diameters than most of the fibers of the focal cell lineage. The other criterion was the relative position of the fiber. When fibers were not in a radial position, similar to the majority of the cells in the lineage, the fiber was assumed to be most likely from another cell lineage. To assign cell lineages, we used two additional criteria. For the first criterion, we looked for the convergence of two parallel cell files into a single cell file, which can occur when fusiform initials divide to form two new ones through an anticlinal division (Figure A1). We coded the convergent cell lineage as a new radial cell file. For the second criterion, we looked for ray parenchyma-fusiform derived cell transitions. VC ray initials can only produce ray cells, but VC fusiform initials can produce VC ray initials. Therefore, cell orderings produced by the VC initials can be of the form *RRFFF*, but not *FFFRR*. If we identified a ray to fusiform derived transition, such as in *RRFFF*, we coded the following fusiform derived cells as a new cell file.

Cell type coding. To consistently code cell types, we used the following criteria:

1. *Vessels.* Vessels in our study had slightly wider diameters than fibers or axial parenchyma. In cases where vessel diameter was similar to fibers or parenchyma, we used perforation plate and pit characteristics to identify them. Perforation plates are holes in the end walls of vessel elements, and can be seen as a ring of cell wall slightly wider than the rest of the cell wall. These wider “rims” of cell wall are not observed in fibers or parenchyma cells. If perforation plates were not visible, lateral wall pitting was often used to distinguish vessels from other cell types. Pits in wood cells are chambers made in the lignified cell walls

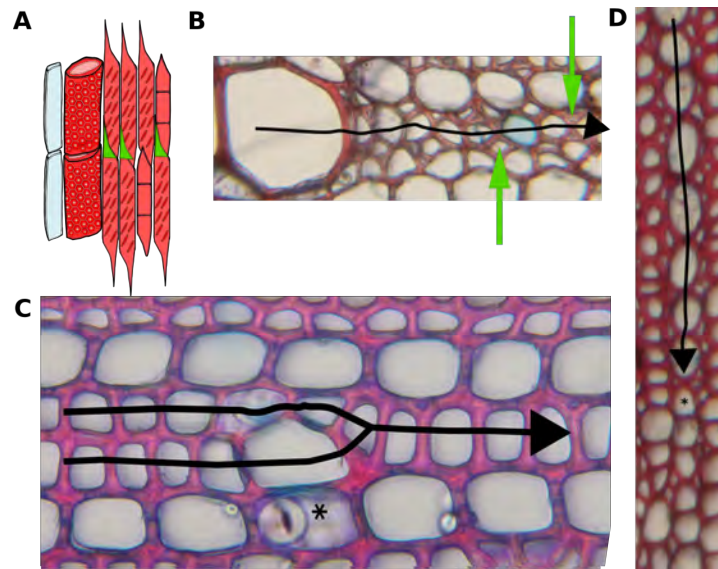


Figure 7: Production of wood cells by cambial progenitor cells, cell lineage assignment. **A**, Occasional interruptions of cell files as a result of fiber intrusive growth. The green tips from fibers represent intrusive growth that can potentially interrupt another cell lineage. **B**, Black arrow showing one cambial cell file and green arrows showing intrusive growth of fibers. **C**, Example of a past anticlinal division, showing two parallel cell files “converging” in one cell file, moving from the outer surface of the stem at left toward the center at right. Asterisk indicates an axial parenchyma ray cell. **D**, Example of fusiform-ray cell transition. Arrow, pointing toward the center of the stem, shows a cell file made up of rays, with the asterisk showing the last differentiated fusiform derived cell. **B** and **C**, *E. coalcomanensis*. **D**, *E. colligata*.

that allow water movement between cells. Vessel pits are characterized by being much more abundant and wider than in fibers and parenchyma cells. The pits can be easily identified in cross-section and were highly diagnostic in distinguishing vessels from fibers and parenchyma.

2. *Fibers*. Fibers usually have thicker cell walls than axial parenchyma. In *Euphorbia* in general and in the *Pedilanthus* clade in particular, most fibers are libriform. Libriform fibers are characterized by having small amounts of pits and they are simple. Simple pits are narrow openings in that lack a chamber. We identified fibers by looking at the thickness of their cell walls, the abundance of pits, and the presence of simple pits. In some cases, libriform fibers in the *Pedilanthus* clade have septa, which are transverse primary walls that divide the cell into compartments. Septa can also be used to identify fibers in cross-section.
3. *Axial parenchyma*. These cells are characterized by having thin cell walls, and in the *Pedi-*

lanthus clade they can be subdivided into strands of cells, each of which is surrounded by a secondary cell wall. The secondary cell walls of these parenchyma cells bear wide pits that lack chambers. Very often, in a cross-section, secondary walls with pittings in axial parenchyma were observed, easily differentiating them from fibers with thin lateral walls.

4. *Ray parenchyma cells.* In the Pedilanthus clade, ray parenchyma cells are uni- or bi-seriate. This means that rays tend to form files one or two-parallel cells wide, going from the periphery of the stem to the center. In a cross-section, rays are more rectangular in shape and longer in the radial direction than in the tangential plane. They have cell walls and pits similar in width and shape to axial parenchyma.

Estimation of ancestral growth form states

To estimate ancestral character states (see TableS3 for species growth form coding) we used the combined data set maximum likelihood tree from (Cacho et al. 2010). We then estimated the equal rate transition model as the best-fit model of character transition rate using the likelihood ratio test and the Akaike information criteria (Table S4) using the fitMk function from the phytools package (Revell, 2012). Based on the MCC tree, we estimated ancestral states using the make.simmap function with 1000 iterations (Table S5). This function uses a stochastic character mapping and a Bayesian inference approach (Bollback, 2006; Huelsenbeck et al., 2000). We used the describe.simmap and count.Simmap functions to summarize the results, and we added the posterior probabilities at nodes of the tree using plot.Simmap function (Revell, 2012).

Supplementary methods

S1. The evolution of ontogenetic “decision-making” in the wood of a clade of tropical plants.

Supplement to Petrone-Mednoza et al., “Evolution of ontogenetic “decision-making” in the woody cells” *Evolution*

S1 Supplementary methods

S1.1 Description of L-systems

As mentioned in the introduction and methods, L-systems can produce a diverse array of strings or words by applying a defined set of rewriting rules. We implemented two probabilistic L-systems with different production rules. In the first, we defined an alphabet, representing the cell types derived from the fusiform initials, with the following elements:

$$\Sigma = FI, F, V, P$$

Where FI = fusiform initial, F = fibers, V = vessels, and P = axial parenchyma. For both stochastic L-systems we used the following axioms or production rules:

$$\begin{aligned} w &: FI \\ P_1 &: FI \xrightarrow{0.33} FIV \\ P_2 &: FI \xrightarrow{0.33} FIF \\ P_3 &: FI \xrightarrow{0.33} FIP \end{aligned}$$

To determine the cell file length distribution of the L-systems with similar length distributions to the *Pedilanthus* clade, we implemented production rules in two different ways. In the first *probL*-system we randomly selected a value n from a range of 10 to 150, which represents the number of times that the production rules are iterated and the cell lineage length. We repeated this process 300 times to create a *probL*-system consisting of 300 hypothetical cell lineages. For the *prob β L*-system we determined the value of n using the β probability distribution with parameter values of $\beta = 2$ and $\alpha = 5$, and scaled the result by a factor of 500, which is the upper range value for cell file length in our sampled individuals. The cumulative distribution function of the β probability distribution for these parameter values is close to 1 at a value of 0.6, meaning that values larger than 0.6 have a probability of less than 10%. By using this distribution to generate values for n , we obtained cell file lengths that closely matched the distribution of lengths observed in our data. We used this method to generate 300 hypothetical cell lineages for the *prob β L*-system.

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Supplementary Figures and Tables

Table S1: Sample information

Species	Sample	Height (m)	Total stem diameter (cm)	Pith diameter (cm)	Latitude	Longitude
<i>E. bracteata</i>	MO845	2.5	2.497		23.202	-106.202
<i>E. calcarata</i>	MO896	2	0.4		19.062	-103.784
	MO892	1.5	2	0.35	19.498	-105.044
	MO939		2.58	0.13	16.705	-93.532
<i>E. coalcomanensis</i>	MO883	3	5		18.711	-103.315
	MO886				18.711	-103.315
<i>E. colligata</i>	MO867	1.7	2.45	0.1	20.294	-104.975
<i>E. conzattii</i>	MO971	2.5	2.7	0.1	15.872	-96.410
<i>E. cymbifera</i>	MO979	0.5	0.6	0.2	18.411	97.431
	EPM15		0.63	0.15	19.31944	-99.192523
	MO973	3	3.07		17.32697	-98.061194
<i>E. cyri</i>	EPM14		1.7	0.9	19.31944	-99.192523
	EPM10		1.5	0.6		
<i>E. diazlanana</i>	EPM11		1.55	0.55		
	EPM12		1.625	0.7		
	MO917	2.5		1.746	17.947	-96.496
<i>E. lomelii</i>	MO853		0.7		27.153	-112.884
	EPM13	0.5	1.2	0.3	24.349	-110.287
<i>E. peritropoides</i>	MO841		3.4	0.25	21.228	-104.998
	MO843		1	0.4	21.642	-105.028
	MO974		2.4		17.132	-97.873
<i>E. personata</i>	EPM7		1.4	0.4		
	EPM9		1.5	0.6		
<i>E. tehuacana</i>	MO981	2	2.1	0.5	18.493	-97.378
<i>E. tithymaloides</i>	EPM5	2	1.25	0.5	16.336	-95.304
	EPM6	2	1.225	0.4	16.336	-95.304
	MO945		2	0.9	16.555	-92.803

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Table S2: Number of cell files coded, total of cell types and total of cells coded per sample.

Species	Sample	Number of cell files	Vessels	Fibers	Axial parenchyma	Ray cells	Total of coded cells
<i>E. bracteata</i>	MO845	145	168	10415	1192	1826	13601
<i>E. calcarata</i>	MO892	93	168	8788	1812	2750	13518
	MO896	123	136	9252	1736	3196	14320
	MO939	281	139	20026	4325	5418	29908
<i>E. coalcomanensis</i>	MO883	224	337	15208	3760	6186	25491
	MO886	190	241	13086	4410	4915	22652
<i>E. colligata</i>	MO867	176	239	13811	2712	4742	21504
<i>E. conzattii</i>	MO971	197	354	19053	4106	5768	29281
<i>E. cymbifera</i>	MO979	161	283	3996	733	1159	6171
	EPM15	249	146	4779	388	1513	6826
<i>E. cyri</i>	MO973	160	302	13817	1618	3447	19184
	EPM14	270	175	6806	655	1925	9561
<i>E. diazlanana</i>	EPM10	139	125	7166	529	2004	9824
	EPM11	135	120	10540	556	2656	13872
	EPM12	141	110	8248	595	2008	10961
<i>E. finkii</i>	MO917	179	261	10823	2033	3144	16261
<i>E. lomelii</i>	MO853	174	59	2685	306	704	3754
	EPM13	183	142	8345	655	2220	11362
<i>E. peritropoides</i>	MO841	219	309	21338	7712	7758	37117
	MO843	172	169	7147	1345	2101	10762
	MO974	206	350	14814	3364	6611	25139
<i>E. personata</i>	EPM7	169	218	8455	909	2892	12474
	EPM9	192	137	7317	714	2882	11050
<i>E. tehuacana</i>	MO981	179	264	17043	1982	4378	23667
<i>E. tithymaloides</i>	EPM5	151	137	6036	368	1639	8180
	EPM6	152	100	5079	436	1283	6898
	MO945	180	271	12418	1444	3256	17389

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Table S3: Number of cell files coded, total of cell types and total of cells coded per sample.

Species	Growth form	Mean cell file length	Standard deviation	Standard error	Confidence level	Group
<i>E. bracteata</i>	succulent	94.8	52.33	5.88	83.3 - 106.3	DE
<i>E. calcarata</i>	tree	138.6	105.67	4.89	129.0 - 148.2	FG
<i>E. coalcomanensis</i>	tree	118.3	77.48	4.69	109.1 - 127.5	EF
<i>E. colligata</i>	tree	132.9	101.89	5.45	122.2 - 143.6	FG
<i>E. konzattii</i>	tree	168.3	137.65	5.26	158.0 - 178.6	H
<i>E. cymbifera</i>	succulent	33.4	17.62	3.54	26.5 - 40.4	A
<i>E. cyri</i>	succulent	71.4	78.27	3.48	64.5 - 78.2	BC
<i>E. diazjunana</i>	succulent	88.9	42.51	3.51	82.0 - 95.7	D
<i>E. finkii</i>	tree	90.8	66.77	5.21	80.6 - 101.0	CD
<i>E. lomelii</i>	succulent	42.2	32.66	3.76	34.8 - 49.5	A
<i>E. peritropoides</i>	tree	131.5	95.45	5.02	121.7 - 141.4	FG
<i>E. personata</i>	succulent	69.8	42.37	3.78	62.4 - 77.2	B
<i>E. tehuacana</i>	succulent	137.5	94.88	5.29	127.2 - 147.9	FG
<i>E. tithymaloides</i>	succulent	69.7	50.98	3.26	63.3 - 76.1	B
<i>proβL-system</i>		144.8	80.82	4	137.0 - 152.7	G

Table S4: Estimation of the best-fitted model of character transition rate using the likelihood ratio test and the Akaike information criteria

model	logL	AIC	Δ AIC
Equal rate	-9.75	21.5	0.00
All rates different	-9.75	23.50	2.00
succ → woody	-10.30	22.61	1.11
woody → succ	-10.81	23.63	2.13

Table S5: Estimation of number of growth form changes along the *Pedilanthus* clade obtained from character mapping with 1000 iterations on MCC tree. Trees have 2.025 changes between states on average

Changes between character states	Number of changes in 1000 iterations
Succulent → woody	1.243
Woody → succulent	0.782

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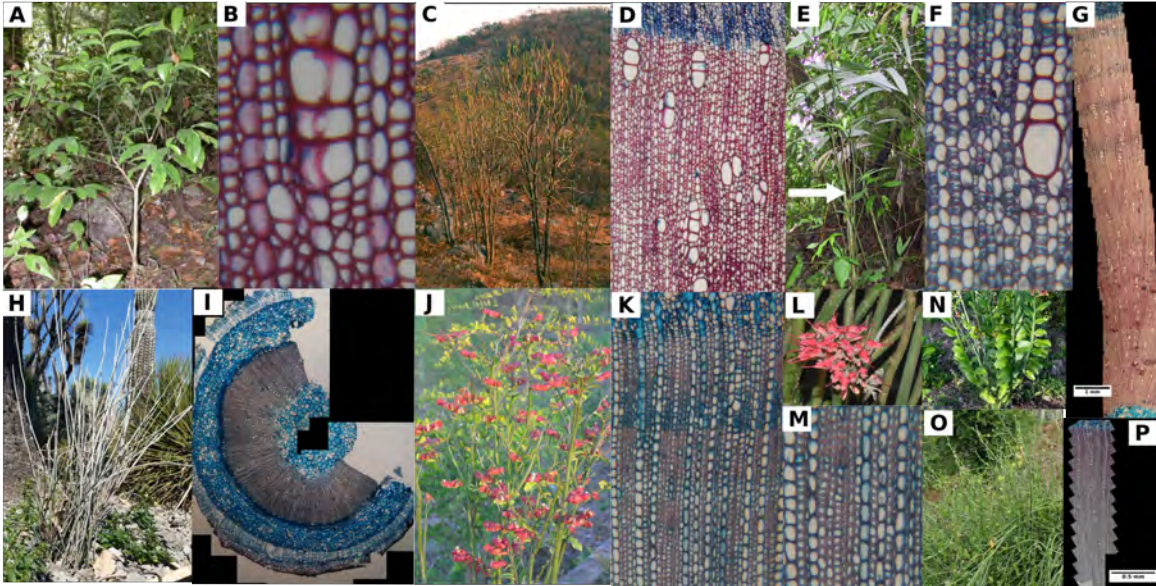


Figure S1: Differences in xylem anatomy and growth form across the Pedilanthus. A-G, Woody trees. H-P, Succulent shrubs. A, *E. conzattii* growing on tropical rain forest. B, Series of “decisions” of cambial initials leading to the formation of consecutive vessels in *E. conzattii* seen in a transversal view. C, *E. colacomansensis* growing on a tropical dry forest. D, Series of “decisions” of cambial initials leading to the formation of series with cells transitioning between vessels, axial parenchyma and fibers in *E. coalcomansensis* seen in a transversal view. E *E. finkii* growing on a tropical rainforest. F, Series of “decisions” of cambial initials leading to the formation of parenchyma-fiber series and series of vessels in the woody *E. calcarata*. Cells with a blue stained cell wall are gelatinous fibers. G Transverse view of the xylem in the woody *E. colligata*. H Habit of growth in the succulent shrub *E. cymbifera*. I Transverse view of the stem in *E. cymbifera*. Note the proportion of xylem with respect to the pith and the cortex. J Growth form of the succulent shrub *E. bracteata*. L Inflorescence of the succulent shrub *E. personata*. K, M Series of “decisions” of cambial initials of *E. personata* predominantly producing series of fibers. Growth form of the succulent shrub *E. diazlanana* shortly after shoot emergence with leaves N, and stems without leaves O. P Total xylem produced by *E. diazlanana*.

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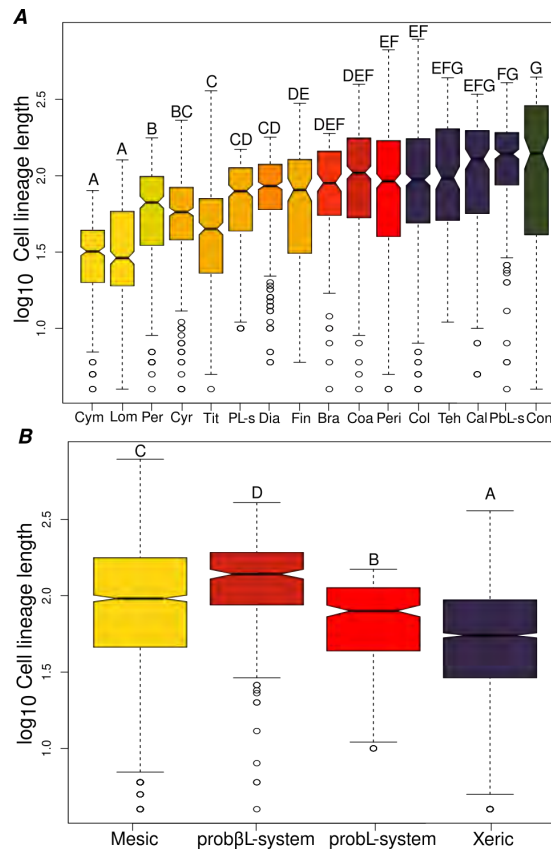


Figure S2: Differences in information content metrics between growth forms of the *Pedilanthus* clade suggest more diverse “decision-making” in VC cells in woody trees than in succulent shrubs. *A*, Boxplots showing the growth form mean and interquartile range of the log-Lempel-Ziv values from cell files, showing that succulent species have lower values than woody treelets. Letters denote different groups estimated from Tukey post-hoc tests. *B*, Boxplot showing the species mean and interquartile range of the Shannon entropy values from cell files, illustrating that succulent species have lower values than woody treelets. Letters denote different groups estimated from pairwise Wilcoxon rank sum tests. Higher values of the Lempel-Ziv and Shannon entropy denote more complicated ordering of cells, therefore a wider array of VC “decisionmaking” during ontogeny.

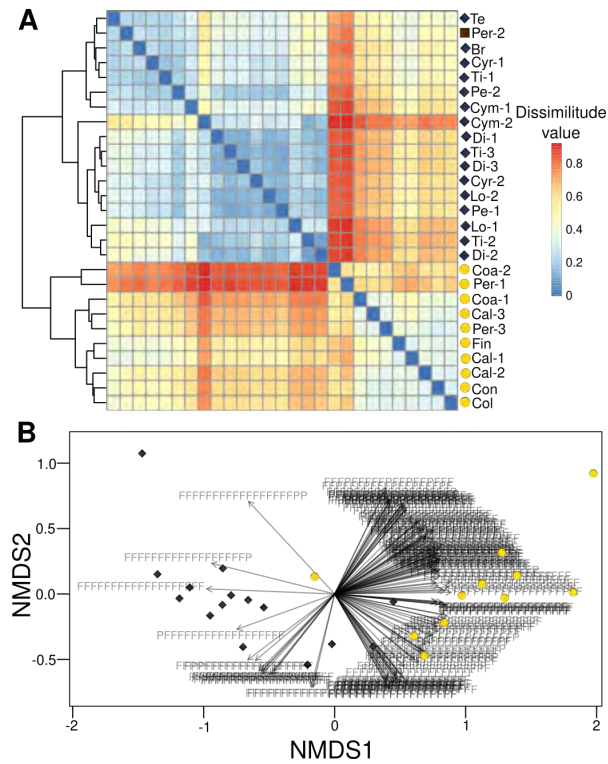
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Figure S3: *Pedilanthus* samples tend to resemble other members of the same growth form as compared to the other growth form. *A*, Heatmap showing the pairwise Bray-Curtis dissimilarity values among individuals estimated from word counts of k -mer length 19, which is a relevant word length because it is the mean value at which the maximum number of words was observed in our samples. Values closer to zero (blue colors) indicate similarity and values closer to one (red colors) indicate dissimilarity. The same values are shown above and below the diagonal. *B*, Non-metric multidimensional scaling plot (NMDS) based on Bray-Curtis dissimilarity values and the word count k -mer length 19 matrix, to visualize the similarity among individuals based on word counts. Points on the plot close to each other indicate more similarity. Words with a significant influence on the NMDS ordination plot ($p < 0.0001$) are shown. Species abbreviations as for Figure 3. Yellow circles denote woody trees while bluish diamonds denote succulent shrubs.

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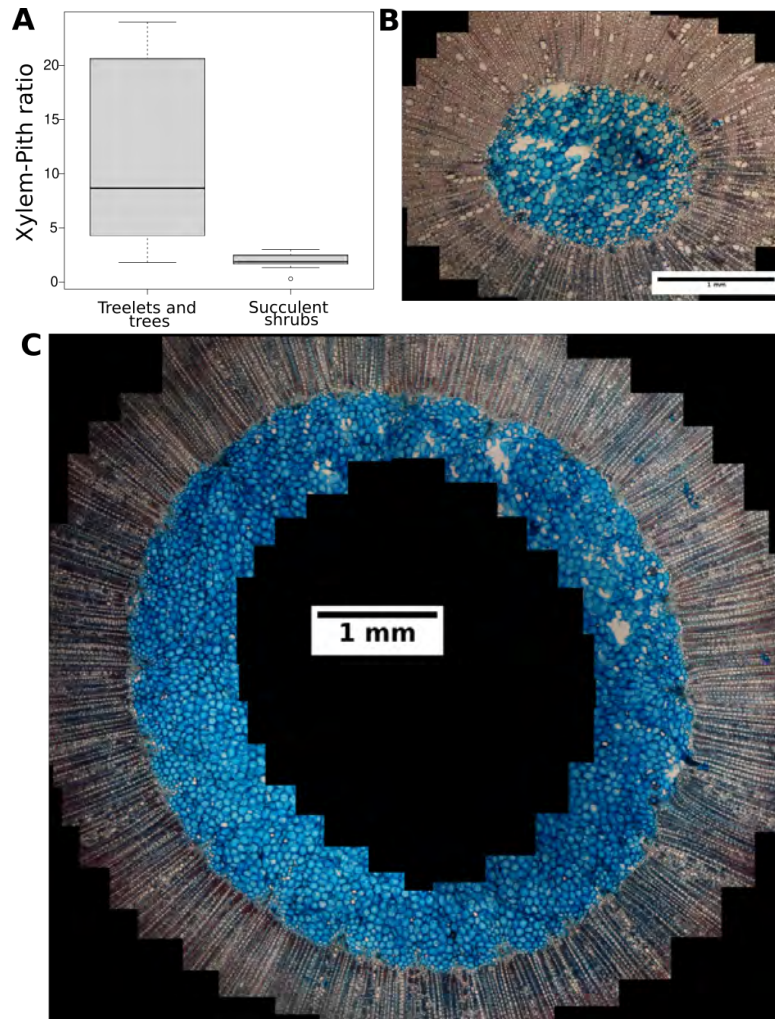


Figure S4: Relationship between pith diameter size (the central tissue in the stem), growth form, and a plausible trade-off in the amount of water and nutrient tissue underlying differences in VC “decisions” across the *Pedilanthus* clade. *A*, Boxplot showing the xylem-pith ratio. Treelets and trees have more xylem in proportion to narrow piths, whereas succulent species have wider piths in proportion to xylem. *B*, Pith diameter of the treelet *E. colligata*. *C*, Pith diameter of the succulent shrub *E. personata*. Scale bar in *B* and *C* = 1mm.

Capítulo 3. The evolutionary accessibility of vascular variants in non self-supporting plants

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The evolutionary accessibility of vascular variants in non self-supporting plants

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Prepared a L^AT_EX template

Abstract

The independent evolution of similar traits, or convergence, appears to occur more frequently in closely related species than in distantly related ones. In closely related species, shared developmental mechanisms or structures, referred to as “trait enablers,” facilitate the subsequent evolution of convergent traits. Identifying these “trait enablers” that enable the “evolutionary accessibility” of the derived traits is a fundamental challenge in evolutionary biology. In the case of plants, the frequent occurrence of the transition from a self-supporting habit to a non self-supporting habit, along with its close association with the evolution of vascular variants, provides an excellent study opportunity to identify the trait enablers that facilitate the evolution of both traits. In this study, I demonstrate a non-random association between these two traits by analyzing data from the InsideWood database. I argue that across Angiosperms, it is more common for the non self-supporting growth form to evolve first, and this form subsequently promotes the evolution of vascular variants. I provide three case studies that support this hypothesis and highlight the potential trait enablers involved in the development of these vascular variants. Finally, I conclude by discussing how a non self-supporting condition can stimulate the development of vascular variants.

Introduction

One remarkable evolutionary pattern is the tendency for closely related species to independently evolve similar traits more frequently than distantly related species. The processes leading to the independent evolution of similar traits is known as replicated evolution (James et al., 2023). Replicated evolution encompasses the evolution of similar traits observed in distantly related species with different organismal structures and ancestral states known as convergent evolution, as well as the evolution of similar traits in closely related species with similar organismal structures but different immediate ancestral states known as parallelism (Arendt & Reznick, 2008; James et al., 2023; McGhee, 2017). In closely related species, multiple instances of trait gains or losses indicates the occurrence of replicated evolution. For example, in the king Protea (*Protea cynaroides*) flowering plant family (Proteaceae), zygomorphic flowers have independently evolved approximately 10-18 times from ancestral actinomorphic flowers, and the reverse transition has occurred four times (Citerne et al., 2017). While similar traits can evolve through different mechanisms in closely related species (Arendt & Reznick, 2008), the higher frequency of replicated evolution in closely related species compared to distantly related species suggests the existence of shared developmental mechanisms or structures that facilitate the subsequent evolution of the replicated trait (Donoghue, 2005). Therefore, it is crucial to unravel the specific sequence of developmental changes, referred to as the evolutionary trajectory, and identify the developmental stages that lead to the higher frequency of replicated trait evolution (Edwards, 2019).

In a lineage with similar evolutionary trajectories, the presence of a developmental mechanism that facilitates the further developmental steps, referred here as “trait enablers,” biases the production of the replicated trait. Therefore, the trait enabler facilitates the “evolutionary accessibility” of the enabled trait, increasing the probability of independent evolution (Edwards, 2019). Phylogenetic groups possessing the enabler trait tend to exhibit higher transition rates of the enabled trait. For example, plant lineages with a high ratio of bundle sheath cells to mesophyll cells are more likely to show increased transition rates toward C4 photosynthesis compared to those with a low ratio (Edwards, 2019; Heyduk et al., 2019; Sage et al., 2014; Williams et al., 2013). The trait enabler concept is different from the exaptation concept which emphasizes the cooption of a trait for a new function that was previously selected for a different function in the past (Gould & Vrba, 1982). It is also different from notions of eco-evolutionary precursors that emphasize past climate and ecological conditions that modify transition rates of trait evolution (Boyle & Conway, 2007; Cacho & Strauss, 2014). I delimit the concept of “trait enabler” to developmental processes that tend to bias the development and evolution of the enabled trait.

The developmental mechanisms that facilitate the subsequent evolution of replicated traits can be difficult to identify because of limited variation in the evolutionary trajectory of closely related species. Closely related species are expected to have more similar ontogenies and in particular developmental trajectories (Furness et al., 2015; Gould, 1985). One initial step to identify enabler and enabled traits, at the phylogenetic level, is to reconstruct ancestral character states on the expectation of first observing the evolution of the enabler trait followed by the evolution of the enabled trait. Identifying the trait enabler becomes challenging when there is limited or no variation in the trait enabler, which is highly probable in lineages that have a higher likelihood of sharing evolutionary trajectories.

To overcome the problem of limited variation in the trait enabler when comparing species within a particular lineage, population-level studies and developmental models can guide the identification of trait enablers that affect the evolutionary accessibility of replicated evolution. If one species exhibits variation in the enabler trait, this variation should be observed in individuals varying in the expression of the enabled trait. A non-random association between the plausible enabler trait and the enabled trait should be expected. Furthermore, selection experiments on variants possessing the enabler trait that more readily induce the enabled trait should result in a higher frequency of the enabled trait in the population. Additionally, developmental models illustrating the connection between evolutionary trajectories serve as additional tools to evaluate the role of hypothetical enabler traits as facilitators of the evolution of replicated traits. Thus, integrating population-level studies and developmental models into the study of evolutionary accessibilities can be a valuable approach for understanding patterns of replicated evolution, such as the evolution of non-self-supporting habit in plants and the coevolution of vascular variants.

The multiple cases of replicated evolution of a non self-supporting habit from a self-supporting

habit and its close association with the evolution of vascular variants provide a suitable study case to unravel their evolutionary trajectories across vascular plants (Angyalossy et al., 2012, 2014; Pace et al., 2009). Vascular variants are forms of vascular stem organizations that exhibit different vascular architectures compared to plants with regular secondary growth (Cunha Neto, 2023). Vascular variants can develop from procambium variations, from cambial variants or from neo-formed ectopic cambia (Carlquist, 2001; Cunha Neto, 2023). Recent advances integrating developmental and phylogenetic frameworks have shed light on the developmental trajectories underlying the evolution of vascular variants and plausible links to non self-supporting habit (Onyenedum & Pace, 2021). However, as far as I know, there is no current hypothesis regarding how one trait may facilitate the evolution of the other or the order in which they tend to evolve within a lineage. Carlquist (2007) suggested that variant secondary growth changes the flexibility of the stems, something that was tested and proven to be true in subsequent biomechanical studies (Isnard & Silk, 2009; Rowe et al., 2004; Rowe & Speck, 2005). Carlquist did not explicitly describe the order for the evolution of both traits but he suggested that changes at the supporting to non self-supporting habit were a consequence of the evolution of vascular variants.

Here, I explore the relationship and plausible evolutionary trajectories between the co-occurrence of non self-supporting habit and vascular variants. First, I demonstrate the preferential association between both traits using data from the inside wood database (Wheeler, 2011). Then, taking advantage of the recent surge in developmental and phylogenetic studies focusing on plant groups exhibiting non self-supporting habit and vascular variants, I provide a brief summary of three plant lineages in which the non self-supporting habit is the ancestral condition, while the cambial variant trait is considered the derived one. In each case, I propose mechanisms through which a non self-supporting habit may contribute to variations in primary or early stages of secondary growth traits, ultimately facilitating the development of vascular variants. I then present a hypothetical scenario involving a population with variation in self and non-self-supporting habit. In this scenario, I discuss the plausible connection between primary growth traits, vascular traits, and the establishment of cambial variants. I conclude by emphasizing the relevance of testing this hypothesis at the population level and identifying species that exhibit both self and non self-supporting habits. Field surveys, genetic analyses, and experimental manipulations could be employed to gather relevant data and evaluate the correlation between non-self-support and the expression of cambial variants. By investigating the potential link between the initial semi-non self-supporting habit and the evolution of cambial variants, we can deepen our understanding of the factors influencing the diversification of plant habit.

Methods

Database construction

To explore the association between vascular variant and growth form, I extracted data from Inside Wood (Wheeler, 2011; Wheeler et al., 2020). To extract all the available data from Inside Wood, I used three different searching criteria. The first criteria was to extract all tree growth forms, the second was to extract all shrub growth forms, and the third one was to extract all vine growth forms. Inside Wood returns a database with columns corresponding to the IAWA Hardwood list of features and if the trait of interest is present it returns a logical value (present, absent, or unknown). A data frame with the variables of our interest was created in R. For vascular variants, we coded presence, absence, or variable, and for the growth form we coded self-supporting, non self-supporting, or variable.

Nomenclature adopted

InsideWood is based on the IAWA Hardwood List from 1989. Given the objective of that list was exclusively plant identification, two categories of cambial variants were then delimited: concentric included phloem and diffuse included phloem. These are both misnomers and have no connection to either their anatomical structure or ontogeny. Subsequent references have dealt with more biologically real entities (Angyalossy et al., 2012, 2014; Carlquist, 1992, 2001) and a better classification scheme was subsequently listed (Angyalossy et al., 2016). However, because Inside Wood still relies on the 1989 categories, I here mention them, indicating between parentheses the actual cambial variant for each taxon mentioned.

Statistical analysis

To evaluate the possible association between the presence of vascular variants and non self-supporting growth form, we tested for an association between both variables by constructing contingency tables. Contingency tables have counts or frequencies resulting from the combination of two or more variables. I constructed a 2x3 contingency table between species with presence or absence of vascular variant on one hand, versus self supporting, non self-supporting, or variable growth form on the other. I used χ^2 tests to test for an association between vascular variants and growth form. I calculated the standardized residuals from the χ^2 tests to quantify the degree of relationship between categorical associations. I performed statistical analyses on R (R Core Team, 2023).

Case studies

To support the hypothesis of a non self-supporting growth form and modifications in stem anatomy and structure as ancestral conditions enabling the evolution of cambial variants we present three clade cases in accordance with our hypothesis. The first case is from the *Paullinia*

genus from the Sapindaceae family, the second case is from the Malpigiaceae family and the third one is from the *Byttneria* genus from the Malvaceae family.

Results

Trait preferential association between non-self supporting habit and the presence of cambial variants.

The data survey of the relationship between non-self supporting and the presence of cambial variants from InsideWood, yielded a total of 7,203 observations, representing 256 families. Based on my searching criteria, Leguminosae, Rubiaceae, and Malvaceae were the families with the highest number of species in our survey from InsideWood. Similarly, Melastomataceae, Nyctaginaceae, Apocynaceae, and Bignoniaceae had the highest number of species with vascular variants (Figure 1A), while the Bignoniaceae, Apocynaceae, Vitaceae, and Menispermaceae were the families with more non self-supporting species in our survey from the InsideWood (Figure 1B). Following the IAWA (1989) categories, the family with more “concentric included phloem” (likely successive cambia) was Chenopodiaceae (Figure 2A), while “diffuse included phloem” was more common in the Melastomataceae and Nyctaginaceae families (probably here a mixture of interxylary phloem and successive cambia)(Figure 2B). Apocynaceae and Sapindaceae were the families with more species having other types of vascular variants across our database sampling (Figure 2C). Polymorphic species that exhibited variation in growth form and possessed cambial variants included *Leptadenia arborea*, *Ruehssia cundurang* (Apocynaceae), *Grewia caffra* (Malvaceae), *Abuta grandifolia* (Menispermaceae), *Neea tristis*, *Pisonia macranthocarpa* (Nyctaginaceae), and *Securidaca virgata* (Polygalaceae).

The non-random association between non self-supporting plants and the presence of cambial variants is shown in table 1. Out of the total plants registered on InsideWood, i.e., 7,203 observations, 6,838 (97%) corresponded to self-supporting plants, while 203 (2.8%) corresponded to non self-supporting plants, and 65 (0.9%) were polymorphic species, being either self and non self-supporting. From these, 202 (2.8%) of the observed species had cambial variants.

Based on the contingency table, the expected value for non self-supporting plants with the presence of cambial variants was 5.7, yet we observed 71 instances, highlighting a significant non-random association (Table 1). Similarly, the expected value for species capable of growing as both self and non self-supporting was 1.8, but we observed 7 instances (Table 1, $\chi^2 = 815.63$, $df = 2$, $P < 0.0001$) (Figure 2). Most of the species having other types of vascular variants (78.3%) were non self-supporting, showing a particular association of these different types of vascular variants with the lianescent habit. I will now present specific clades that have non self-supporting growth form as ancestral character state. I will emphasize the trait enablers promoting the evolution of cambial variants.

Table 1: Contingency table showing the preferential association between the non self-supporting growth form habit and the occurrence of cambial variants based on data from InsideWood.

		Cambial variant		Total
		absent	present	
Growth form	Non-self supporting	132 (197.3) -28.2	71 (5.7) 28.2	203
	Self supporting	6838 (6767.5) 26.6	124 (194.5) -26.6	6962
	Self and non-self supporting	58 (63.2) -3.9	7 (1.8) 3.9	65
	Total	7028	202	7230

Note the difference between observed values and expected values in parenthesis. Standardized values in bold greater than two show the preferential association between the non self-supporting growth form and the presence of cambial variants.

Table 2: Frequency of vascular variant types based on the IAWA (1989) classification and InsideWood data and growth form type.

Cambial variant type	Self supporting	Non-self supporting	Variable
Included phloem concentric	42	23	2
Included phloem diffuse	85	10	3
Other cambial variant	10	47	3

The Paullinia case (probably Paullinia + Urvillea, Sapindaceae)

One crucial factor determining the developmental accessibility to cambial variants relies on the primary to secondary growth transition in plants. The transition involves the production of secondary xylem and phloem by fascicular cambium derived from procambium from within vascular bundles, and the differentiation of interfascicular cambium from adjacent parenchyma and subsequent formation of a continuous vascular cambium ring. Coordination between fascicular and interfascicular cambium, in terms of production rate of secondary phloem and xylem, result in the formation of the vascular cambium. Therefore, changing the position, distance and signaling between fascicular and interfascicular cambium during the transition can result in different anatomies, as exemplified in species of the genus *Paullinia*.

Primary lobed stems have been suggested as trait enablers for the development of cambial variants in non self-supporting plants of the genus *Paullinia* (Sapindaceae) (Chery et al., 2020). This clade of tropical lianas has different types of stem anatomies, being perhaps the richest in different, and many unique, vascular variants (Chery et al., 2020; Pace et al., 2022; Schenck,

1892). Some species have the typical, regular stem growth, while other species have different types of vascular variants (Pace et al., 2022). Some species of the clade have primary lobed stems and after performing character state reconstruction, primary lobed stem was supported as the ancestral condition of the clade (Chery et al., 2020). Additionally, vascular bundles tend to be more abundant in ridges than in valleys (Chery et al., 2020). During the transition to secondary growth, the interfascicular cambium of some species with primary lobed stems produce more secondary phloem than secondary xylem in relation to the activity of the fascicular cambium, filling the valleys and forming phloem wedges (Figure 4Aii). If the fascicular cambium produces more xylem than phloem with respect to the interfascicular cambium, secondary lobed stems are produced (Figure 4Aiv). Another vascular variant forms when the vascular bundles associated with lobed primary stems are not arranged in a circumferential pattern. If some vascular bundles are spatially isolated from the rest, they will form an independent vascular cylinder, leading to the formation of compound stems, which is a vascular variant unique of this family, and present in both *Paullinia* and *Serjania* (Figure 4Aiii). All *Paullinia* species are non self-supporting plants, so that the appearance of a primary lobed stem could be facilitated by the non self-supporting habit. The hypothesis of primary lobed stem facilitating the evolution of vascular variants is also supported in the genus *Urvillea* (Paullinieae, Sapindaceae) in which most species start growth with a primary lobed stems leading to the formation of vascular variants and in which reversal to self-supporting growth is associated with the loss of vascular variants (Cunha Neto et al., 2023).

The Byttneria case

Further support for the primary lobed stems as trait enablers for the development of cambial variants in non self-supporting plants has been observed in one of the only non self-supporting plant genus from the Malvaceae family, *Byttneria* (Luna-Márquez et al., 2021). *Byttneria s.l.* (Malvaceae) is a paraphyletic group (*Ayenia*, *Byttneria*, *Rayleya* and *Megatritheca*) (Bayer & Kubitzki, 2003) in which the non self-supporting habit has evolved twice and primary lobed stems has evolved at least three times (Luna-Márquez et al., 2021). Non self-supporting species with primary lobed stems have vascular variants in which the fascicular cambium cells divide more frequently than interfascicular areas giving rise to the lobes in the mature stems (Luna-Márquez et al., 2021). In contrast, self-supporting species such as *Byttneria morii*, *B. fruticosa*, or species from the *Ayenia* genus have primary lobed stems but they become cylindrical in the adult transition (Luna-Márquez et al., 2021). This suggests that if primary lobed stem is ancestral and a non self-supporting growth form is favored, adult lobed stems will also be favored and developmentally accessible, but if a transition towards self-supporting happens, the activity of the fascicular and interfascicular cambium will be coordinated towards a cylindrical adult stem. The *Byttneria* lobed stem evolution also points to a tight interaction between mechanical demands and the activity of the fascicular and interfascicular cambium.

Malpighiaceae case

In Malpighiaceae, it was inferred that the self-supporting growth form and regular cambium were ancestral to the family, and non self-supporting and cambial variants were derived, with at least two independent evolutions of the lianescent habit. Non self-supporting habit without vascular variants has evolved in some species (e.g., *Cordobia*, *Psychopterys*, *Tetrapterys*), but most of the non self-supporting plants evolved some type of vascular variants, the most common of which, the formation of phloem wedges with a continuous cambium (Quintanar-Castillo & Pace, 2022), are positioned in directly relation to the decussate leaves (Cabanillas et al., 2017; Quintanar-Castillo & Pace, 2022). The reversibility of both the lianescent habit and the presence of cambial variants also points to a tight selective process towards the favored mechanical stem configuration given a non-self or self-supporting habit. In fact, some ancestrally lianescent genera of Malpighiaceae has reversed to the self-supporting habit when occupying drier areas (e.g., the Brazilian cerrado), which is the case of several *Peixotoa* and *Stigmaphyllon paralias*, and in these shrubs the vascular variants disappeared altogether. Within Malpighiaceae two elements act as trait enablers, the common formation of non-lignified secondary xylem parenchyma and the formation of phloem wedges. The non-lignified axial and ray parenchyma, for instance, in many genera originate new cambia within the secondary xylem, such as in *Stigmaphyllon*, forming interxylary cambia, which produce new tissues within the wood cylinder (Pace et al., 2018). Other Malpighs have non-lignified parenchyma in combination to the phloem wedges; in these cases, the multiplication of the non-lignified parenchyma cells next to the phloem wedges promote the complete disruption of the lignified portions of the secondary xylem forming Malpighiaceae's most conspicuous vascular variant, the fissured stems (Cabanillas et al., 2017), which is common to the entire clade Christianelloid, the genus *Diplopterys*, and some *Banisteriopsis* (e.g., *Banisteriopsis caapi*, commonly known as the psychoactive ritualistic ayahuasca; (Nagamine-Pinheiro et al., 2021; de Oliveira et al., 2023)).

Self to non-self supporting transition leads to different mechanical demands favoring vascular variant development

Self-supporting and non self-supporting plants are subject to distinct mechanical demands and thus exhibit different mechanical properties. Specifically, when comparing the increase in stem diameter, non self-supporting growth forms tend to show a steeper increase in stem length compared to self-supporting growth forms (Olson & Rosell, 2013). This increase in stem length in non self-supporting forms is characterized by tissues that do not become stiffer but instead become more flexible (Castorena et al., 2015; Rowe & Speck, 2005; Speck & Burgert, 2011). Cylindrical concentric wood rings are generally stiffer than lobed or fissured stems (Speck & Burgert, 2011). Consequently, if natural selection favors non self-supporting growth forms with more flexible stems, it is likely to also favor the development of vascular variants. For example, in the genus *Condylocarpon* (Apocynaceae), the initial developmental stages are characterized by growth of

self-supporting stems with concentric wood (Hoffmann et al., 2003; Rowe & Speck, 1996a). However, as these stems become attached to other trees, secondary growth changes, leading to the development of lobed stems (Hoffmann et al., 2003; Rowe & Speck, 1996b). The same has been reported to *Heteropterys* subsect. *Aptychia* (Amorim 2003; Pace 2015). It will be crucial to gain a more precise understanding of the anatomical changes associated with the transition from self-supporting to non self-supporting growth, including during primary growth.

Additional support for the self-supporting mechanical release function can also be observed in roots. In several species, roots are distinguished by having more storage tissues and a higher abundance of non-structural carbohydrates compared to stems (Baer et al., 2021; Pratt & Jacobsen, 2017; Würth et al., 2005). When roots and stems of the same species are compared, roots are found to be more flexible and have lower resistance to bending and twisting (Plavcová et al., 2019). Furthermore, there have been reports suggesting that the presence of vascular variants is very frequent in roots (Avetta, 1887), and some studies have even proposed a Turing-like developmental mechanism for tuberous root having vascular variants (Hearn et al., 2013). Therefore, the evidence of the development of vascular variants in situations where the mechanical demands for self-supporting growth are not favored by natural selection supports the hypothesis.

Given variable environmental conditions that can favor both non self-supporting and self-supporting growth forms in a population, it is likely that natural selection acts on individuals to optimize their growth strategies in a context-dependent manner. This suggests that developmental plasticity in both self-supporting and non self-supporting forms may be accompanied by the presence of regular and vascular variants. For instance, species such as *Machaerium floribundum* have the capacity to grow as shrubs, trees, and lianas. One study reported the presence of a vascular variant (interxylary phloem) in this species, but only non self-supporting growth forms were sampled (Pattsi, 2016). Sampling more species with variable growth form will be very valuable. In the genus *Thunbergia* of Acanthaceae, all lianas have interxylary phloem produced exclusively by the interfascicular cambium, while the cambial variant is lacking in the self-supporting species *Thunbergia erecta* (Carlquist & Zona, 1988; Pace, 2015).

In summary, our data strongly suggests a close association between non self-supporting growth and the development of vascular variants in plants. Conducting detailed anatomy studies with a clear phylogenetic framework across all vascular plants will be crucial to determine if there is an evolutionary sequence of both traits.

Figures

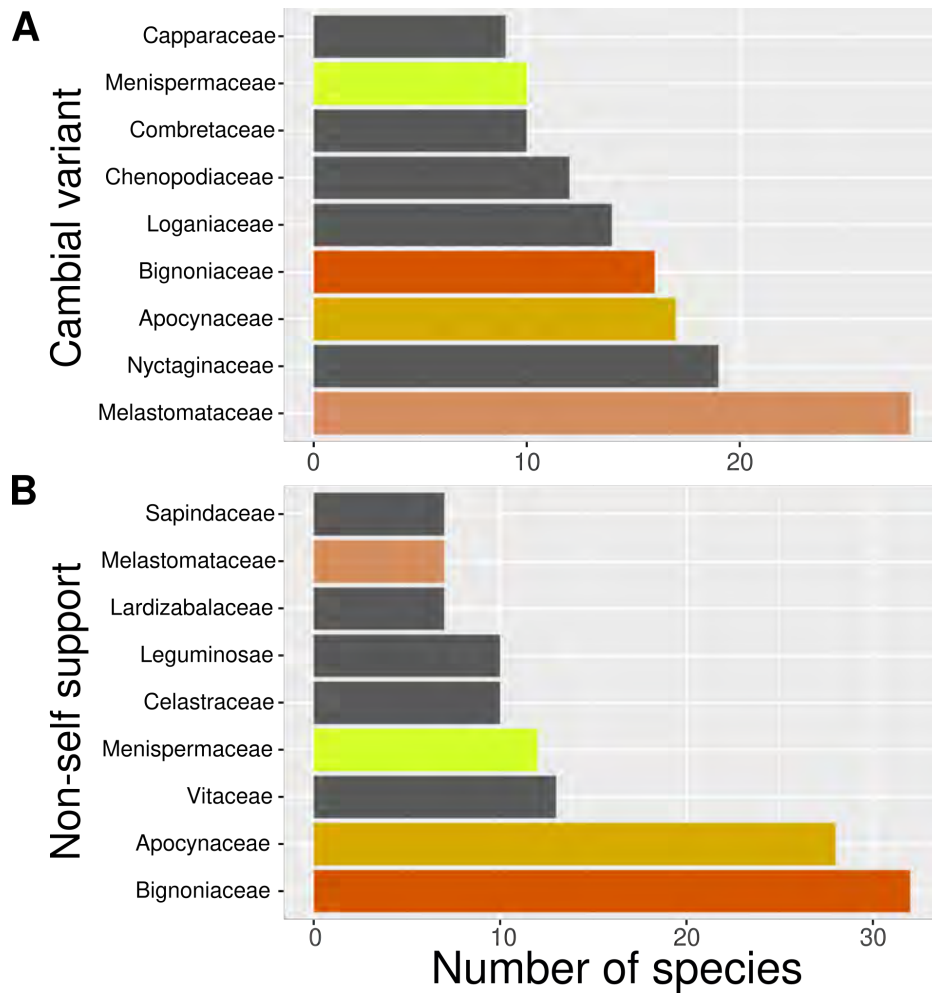


Figure 1: Families with a greater number of species based on our InsideWood database survey exhibiting (A) cambial variants and (B) a higher number of species with a non self-supporting growth form. Bars with colors other than gray denote the families (Bignoniaceae, Apocynaceae, Melastomataceae, and Menispermaceae) that have more species with cambial variants and more non self-supporting species.

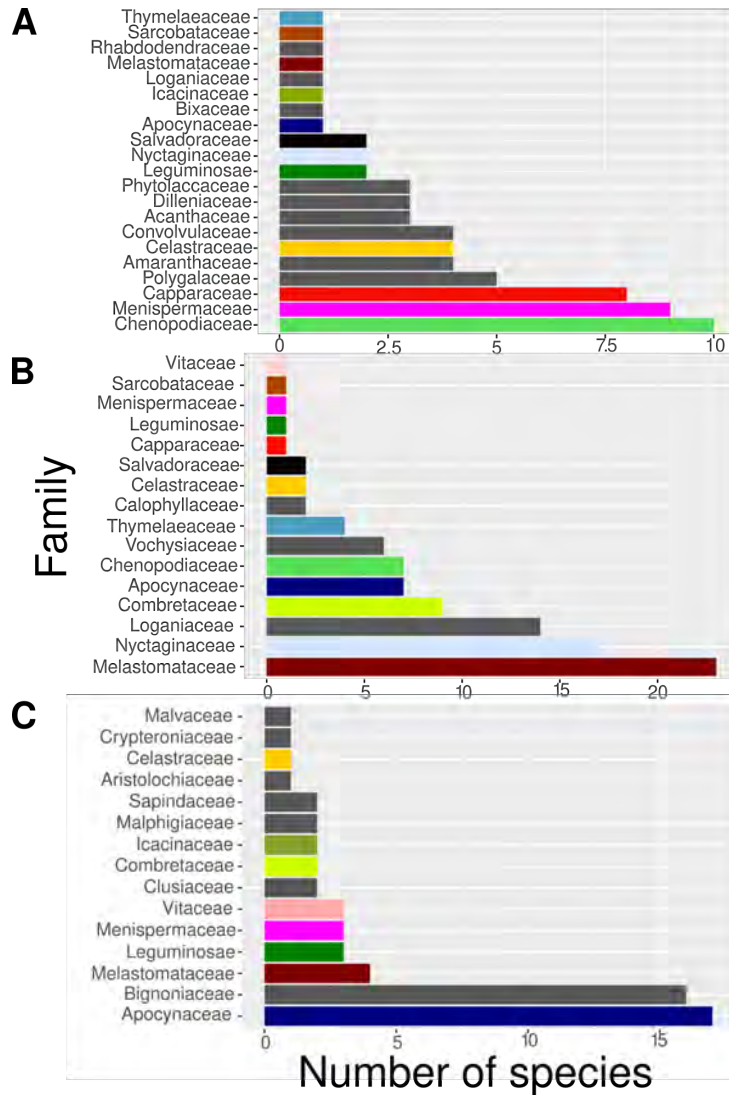


Figure 2: Families with the greatest number of species with different types of vascular variants based on IAWA classification (1989). Families with the greatest number of (A) concentric included phloem, (B) diffuse included phloem, and (C) other types of vascular variants. Families with bar with color different than gray are represented in more than one category.

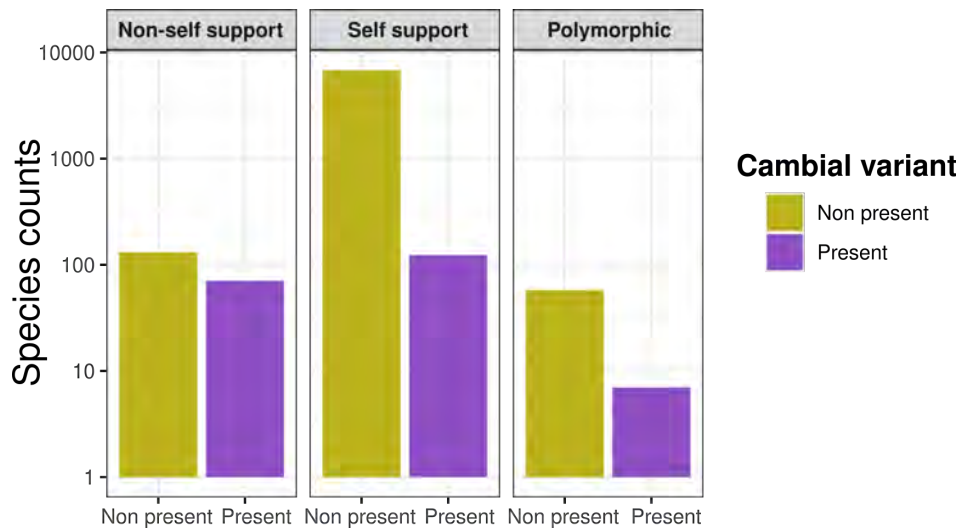


Figure 3: Preferential association of the non-self support growth form and the presence of cambial variants. The proportion of non-self support species having cambial variants is higher (35%) than the proportion of self support species with cambial variants (2%). The proportion of polymorphic species having cambial variants (10%) is higher than self support species. The y-axis count is in logarithmic scale.

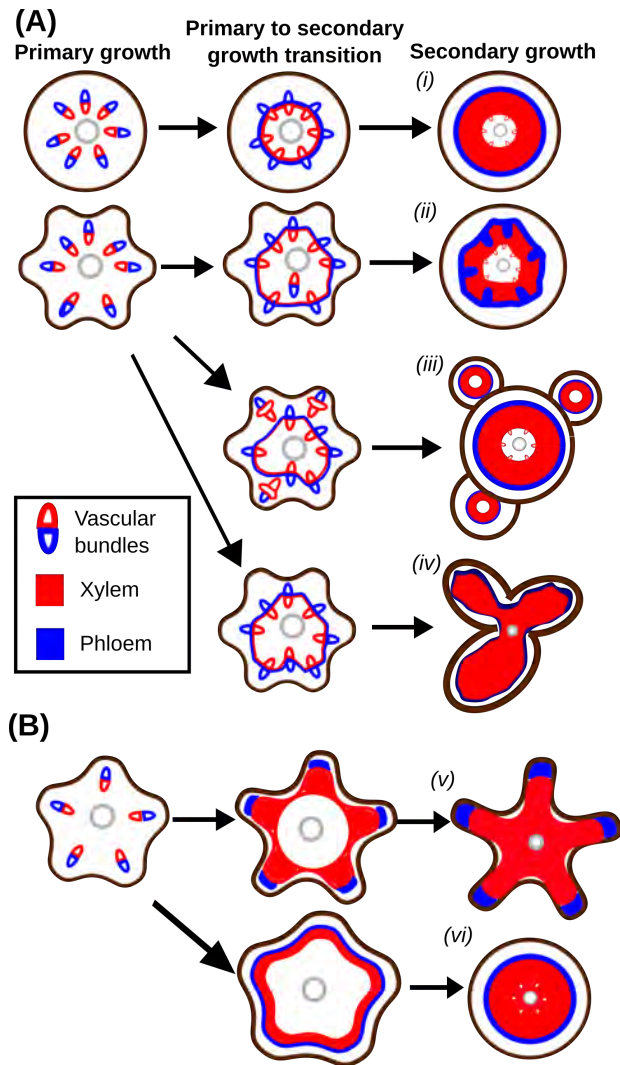


Figure 4: Evolutionary accessibility of cambial variants facilitated by primary lobed conditions. (A) In the *Paullinia* genus, during primary growth, plants with regular cambium have vascular bundles arranged in a circumferential position approximately at equidistant positions. In contrast, species from the *Paullinia* group having vascular variants have vascular bundles not arranged in circumferential positions with probably not equidistant positions. Lobed stems can form (ii) phloem wedges, (iii) compound stems, or (iv) non cylindrical stems. (B) In the genus *Byttneria*, non self-supporting species have primary lobed stem with fascicular cambium having more activity than interfascicular cambium. Lobes in lobed stems (v) correspond with the fascicular cambium. Self-supporting species with primary lobed stems, such as *Byttneria morii*, become cylindrical (vi) during adult stem morphologies.

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Discusión general y conclusiones

La extensa diversidad anatómica y el valioso registro ontogenético del xilema secundario en las plantas son una fuente invaluable de datos para la realización de estudios de evo-devo a nivel morfológico. En la mayoría de los casos, el xilema secundario actúa como un archivo donde queda documentado su propio desarrollo, lo que permite rastrear, a nivel de linaje celular, decenas e incluso cientos de eventos de diferenciaciones celulares o “decisiones” ontogenéticas. En esta tesis, aproveché esta ventaja para proporcionar información detallada sobre el efecto que tienen las variaciones en las “decisiones” de las células cambiales en la diversificación morfológica de un clado de plantas tropicales del género *Euphorbia*. Además, exploré la relación preferencial entre el hábito de crecimiento en el cual las plantas requieren algún tipo de soporte para sostenerse, o ‘no auto-sostén’, y la presencia de variantes vasculares utilizando una base de datos pública. Ambos enfoques contribuyen al campo de la evo-devo, y en particular al de la “morfo-evo-devo.”

A continuación, discutiré brevemente las ventajas del enfoque “morfo evo-devo” en el contexto de la investigación científica desde un país del “sur-global.” Posteriormente, discuto las perspectivas metodológicas y conceptuales relacionadas con el seguimiento de las “decisiones” ontogenéticas de las células cambiales y su representación como letras. También abordaré la estrecha relación que existe entre la diversidad ontogenética y la diversidad funcional. Posteriormente, analizo las perspectivas y limitantes de los resultados encontrados entre la relación del hábito de crecimiento de no auto-sostén y la presencia de variantes vasculares. Finalmente, sugiero posibles mecanismos de desarrollo que podrían mostrar una relación entre el desarrollo de variantes vasculares en determinadas condiciones durante el crecimiento primario de las plantas.

El enfoque “morfo evo-devo” como un aporte enriquecedor de la evo-devo.

La revisión y propuesta del primer capítulo, me permitió establecer un marco teórico para hacer estudios de evo-devo a nivel morfológico basados en características que dejan un registro ontogenético. En este capítulo, se muestran ejemplos de cómo los datos morfo evo-devo se pueden utilizar para hacer inferencias sobre evolución por heterocronía, modularidad, evolución de fenotipos temporales, o inclusive evolución del tipo *fenotipo primero* (“phenotype-first evolution” en inglés). La riqueza de los datos morfo evo-devo adquieren una mayor relevancia en países que albergan una gran diversidad de especies, como es el caso de México.

Los sesgos en la investigación en evo-devo centrada en preguntas moleculares también influyen en la selección de especies y, por lo tanto, limitan la diversidad ontogenética y morfológica estudiada. Un análisis de la literatura científica reveló que la mayoría de los estudios en el área de botánica general realizados en las últimas dos décadas se concentran en cultivos que son de importancia económica, mientras que hay una falta de representación de la biodiversidad (Marks et al., 2023). También se identificó una correlación entre el producto interno bruto por país y el número de publicaciones, así como entre la inversión en investigación por país y el número de publicaciones (Marks et al., 2023). Una de las ventajas del enfoque morfo-evo-devo radica en la posibilidad de hacer preguntas de investigación más accesibles en términos presupuestales, al mismo tiempo que se investigan las causas de los patrones de diversidad morfológica, abarcando una mayor variedad de especies. Por ejemplo, después de analizar el desarrollo de variantes cambiales en especies de la tribu Paullinieae, se encontró la existencia de más de seis variantes vasculares (Chery et al., 2020; Cunha Neto, 2023). Es importante destacar, como se menciona en el segundo capítulo, que el enfoque morfo evo-devo no excluye los estudios de biología molecular del desarrollo; por el contrario, puede complementarlos. Por ejemplo, las propuestas de modelos espaciales y moleculares son fundamentales para entender la relación causal entre la condición de tallos primarios lobados y el desarrollo de variantes vasculares. A continuación, exploraré las posibles perspectivas asociadas a la investigación de las “decisiones” ontogenéticas en otros grupos de plantas leñosas.

Decisiones ontogenéticas de las células del cambium vascular.

En el segundo capítulo identificamos diferencias en las “decisiones” de las células progenitoras del xilema secundario entre las especies con tallos suculentos y las especies con tallos leñosos del clado *Pedilanthus*. Una de las diferencias en estas “decisiones” fue que las especies con tallos leñosos tienen una mayor diversidad de “palabras” en comparación con sus contrapartes suculentas, lo que refleja una mayor diversidad ontogenética. La diversidad ontogenética se refiere al conjunto de procesos ontogenéticos observados en una especie, que pueden ser descritos, y deseablemente cuantificables y comparables entre especies. Esto puede abarcar desde patrones y tasas de crecimiento relativas que derivan en la formación de rasgos paedomórficos o peramórficos (Alberch et al., 1979; Bonett, 2021), hasta la diversidad de tipos celulares que componen un tejido. Una posible explicación de la mayor diversidad ontogenética en el xilema de las plantas con tallos leñosos podría estar relacionada con una mayor diversidad funcional en estas plantas en comparación con las especies de tallos suculentos. La diversidad funcional se refiere al conjunto de funciones o roles ecológicos que un organismo o sus “partes” pueden desempeñar. Mientras que las plantas con tallos suculentos se caracterizan por tener médulas más grandes en donde se almacena agua, las plantas con tallos leñosos tienen médulas pequeñas y por lo tanto producen más células que se encargan de almacenar agua dentro del xilema. Además, algunas plantas leñosas son perennifolias o caducifolias, en contraste con las plantas suculentas que en muchos casos se mantienen con tallos sin producir más hojas una vez que las pierden. Estas diferencias contribuyen a que el xilema de las plantas leñosas exhiba una mayor diversidad de “palabras” y, por lo tanto, una mayor diversidad de “decisiones” por parte de las células del cambium que deriva en una posible mayor amplitud funcional del tejido.

La estrecha relación entre la diversidad ontogenética y la diversidad funcional es clave para entender la evolución del desarrollo de los organismos. La coordinación de las diferentes trayectorias ontogenéticas que ocurren entre partes de un organismo, determinan la diversidad ontogenética de un órgano y el intervalo funcional dentro del ambiente. Una mayor diversidad ontogenética puede dar lugar a una variedad más amplia de atributos funcionales, permitiendo a las especies adaptarse y responder a condiciones ambientales cambiantes. Por ejemplo, en algunas especies de renacuajos sapos del género *Spea* (Scaphiropodidae) se desarrollan dos fenotipos contrastantes dependiendo de

la dieta (Levis and Pfennig, 2020). Los renacuajos con dieta omnívora se caracterizan por tener músculos mandibulares pequeños, partes bucales lisas, numerosas hileras de denticulos y un intestino largo; mientras que los renacuajos con dieta carnívora tienen músculos mandibulares más grandes, partes bucales con muezcas, pocas hileras de denticulos y un intestino corto (Ledon-Rettig et al., 2008). Los cambios coordinados entre los órganos de los dos fenotipos de las especies polifénicas del género *Spea* reflejan una mayor diversidad ontogenética que contrasta con especies como *Scaphiopus holbrookii* (Scaphiopodidae) en donde solo se observa el fenotipo omnívoro (Levis et al., 2018). Al realizar experimentos con *S. holbrookii* e inducir el fenotipo carnívoro se observó que habían cambios en ciertas características fenotípicas como un intestino más corto y un menor número de hileras de denticulos, al igual que en formas carnívoras de *Spea*. Sin embargo, se observó que no había un aumento claro en los músculos mandibulares y las partes bucales eran más lisas y estos cambios se identificaron como maladaptativos (Levis et al., 2018). Esto muestra que la interacción entre partes es fundamental para entender la diversidad ontogenética y funcional observada a nivel de los órganos.

Existen casos donde se logra una mayor diversidad funcional sin necesariamente una mayor diversidad ontogenética subyacente. Por ejemplo, las lagartijas del género *Liolaemus* (Liolaemidae) que habitan sitios abiertos y que corren largas distancias, generalmente, tienen extremidades más largas en comparación con sus contrapartes que habitan en lugares cerrados (Schulte II et al., 2004). Mayor proliferación celular en las extremidades de lagartijas ocurre durante el desarrollo embrionario (Feiner et al., 2021) y es mi proxy de mayor diversidad ontogenética. Algunas especies con extremidades cortas se caracterizan por correr largas distancias y además por cambiar de hábitat de espacios abiertos a los troncos de árboles. Posiblemente, el cambio de comportamiento sin generar mayor diversidad ontogenética en forma de menos proliferación celular que resulta en extremidades cortas fue favorecido por la selección natural (Schulte II et al., 2004). La relación entre diversidad ontogenética y funcional resalta la relevancia de desentrañar los intrincados procesos del desarrollo y el ambiente que moldean las capacidades funcionales de los organismos, el potencial ontogenético y la no trivialidad en la relación entre diversidad ontogenética y funcional.

En lo que respecta a las perspectivas de nuestro método de codificación de los tipos celulares en letras y la comparación de palabras, se requerirá una expansión del estudio a más grupos y la incorporación de métodos semiautomatizados para agilizar la recopilación de los datos. Estudios previos han intentado delimitar de forma semiautomática

las hileras de células derivadas de una célula progenitora del cambium (Brunel et al., 2013, 2014). Estos análisis producen imágenes en las que cada hilera de células se identifica con un color particular sin embargo la correcta identificación de hileras es muy baja (observación personal). Será fundamental utilizar y optimizar el método y el software ya desarrollado por Brunel et al. 2014, para dos aspectos diferentes. El primero deberá enfocarse en mejorar la correcta identificación de hileras de células. El segundo deberá enfocarse en codificar los tipos celulares de esas hileras de manera semiautomática. Por otra parte, las mediciones de número máximo de palabras, los índices de Shannon Entropy y de Lempel-Ziv, se podrán estandarizar fácilmente en especies con anillos de crecimiento claramente delimitados. Esto permitirá la comparación de cambios de palabras producidas tanto dentro de una misma planta como entre plantas. A medida que ampliamos el número de especies estudiadas, podremos construir un morfoespacio de palabras producidas por las plantas leñosas. Esto nos proporcionará un muestreo más amplio para la identificación de posibles restricciones ontogenéticas (palabras no observadas) y del potencial ontogenético de las células cambiales.

Relación entre el hábito de crecimiento de “no auto sostén” y las variante vasculares.

En el capítulo tres, presenté evidencia de una relación preferencial entre el hábito de crecimiento de “no auto-sostén” y la presencia de variantes vasculares. Esta asociación podría estar vinculada al hecho de que en poblaciones ancestrales con hábito de crecimiento de “no auto-sostén,” las variantes que exhiben configuraciones de tallos diferentes de la organización de cambium vascular “típica,” que potencialmente es la configuración con mayor adecuación en términos mecánicos para una planta de “auto-sostén,” son favorecidas por la selección natural. Las trayectorias ontogenéticas que dan lugar a variantes vasculares no parecen ser desfavorecidas por la selección cuando, en el contexto de una población, los individuos que crecen más fácilmente sosteniendo su peso sobre otras plantas son los predominantes.

Por otra parte, las especies evolucionan a partir de la variación existente y esto en cierta medida restringe las trayectorias ontogenéticas. Por ejemplo, especies del género *Aristolochia* (Aristolochiaceae), subgénero *Isotrema* son plantas de “auto-sostén” cuya forma ancestral es de no auto-sostén (Wagner et al., 2012). Las plantas del subgénero *Isotrema* se caracterizan por ser pequeños arbustos que ha diferencia de las especies

de no auto-sostén del género *Aristolochia* producen una mayor cantidad de madera (Wagner et al., 2012). Sin embargo, las especies del subgénero *Isotrema* tienen tallos y ramas que se doblan fácilmente por la retención de características que promueven la flexibilidad de los tallos, como los radios multiseriados grandes que caracterizan a muchas especies de no auto sostén (Trueba et al., 2015). Por estas razones es importante considerar la variación pasada y presente que tienen las poblaciones para entender mejor las trayectorias ontogenéticas posibles. Un ejemplo de trayectorias ontogenéticas que podrían facilitar la formación de variantes cambiales es la formación de tallos primarios lobados.

Para poner a prueba la hipótesis de tallos primarios lobados que favorecen la evolución de variantes vasculares será necesario identificar los cambios en las proporciones de divisiones del cambium que derivan en diferentes proporciones de células del floema y xilema, así como las redes de regulación genética que participan en la regulación de estas divisiones. En años recientes, se ha avanzado en la elucidación de algunas de estas redes de regulación que participan tanto en el establecimiento como en el mantenimiento del cambium vascular (Fischer et al., 2019; Ruonala et al., 2017; Shi et al., 2019; Wang et al., 2021). Una de estas redes de genes es la involucrada en el plano de división anticlinal del cambium y está mediada por la actividad del gen *PXY*. El gen *PXY* se expresa en las células del procambium, mientras que el gen *CLE41*, produce un ligando que se une a la proteína de *PXY*, y se expresa en las células adyacentes del floema (Etchells and Turner, 2010). La orientación del plano de división periclinal en las células del cambium depende, en parte, de la interacción de estos dos genes y variaciones en las tasas de difusión podrían dar lugar a la variación en las proporciones de células del xilema y floema secundario en distintos sectores del cambium. Será crucial determinar la interacción de los genes que actúan en el desarrollo del cambium fascicular con respecto a las del cambium interfascicular. Por ejemplo, las plantas mutantes de *Populus trichocarpa* (Salicaceae) para el gen HD-ZIP III, *PtrHB4*, tienen deficiencias en la formación del cambium interfascicular (Zhu et al., 2018). Entender las redes de regulación que participan en la transición de cambium fascicular e interfascicular a un cambium conjunto será fundamental para comprender cómo cambios en las posiciones de los haces vasculares, como sucede en plantas con tallos lobados, podría ser una forma de generar sectores del cambium con actividades diferenciadas que resultan en la formación de variantes vasculares.

En conclusión, los capítulos de esta tesis representan una modesta contribución al

campo de la evolución del desarrollo vascular de las plantas y, en general, al campo de la evo-devo. Los datos obtenidos a través del enfoque morfo evo-devo son una valiosa fuente de información para plantear preguntas de investigación en cualquier parte del mundo, pero adquieren una importancia particular en lugares con una alta biodiversidad donde la realización de experimentos moleculares puede no ser de fácil acceso. Contar con una perspectiva amplia y diversificada de los estudios en evo-devo se revela esencial para comprender y abordar los diversos fenómenos evolutivos que caracterizan a los seres vivos.

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Anexo 1. Análisis bibliométrico

Para determinar los principales temas de investigación en evo-devo, realicé una análisis bibliométrico. Uno de los servidores que tiene menos sesgos y mayor acceso a artículos es “Web of Science.” Utilicé “Web of Science” buscando artículos que tuvieran la palabra “evo-devo” en el título, el abstract o en las palabras clave, desde 1997 hasta la actualidad. La búsqueda resultó en un total de 3007 artículos. Para resumir y graficar los resultados de la búsqueda bibliográfica, descargué los artículos en un archivo .bib y utilicé el paquete *bibliometrix* de R ([Aria and Cuccurullo, 2017](#)). Para identificar estructuras conceptuales dentro del campo, utilicé la función *biblioNetwork* de *bibliometrix*. *biblioNetwork* construye una matriz de las palabras clave, asignando un valor en función de las palabras clave que co-ocurren más frecuentemente. Para visualizar el resultado de la matriz de palabras clave utilicé la función *networkPlot*. *networkPlot* grafica una red asignando el mismo color a las palabras que co-ocurren más frecuentemente y asigna un tamaño en proporción al número de palabras.

Para identificar si las mismas estructuras conceptuales y temas de investigación ocurren solo dentro del campo de biología de plantas, utilicé un filtro en “Web of science” utilizando la misma palabra clave que utilicé para todo el campo de evo-edvo. El resultado de aplicar este filtro fue de un total de n artículos. El análisis posterior de esta búsqueda bibliográfica fue el mismo que para el análisis general. Además de las tablas con los artículos más citados y del análisis de redes sobre la co-ocurrencia de palabras clave, en la tabla A1 se muestran las palabras clave más frecuentes provistas por los autores más frecuentes, así como las palabras clave más frecuentes determinadas por la “Web of science” en el campo de la evo-devo. En la tabla A2 se muestra lo mismo pero para la búsqueda bibliográfica realizada sólo en el campo de evo-devo de plantas. El código utilizado para realizar este análisis se encuentra disponible en el repositorio [BibliometricAnalysisEvoDevo](#).

Cuadro 3: Palabras clave más frecuentes en artículos publicados desde 1997 hasta la fecha en el campo de evo-devo.

Palabra clave provistas por Clearview	Número de artículos	Palabra clave provistas por los autores	Número de artículos
Evo-devo	599	Evolution	827
Evolution	327	Evo-devo	669
Development	187	Expression	380
Evodevo	79	Origin	189
Morphogenesis	60	Gene-expression	178
Heterochrony	58	Genes	146
Homology	53	Patterns	134
Morphology	46	Drosophila	126
Ontogeny	46	Mechanisms	117
Adaptation	45	Diversification	116

Cuadro 4: Palabras clave más frecuentes en artículos publicados desde 1997 hasta la fecha en el campo de evo-devo de plantas.

Palabra clave provistas por Clearview	Número de artículos	Palabra clave provistas por los autores	Número de artículos
Evolution	138	Evolution	185
Evo-devo	113	Expression	139
Development	92	Arabidopsis	136
Arabidopsis	30	Arabidopsis thaliana	128
Phylogeny	28	Gene-expression	69
Flower development	25	Plants	69
Heterochrony	21	Mads-Box genes	66
Plant development	21	Growth	62
Flower	20	Flower development	54
Inflorescence	19	Phylogeny	52