Insight

Unusual spermine-conjugated hydroxycinnamic acids on pollen: function and evolutionary advantage

Thomas Vogt
Leibniz Institute of Plant Biochemistry, Department of Cell and Metabolic Biology, Weinberg 3, D-06120 Halle (Saale), Germany
Correspondence: tvogt@ipb-halle.de

Conjugates between polyamines and hydroxycinnamic acids are found on the pollen surface of all higher plants, both mono- and dicots. But we don’t know why they are there. Delporte et al. (2018) have now shown that in the tapetum of the Asteraceae (sunflower family) a new type of BAHD-acyltransferase is expressed, able to transfer coenzyme A-activated coumaric acid to all four primary and secondary amine groups of the polyamine spermine. In the case of chicory this sequential addition results in a fully substituted tetracoumaroyl–spermine conjugate and points to an evolutionary advantage of these functionally enigmatic compounds.

This story begins 40 years ago, when large amounts of unusual conjugates were detected in the male reproductive organs of maize (Martin-Tanguy et al., 1978). These were hydroxycinnamic acids (HCAs), like ferulic acid, linked to the individual amino groups of putrescine and spermidine. Putrescine and its biosynthetic descendant spermidine and spermine, which are all polyamines, are derived from ornithine and arginine by decarboxylation and sequential aminopropyltransfer from decarboxylated adenosylmethionine via spermidine and spermine synthase, respectively (Martin-Tanguy, 2001). The high content of these HCA–amide (HCAA) conjugates in maize pollen was initially linked to plant fecundity, until it was shown that they are present throughout the plant kingdom. Their high concentration specifically in pollen grains remains a mystery.

Further characterization followed in the 1980s with the rise of chromatographic and analytical tools and an increased interest in chemotaxonomy. Several studies using mass spectrometry and NMR of a variety of spermidine–conjugated HCAs from pollen of wind-pollinated species like birch and hazel showed localization on the surface of pollen grains and a more precise structural identification (Meurer et al., 1988). Initially, only mono- and bis-substituted conjugates were identified which were later shown to be present in flowers, leaves, seeds or roots of all plant species investigated (Luo et al., 2009). The first fully substituted HCAA, \( N_1^1, N_2^2, N_3^3 \)-tricoumaroyl spermidine, was isolated and described from the pollen of *Crataegus* (hawthorn) and was also detected in several other Rosaceae (Strack et al., 1990). Initial speculation that these compounds could be used as a chemotaxonomic marker failed, but it turned out that tris-substituted conjugates were indeed pollen specific and apparently exclusively synthesized in the tapetum of developing flowers through the action of a new type of a BAHD-type acyltransferase, spermidine hydroxycinnamoyltransferase (SHT) (Grienenberger et al., 2009).

Although not of chemotaxonomic significance, in a comprehensive study Elejalde-Palmett (2015) showed that the products of SHT, tris-substituted conjugates, can be regarded as a marker of the pollen exine of eudicotyledons: they are always present. Neither these compounds nor any corresponding SHT-like sequences have so far been reported from monocots or gymnosperms. While the product profile of SHTs of Rosaceae appeared restricted to uniform coumaric acid derivatives of spermidine, Arabidopsis SHT is co-expressed with tapetum-specific CYP98A8 and an O-methyltransferase, AtTSM1 (Ehiting et al., 2008; Fellenberg et al., 2008; Matsuno et al., 2009), resulting in downstream hydroxylations and a single methylation towards a diverse pattern of mono-, bis- and tris-substituted HCAAs which is characteristic for the Brassicaceae (Matsuno et al., 2009; Handrick et al., 2010).

Two additional BAHD-like acyltransferases, denoted SCT and SDT, are expressed in the Arabidopsis tapetum, but only SHT acts to synthesize the major tris-feruloyl and sinapoyl-based spermidine conjugates (Grienenberger et al., 2009). Similar to the biosynthesis of sporopollenin polymers of the exine, phenylalanine metabolism is probably associated in a metabolon and links polyamine to phenylpropanoid metabolism (Bassard et al., 2010; Lallemant et al., 2013). Although spermine is also present in Arabidopsis anthers in high concentrations Arabidopsis SHT is selective for spermidine. SHT knockout mutants, which are virtually devoid of HCAAs, showed no effect on the level of free polyamines and do not result in an increase of pollen-specific flavonol sophorosides (Fellenberg et al., 2012; Yonekura-Sakakibara et al., 2014) pointing to different pools of precursors and independent pathway organization for both types of phenylpropanoids in the tapetum.
New BAHD-type enzyme in the tapetum of Asteraceae

Asteraceae (sunflower family) were the focus of the work by Delporte et al. (2018). It is the most-rich angiosperm family, with rapid diversification over the last 60 million years specifically in open habitats all over the world (Barreda et al., 2015). Besides sunflower, many other Asteraceae have been used for nutrition (e.g. artichoke, Cynara scolymus), as herbal medicines (Arnica and Echinacea spp.) or, most recently, as an alternative source of rubber (dandelion, Taraxacum officinale). Roasted chicory (Cichorium intybus) rootstocks were used extensively in the last century as a cheap coffee replacement. Due to its high content of inulin, a 1,3-linked carbohydrate, it is currently considered as a source of dietary fiber and promoted as ‘functional food’ (Stolze et al., 2017).

Asteraceae are usually pollinated by insects and attractive floral organs have contributed to their worldwide success and promoted the evolution of insects, such as solitary bees (Barreda et al., 2015). Delporte et al. (2018) identified two new members of the SHT clade of BAHD-acyltransferases in Asteraceae, CiSHT1 and CiSHT2, and extend the biosynthetic diversity of HCAAs in reproductive organs of plants (Delporte et al., 2018). LC-MS-based analysis identified a tetra-substituted coumaroyl-CoA spermine as the major phenolamide in methanolic pollen washes of chicory besides minor spermidine conjugates which can be bis- or tris-substituted (Box 1).

At first glance this extended acceptor specificity may not seem spectacular, but requires multiple changes in terms of substrate availability, transport, regulation and (last but not least) changes in the amino acid sequence of the two chicory CiSHTs identified by Delporte et al. (2018). New enzyme functions are based on divergent evolution, gene duplication and subsequent random functional re-differentiation of established features (Lynch and Katju, 2004; Aharoni et al., 2005). A closer inspection of the active site and site-directed mutagenesis of this enzyme might reveal that only a single amino acid change leading to a somewhat larger hydrophobic cavity, capable of hosting bulky acylated spermine derivatives, could result in this minor shift of substrate preference. Recombinant

Box 1. Cladogram of individual clusters of enzymes required for differentially substituted HCAA

The cladogram shows the difference between the new tetracoumaroyl spermine conjugates (1) synthesized via CiSHT1 and CiSHT2 compared to the previously identified clusters of tris (2) and bis (3)-substituted spermidine derivatives. Functionally characterized SHTs and SDT are marked in red. Based on sequence identities of the SHTs, the set of tetra-substituted compounds appears to be restricted to the Asteraceae, but further analysis of metabolites and functional characterization of the corresponding enzymes is required.
BAHDs are usually difficult to express in microbial systems and the currently solved BAHD-crystal structures belong to only distantly related BAHD subclades compared to CsSHTs (Unno et al., 2007). In functionally related serine carboxypeptidase-like enzymes (SCPLs) a simple Glu to Asp change in a catalytic triade resulted in a surprisingly complete functional change from a peptide hydrolase to a 1–O-glucose ester transferase. Instead of water as in the case of a hydrolase, the sinapoyl residue is now transferred to malic acid resulting in sinapylmalate, a typical metabolite in Brassicaceae leaves (Stehle et al., 2009). Nature seems to favour simple and elegant solutions.

Crystallization of the enzyme and a close-up view of the active site by modelling and docking studies of CsSHT should shed light on the new specificity and might also reveal the differences in CsSHT1 and CsSHT2 in terms of functional expression. All other features of the chicory BAHDs appear conserved compared to other BAHDs, including high activity at alkaline pH, promiscuous substrate CoA-donor and amine-acceptor preference in vitro and in vivo, even when expressed ectopically in Arabidopsis, and localization in the cytoplasm, as demonstrated by YFP-fusions. A tight association of SHT in a complex appears plausible, since only the fully substituted HCAA is detected in large quantities in vivo and no isomerization of the product is observed.

Compared to the complex substitution pattern of Arabidopsis, the simple tetra-coumaroyl spermine pattern in chicory already points to evolutionarily independent diversification in the Brassicaceae and Asteraceae. Additional downstream modifications, like hydroxylation and subsequent methylation, appear to be missing in chicory and presumably are less relevant for their biological function. The modified acceptor specificity, of course, now requires verification in other tribes of the Cichorioideae and Asteroideae, since the reported sequence identities are insufficient to draw precise conclusions on acceptor preference. It appears plausible that gene duplication and neo-functionalization in the Asteraceae occurred before the divergence of the eudicots, but after the tapetal SHT and the accumulation of tris-substituted HCAAs were established. Now, with emerging sequencing of complete genomes of ‘primitive’ dicots like Amborella a more precise annotation of SHT divergence and evolution might be possible (The Amborella Genome Project, 2013).

### Enigmatic roles of SHT and resulting metabolites: many questions

The universal deposition of HCAAs late in pollen development is usually regarded as a final decoration step in the highly coordinated assembly of the male gametophyte (Arizumi and Toriyama, 2011). After mechanistic and regulatory steps in this process have been solved, the most intriguing question remains unanswered: what is the function of these compounds on the pollen surface and what exactly is the advantage to pollen fitness? Also, why is there conserved expression of SHT-type BAHDs specifically in the male reproductive organs of all higher dicots? And finally, is there any advantage of the shift from spermidine– to spermine-conjugated HCAs? To quote the famous biochemist Erwin Chargaff (1905–2002): ‘science is wonderfully equipped to answer the question “how?” but it gets terribly confused when you ask the question “why?”’ Are we confused or is it just the lack of suitable tools or the complexity of the problem which impedes identification of one or several functions?

Mo et al. (1992) unexpectedly restored the fertility of chalcone synthase deficient and conditionally sterile petunia plants (Napoli et al., 1990) by including micromolar concentrations of flavonols to the germination medium. Therefore, the flavonol diglycosides which, besides HCAAs, accumulate on the exine of higher dicots, could at least be associated with pollen fertility in these two species. However, in all other species tested so far, including Arabidopsis, this requirement could not be verified. How exactly these flavonols contribute to pollen germination remains enigmatic even today. Fertility in SHT and HCAA-deficient Arabidopsis plants appears unimpaired, although cracks in a small percentage of sht pollen grains could indicate a minor structural role of HCAAs in the pollen wall (Grienenberger et al., 2009). Based on the current data, a general requirement of phenylpropanoids for fecundity of eudicot pollen is not evident, although a supportive role in the numerous factors regulating pollen germination and pollen tube guidance cannot be ruled out (Hafid et al., 2016).

A universal and most obvious function of phenylpropanoids in general is UV protection (Youhnovski et al., 2001). HCAAs, either spermidine- or spermine-linked, show absorbance maxima of 315–330 nm, flavonol glycosides absorb at 354 nm, and a combination of both covers the UV-B and UV-A spectrum of sunlight perfectly. Protection of the genetic information of the pollen grains from damaging UV radiation is consistent with an established role of phenylpropanoids in UV protection (Jansen et al., 1998). Decoration of all four nitrogen atoms of spermine with HCAs increases the UV absorbance of a single molecule by roughly 30% compared to spermidine (which only contains three nitrogen atoms) without affecting the concentration of the metabolites, since the extinction coefficients of individual phenylpropanoids add up. If transport to the pollen surface by an ABC-transporter can be demonstrated for hydrophobic HCAAs as postulated for polyketide-derived exine polymers (Quilichini et al., 2014; Lefèvre and Boutry, 2018), then the same number of transported molecules, in an ATP-dependent process, would result in a more efficient way to generate a pollen surface of increased UV absorbance, independent of the decorations of individual phenylpropanoids, which all show similar extinction coefficients. The identification of SHTs in the Asteraceae by sequence identities in this report requires support by identification of the corresponding HCAA-pattern and analysis of SHT-product specificity in other Asteraceae.

In addition to a very plausible role of HCAAs in UV protection, and the usual claims of antioxidant and free radical scavenger potential, a biological role in plant–microbe and plant–pollinator interaction, although difficult to prove, deserves more attention. Although antimicrobial activities of phenylpropanoids and specifically HCAAs have been reported...
(Daglia, 2012; Tanabe et al., 2015), a specific advantage of the new HCAA pattern in Asteraceae pollen compared to pollen of other species is not immediately obvious. In contrast, a much more fascinating and largely unexplored area is the potentially beneficial effect of these types of compounds in plant–pollinator interactions. The flavonol content and resulting high UV absorbance in flowers of various Petunia spp. were already linked to pollinator preference (Sheehan et al., 2016). Therefore, dark spots in flowers could be attractive to some insects, such as bees, which can perceive UV light (Briscoe and Chittka, 2001; Barreda et al., 2015). Most intriguing, tris-coumaroyl spermidine, besides lipids and flavonols from sunflower pollen (Asteraceae), was reported as an insect feeding stimulant (Lin and Mullin, 1999). One should remember that a spermine molecule contains four nitrogen atoms which might be reused by insects upon cleavage of the amide bond. If harvest and distribution of pollen grains could be enhanced by an optimized blend of ‘tasty’ metabolites, this could at least in part account for the rapid success and evolutionary advantage of angiosperms (and specifically the Asteraceae) in their coevolution with insects, potentially accelerating the adaptive radiation of this family of angiosperms and leading to their current diversity. The biological story may be more complex. A clear proof of these assumptions requires precise analytics, mutant analyses, pollen viability studies under enhanced UV light, and insect feeding studies, preferentially under natural, non-greenhouse light conditions.

Keywords: Acylated spermine, BAHD acyltransferases, Cichorium intybus, metabolic diversification, pollen coat, phenolamides, SHFs

References
Aharoni A, Gaidukov L, Khersonsky O, McQ Gould S, Roodveldt C, Tawfik DS. 2005. The ‘evolvability’ of promiscuous protein functions. Nature Genetics 37, 73–76.
Arizumi T and Toriyama K. 2011. Genetic regulation of pollen sporopollenin biosynthesis and pollen exine development. Annual Review of Plant Biology 62, 437–460.
Barreda VD, Balazs N, Tellería MC, Oliverod EB, Rainee JI, Forest F. 2015. Early evolution of the angiosperm clade Asteraceae in the Cretaceous of the Antarctic. Proceedings of the National Academy of Sciences, USA 112, 10989–10994.
Bassard JE, Ullmann P, Bernard F, Werck Reichardt D. 2010. Phenolamides: bridging polyamines to the phenolic metabolism. Phytochemistry 71, 1808–1824.
Briscoe AD, Chittka L. 2001. The evolution of color vision in insects. Annual Review of Entomology 46, 471–510.
Elejalde-Palmett C, Dugé de Bernonville T, Glevarec G, Pichon O, Papon N, Courdavault V, St-Pierre B, Giglioli-Guivarc’h N, Lanoue A, Besseau S. 2015. Characterization of a spermine hydroxycinnamoyltransferase in Malus domestica highlights the evolutionary conservation of trihydroxycinnamoyl spermidines in pollen coat of core Eudicotyledons. Journal of Experimental Botany 66, 7271–7285.
Daglia M. 2012. Phenolypens as antimicrobial agents. Current Opinion in Biotechnology 73, 174–181.
Delporte M, Bernard G, Legrand G, Hielscher B, Lanoue A, Molinié R, Rambaud C, Mathiron D, Besseau S, Linka N, Hilbert J-L, Gagneud D. 2018. A BAHD neofunctionalization promotes tetrahydroxycinnamoyl spermine accumulation in pollen coat of the Asteraceae family. Journal of Experimental Botany 69, 5355–5371.
Ehlting J, Sauveplane V, Ötvös A, Gaudinger JP, Provart NJ, Werck-Reichhart D. 2008. An extensive (co-)expression analysis tool for the cytochrome P450 superfamily in Arabidopsis thaliana, BMC Plant Biology 8, 1–19.
Fellenberg C, Milkowski C, Hause B, Lange PR, Böttcher C, Schmidt J, Vogt T. 2008. Tapetum-specific localization of a cation-dependent O-methyltransferase in Arabidopsis thaliana. The Plant Journal 56, 132–145.
Fellenberg C, Ziegler J, Handrick V, Vogt T. 2012. Polyamine homeostasis and wildtype and phenolamiddeficient Arabidopsis stamens. Frontiers in Plant Science 3, 180.
Grienerberger E, Besseau S, Geoffroy P, Debaye D, Heintz D, Lapierre C, Pollet B, Heitz T, Legrand M. 2009. A BAHD acyltransferase is expressed in the tapetum of Arabidopsis anthers and is involved in the synthesis of hydroxycinnamoyl spermidines. The Plant Journal 58, 246–259.
Hafid S, Fila H, Honeys D. 2016. Male gametophyte development and function in angiosperms: a general concept. Plant Reproduction 29, 31–51.
Handrick V, Frolov A, Fellenberg C, Vogt T. 2010. Profiling of hydroxycinnamic amides in Arabidopsis thaliana pollen by tandem mass spectrometry. Analytical and Bioanalytical Chemistry 398, 2789–2801.
Jansen, MAK, Gaba V, Greenberg BM. 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends in Plant Science 3, 131–135.
Lallemand T, Erhardt M, Heitz T, Legrand M. 2013. Sporopollenin biosynthetic enzymes interact and constitute a metabolon localized to the endospermic cellulum of tapetum cells. Plant Physiology 152, 616–625.
Lefèvre F, Boutilier M. 2018. Towards identification of the substrates of ATP-binding cassette transporters. Plant Physiology 178, 18–39.
Lin S, Mullin CA. 1999. Lipid, polyamide, and flavonoid phagostimulants for adult western corn rootworm from sunflower (Helianthus annuus L.) pollen. Journal of Agriculture and Food Chemistry 47, 1223-1229.
Luo J, Fuell C, Parr A, Hill L, Bailey P, Elliott K, Fairhurst SA, Martin C, Michael AJ. 2009. A novel polyamine acyltransferase responsible for the accumulation of spermidine conjugates in Arabidopsis seed. The Plant Cell 21, 318–333.
Lynch M, Katju V. 2004. The altered evolutionary trajectories of gene duplicates. Trends in Genetics 20, 544–549.
Martin-Tanguy J, Cabanne F, Perdrizet E, Martin E. 1978. The distribution of hydroxycinnamic acid amides in flowering plants. Phytochemistry 17, 1927–1928.
Martin-Tanguy J. 2001. Metabolism and function of polyamines in plants: recent development (new approaches). Plant Growth Regulation 34, 135–148.
Matsuno M, Compagnon V, Schoch GA, Schmitt M, Debaye D, Bassard JE, Pollet B, Hehn A, Heintz D, Ullmann P, Lapierre C, Bernier F, Ehlting J, Werck-Reichhart D. 2009. Evolution of a novel phenolic pathway for pollen development. Science 326, 1688–1692.
Meurer B, Wiermann R, Strack D. 1988. Phenylpropanoid pattern in Fagales pollen and their phylogenetic relevance. Phytochemistry 27, 823–828.
Mo Y, Nagel C, Taylor, L.P. 1992. Biochemical complementation of chalcone synthase mutants defines a role for flavonoids in functional pollen. Proceedings of the National Academy of Sciences, USA 89, 7213–7217.
Napoli C, Lemieux C, Jorgenson R. 1990. Introduction of a chimeric chalcone synthase gene into Petunia results in reversible co-suppression of homologous genes in trans. The Plant Cell 2, 279–289.
Quilichini TD, Samuals L, Douglas C. 2014. ABCG26-Mediated polyketide trafficking and hydroxycinnamoyl spermidines contribute to pollen wall exine formation in Arabidopsis. The Plant Cell 26, 4483–4498.
Sheehan H, Moser M, Klahre U, Esfeld K, Dell’Olivo A, Mandel T, Metzger S, Vandenbussche M, Freitas L, Kuhlmeier C. 2016, MYB-FL controls gain and loss of floral UV absorbance, a key trait affecting pollinator preference and reproductive isolation. Nature Genetics 48, 159–166.
Stehle F, Brandt W, Stubbs MT, Milkowski C, Strack D. 2009. Sinapoyltransferases in the light of molecular evolution. Phytochemistry 70, 1652–1662.
Stolze A, Wanke A, van Deenen N, Geyer R, Prüfer D, Schulze Gronow C. 2017. Development of rubber-enriched dandelion varieties by metabolic engineering of the inulin pathway. Journal of Biotechnology 15, 740–753.

Strack D, Eilert U, Wray V, Wolff J, Jaggy H. 1990. Tricoumaroylspermidine in flowers of Rosaceae. Phytochemistry 29, 2893–2896.

Tanabe K, Hojo Y, Shinya T, Galis I. 2015. Molecular evidence for biochemical diversification of phenolamide biosynthesis in rice plants. Journal of Integrative Plant Biology 58, 903–913.

The Amborella Genome Project. 2013. The Amborella genome and the evolution of flowering plants. Science 342. DOI: 10.1126/science.1241089.

Unno H, Ichimaida F, Suzuki H, Takahashi S, Tanaka Y, Saito A, Nishino T, Kusunoki M, Nakayama T. 2007. Structural and mutational studies of anthocyanin malonyltransferases establish the features of BAHD enzyme catalysis. Journal of Biological Chemistry 282, 15812–15822.

Yonekura-Sakakibara K, Nakabayashi R, Sugawara S, Tohge T, Ito T, Koyanagi M, Kitajima M, Takayama H, Saito K. 2014. A flavonoid 3-O-glucoside:2′-O-glucosyltransferase responsible for terminal modification of pollen-specific flavonols in Arabidopsis thaliana. The Plant Journal 79, 769–782.

Youhnovski N, Werner C, Hesse M. 2001. N,N,N’-triferuloylspermidine, a new UV-absorbing polyamine derivative from pollen of Hippeastrum x hortorum. Zeitschrift für Naturforschung 56c, 526–530.

Insight

Mesophyll conductance and accurate photosynthetic carbon gain calculations

Tiina Tosens* and Lauri Laanisto

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Estonia

* Correspondence: tiina.tosens@emu.ee

Mesophyll conductance ($g_m$) is now known to be an important limiting factor of photosynthetic resource use efficiency in C_3 plants, with significant implications for crop improvement. Non-leaf green organs of plants only add to the complexity, but are another crucial part of the equation. Han and co-authors (2018) examined cotton bracts, and provide comprehensive analysis of limitations to photosynthesis and underlying structural correlates, including mesophyll anatomical traits. The work provides an object lesson in the level of detail essential for accurate photosynthetic carbon gain estimations.

There is a massive, 36-fold variation in net photosynthetic rate across C_3 species (Wright et al., 2004), and knowing exactly how this comes about is vitally important. Significantly, it cannot simply be explained by stomatal restriction and biochemical potential. Technological innovation at the end of the last century, including pulse-modulated fluorometry and concurrent measurement of carbon isotope discrimination, led to the discovery that there is significant CO_2 drawdown from intercellular airspaces to chloroplasts. Hence the CO_2 diffusion efficiency from substomatal cavities to chloroplasts has to be considered as well, and plays an important role in shaping photosynthetic capacity and leaf resource use efficiency across Earth’s ecosystems (Veromann-Jürgenson et al., 2017). Over the past decade great progress has been made in quantifying $g_m$ across plant groups and, according to quantitative photosynthetic limitation analysis, it can limit photosynthesis across a range of 16–80% (Tomas et al., 2013; Tosens et al., 2016).

Stress is another important consideration. During these episodes, $g_m$ becomes proportionally more significant, with the plant forced to modify carbon and nutrient investments. This makes understanding $g_m$ even more important in globally changing conditions. However, even though this is now widely recognized, $g_m$ is still generally ignored in large-scale carbon gain estimations due to lack of knowledge about its variability among different species and how best to model it (e.g. Niinemets et al., 2009).

Mesophyll conductance is linked to the structure of the tissue, and the most important traits behind its inherent variability across species are cell wall thickness and chloroplast surface area exposed to intercellular airspaces (see Peguero-Pina et al., 2017b). But what about the many non-leaf green organs of plants? These have been less studied despite their importance in regulating whole-plant carbon gain. Taking the area forward, Han and co-workers (2018) have now demonstrated that stomatal, mesophyll and biochemical limitations are of similar magnitude in cotton bracts and leaves, and significantly improving photosynthetic capacity in crops cannot be achieved unless all these limitations are manipulated. Dissecting the CO_2 diffusion pathway and modelling it from mesophyll structural traits allowed them to determine the main anatomical features responsible for extremely low CO_2 diffusion efficiency within cotton bracts. Furthermore, their results add to the growing body of evidence supporting the significance of $g_m$ in large-scale vegetation models when estimating future vegetation responses.

Predictability of mesophyll conductance

One way of adding $g_m$ to models of photosynthesis is to relate it to structure. Leaf mass per area, an integrative trait, is easy to measure, but fails to explain $g_m$ across species. However, $g_m$ is explained by cellular traits (Onoda et al., 2017). The 1D model