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Remote evolutionary constraint to the vascular cambium of seed plants

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Summary: The non-storeyed cambium of very long fusiform initials is the original type of the vascular cambium of seed plants. The additive and transformative divisions of its fusiform initials seem more resource- and time-consuming than those in the storeyed cambium. The multiplicative divisions of the fusiform initials of non-storeyed cambium are inevitably accompanied by subsequent resource- and time-consuming intrusive elongation of resulted new fusiform initials. In addition, these divisions result in highly excessive fusiform initials, most of which are doomed to be lost. These disadvantages of the non-storeyed cambium caused the evolutionary trend to the storeyed cambium of very short fusiform initials. Water transport through the longitudinal tracheids in the vesselless xylems and between the vessels in most vessel-bearing ones absolutely averts the implementation of this trend. Having arisen, the direct intervessel communications excluded tracheid-mediated transport of water and eliminated the evolutionary constraint of occurrence of the storeyed cambium.

Keywords: vascular cambium, storeyed cambium, non-storeyed cambium, additive divisions, transformative divisions, multiplicative divisions, cambium evolution, evolutionary constraints

Since the late 19th century, the vascular cambia of seed plants have usually been categorized into 2 main types, the storeyed and non-storeyed ones (Fig. 1), according to the arrangement of their fusiform initials in the tangential plane (von Höhnel 1888a,b; Bailey 1923; Beijer 1927; Philipson & Ward 1965; Philipson et al. 1971; Larson 1994; Iqbal 1995; Evert 2006; Crang et al. 2018; etc.). Tokomatsu's (1928) 4 types of the vascular cambium can reasonably be considered only a more detailed habitual classification of this tissue. The 'regularly stratified' and 'irregular' types he described are quite comparable with the traditional storeyed and non-storeyed types, respectively, whereas his 'slightly stratified' and 'non-stratified' types are intermediate between the 2 main acknowledged types. Various intermediate types of cambium have repeatedly been described elsewhere without being termed (Bailey 1923; Krawczyszyn 1977; Larson 1994; etc.).

The non-storeyed cambia (Fig. 1A) have irregularly greatly overlapping fusiform initials. Their pseudotransverse multiplicative divisions are variably oblique and result in paired fusiform initials which are much shorter than their mother fusiform initial (Fig. 2A, a,b). Therefore, the newly formed fusiform initials elongate intrusively (Fig. 2A, c,d) to equal their mother cells (Bailey 1923; Philipson & Ward 1965; Philipson et al. 1971; Larson 1994; Iqbal 1995; Evert 2006). The xylem derivatives of the fusiform initials elongate no more than 10% if at all; therefore, they quite accurately render the arrangement of their mother fusiform initials (Klinken 1914; Bailey 1920; Bannan 1965; Philipson et al. 1971; Larson 1994; Lachaud et al. 1999; Evert 2006).

The storeyed cambia (Fig. 1C) have fusiform initials in more or less long strata and the fusiform initials of adjacent strata are much less overlapped. Multiplicative divisions of the fusiform initials

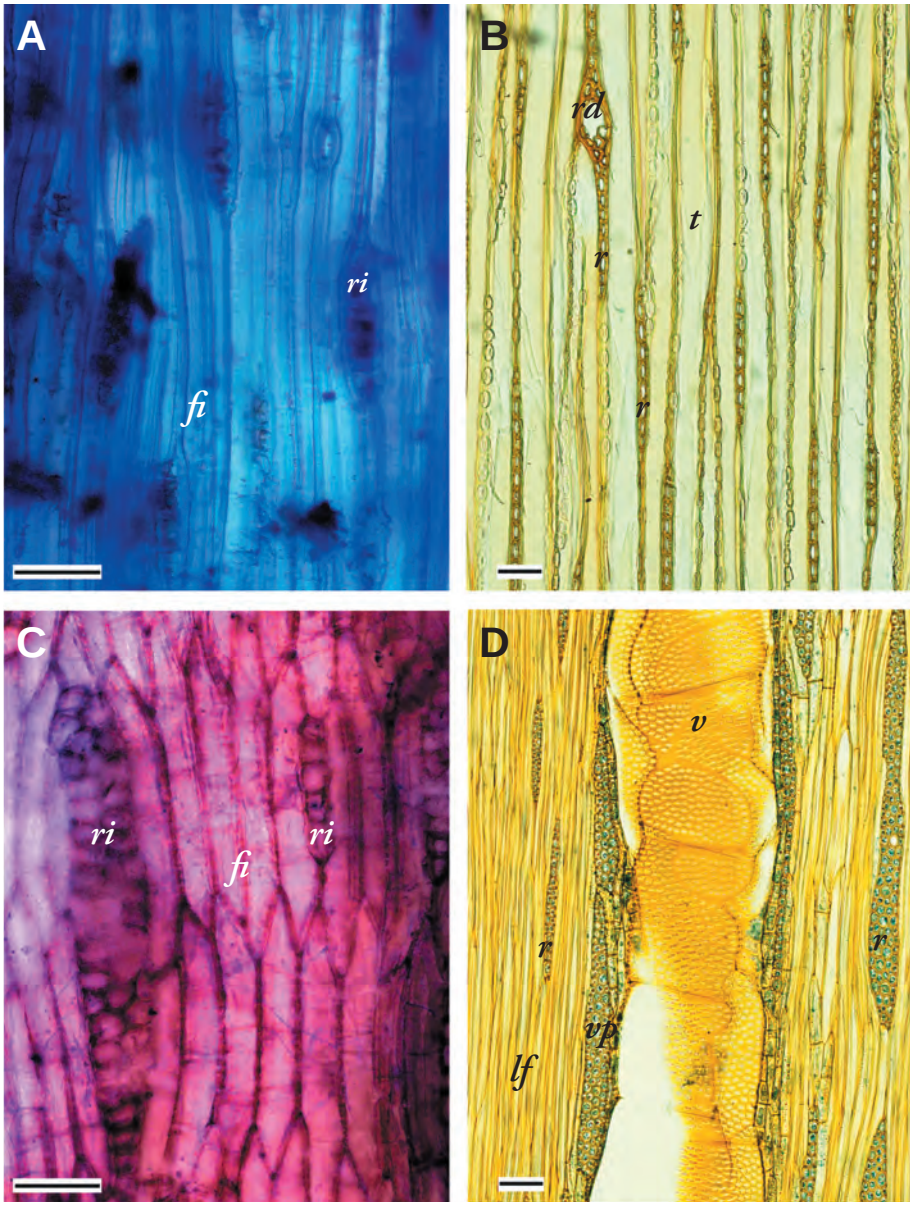

Figure 1. Cambium and its derivative xylem. A – non-storeyed cambium of *Larix* sp.; B – vesselless xylem of *Larix* sp.; C – storeyed cambium of *Robinia pseudoacacia* L.; D – vessel-bearing xylem of *Robinia pseudoacacia* L. *fi* – fusiform initial; *lf* – libriform fibre; *r* – ray; *rd* – resin duct; *ri* – ray initial; *t* – tracheid; *v* – vessel; *vp* – vasicentric parenchyma. Scale bars = 50 µm.

are generally believed to be radial (Fig. 2B, b_1) and resulting in a pair of new fusiform initials as long as their mother cell (von Höhnel 1888a; Beijer 1927; Metcalfe & Chalk 1950; Philipson & Ward 1965; Larson 1994; Carlquist 2001; Evert 2006; Krishnamurthy et al. 2015; Schmitt et al. 2016; etc.) (Fig. 2B, b). Accordingly, the newly formed fusiform initials are usually credited with an absence of intrusive elongation (Bailey 1923; Carlquist 2001; Krishnamurthy et al. 2015; Schmitt et al. 2016; etc.). The vessel elements and parenchyma strands mostly remain as long as the fusiform initials they originated from, whereas the derivative fibres usually highly elongate intrusively and often completely mask their original storeyed

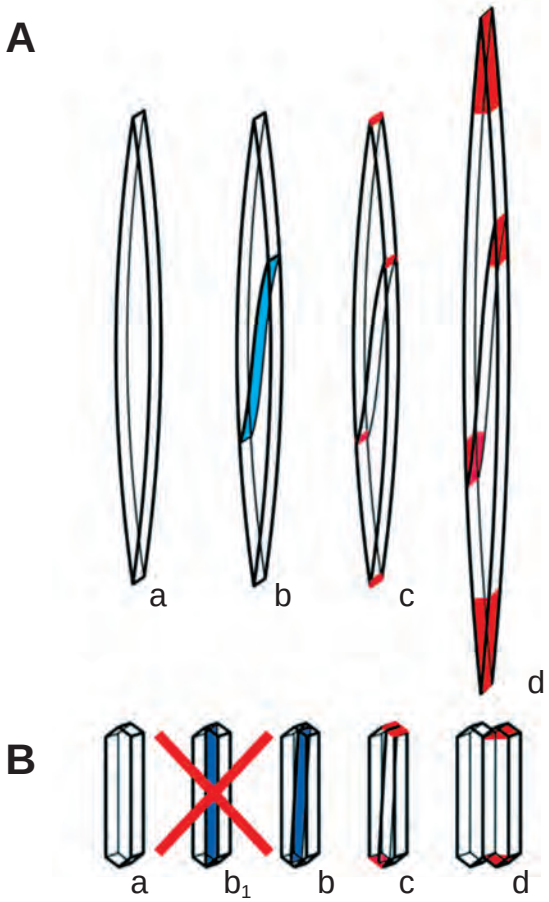

Figure 2. Multiplicative divisions of fusiform initials in the non-storeyed (A) and storeyed (B) cambia. a – original fusiform initial; b_1 – commonly described division; b – real division; c – initiation sites of the intrusive elongation; d – intrusively elongated fusiform initials.

arrangement (Neef 1920; Chattaway 1936; Larson 1994; Carlquist 2001; Zhang et al. 2002) (Fig. 1D).

Bailey (1923) interpreted the storeyed cambium as evolutionary advanced for the first time. Beijer (1927) questioned Bailey's (l.c.) interpretation, but it become generally accepted thereafter (Metcalfe & Chalk 1950; Philipson et al. 1971; Larson 1994; Iqbal 1995; Carlquist 2001; Evert 2006; etc.).

Advantages of the storeyed cambium

Bailey (1923) argued the storeyed cambium to be evolutionary advanced, because it was coupled with the shortness of the fusiform initials (this conjunction was disputed by Zhang et al. 2002), which in turn were correlated with the short wide vessel elements with only simple perforation plates. As such vessels are more efficient, then he conceptualized an evolutionary shortening of the fusiform initials. This shortening caused the (nearly longitudinal) pseudotransverse multiplicative divisions to change into radial ones resulting in transformation of the non-storeyed into the storeyed cambium. The latter is thus considered a by-product of evolutionary shortening of the fusiform initials.

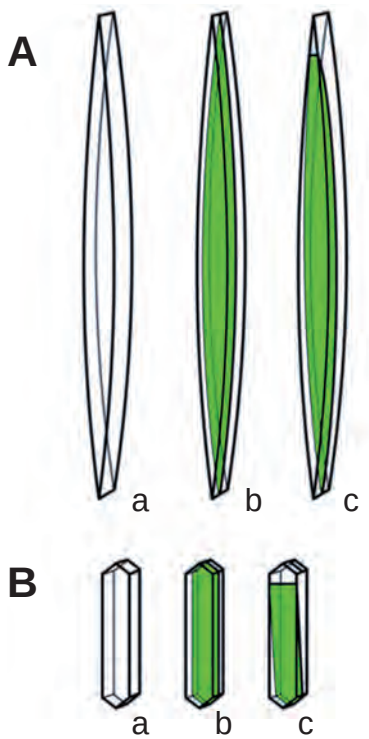

Figure 3. Additive divisions of fusiform initials in the non-storeyed (A) and storeyed (B) cambia. a – original fusiform initial; b – tangential longitudinal division; c – tangential oblique division.

Additional arguments can be assumed in favour of the evolutionary advancement of the storeyed cambium.

The storeyed cambia sporadically occur among the heteroxylic dicotyledons. They are found in a few primitive taxa (Record 1936; Carlquist 2001), but in general they clearly gravitate toward more advanced families (Bailey 1923; Record 1936; Philipson et al. 1971; Larson 1994; Carlquist 2001; Evert 2006). These cambia have been found neither in homoxylic dicotyledons nor in gymnosperms (Larson 1994; Carlquist 2001). Although absence of the vessels in dicotyledons could be a derived feature (Young 1981; Baas & Wheeler 1996; Field et al. 2002), the gymnosperms are certainly initially vesselless (Chamberlain 1935; Boureau & Marguerier 1985). Accordingly, the non-storeyed cambium should be considered as initial type of the vascular cambium of seed plants. Therefore, the storeyed cambium is a derived type which has independently evolved in some heteroxylic dicotyledons.

The fusiform initials of the non-storeyed cambia are on average much longer than those of the storeyed cambia (Bailey 1923; Metcalfe & Chalk 1950; Philipson et al. 1971; Larson 1994). Therefore, intracellular transportation and regulation are carried out over longer distances and seem to be more time consuming and less efficient in long fusiform initials of the non-storeyed cambia than in shorter fusiform initials of the storeyed ones.

The phragmoplast of the additive division runs throughout the fusiform initial length and forms a long cell plate (Fig. 3A,B, b). Accordingly, the additive divisions of the longer fusiform initials of non-storeyed cambia are likely to be more time and resource consuming than those of the shorter fusiform initials of storeyed cambia.

The phragmoplast was reported to migrate also a rather long distance, up to several millimetres, to complete the multiplicative division of the fusiform initial of non-storeyed cambium (Romberger 1963). Apparently, this is not true, because the multiplicative divisions of the fusiform initials of non-storeyed cambia are typically hard oblique to nearly transverse (Bailey 1920; Larson 1994; Iqbal 1995; etc.) and the distance the phragmoplast has to run through seems quite comparable with that the phragmoplast passes during the multiplicative division of the fusiform initials of storeyed cambia (Fig. 2A,B). However, every multiplicative division in the non-storeyed cambia is followed by the intrusive elongation of both newly formed fusiform initials (Fig. 2A, c,d) which dissolve the middle lamellae of adjacent cells, wedge between them and form new plasmodesmata and pit fields (Klinken 1914; Iqbal 1995; etc.). These processes must surely be very resource intensive. None of these processes is commonly thought to accompany the multiplicative divisions in the storeyed cambia. If so, the multiplication of the fusiform initials would be more energy efficient in the storeyed cambia.

Additional disadvantages of the non-storeyed cambia are associated with the tracheary elements they produce.

The storeyed cambia form wide to very wide vessel elements, whose width even exceeds their length in some species (Evert 2006). The vessels and tracheids formed by the non-storeyed cambia are usually much narrower (Fig. 1B, D) and their water conductance is much lower in accordance with the Poiseuille's law (Niklas & Spatz 2012).

The fusiform initials are right or left tilted (Larson 1994). Regular changing of the fusiform initial tilting results in an interlocked grain of the wood (Larson 1994; Zagórska-Marek 1995) which increases wood coherency and strength (Kojs et al. 2003; Kretschmann 2010). Tilt changing of the fusiform initials of non-storeyed cambia are feasible only by means of their infrequent multiplicative divisions (Bannan 1963; Larson 1994; Evert 2006; Włoch et al. 2013), whereas tilt changing of the fusiform initials of storeyed cambia are mostly realized by their slightest intrusive elongation irrespective of the multiplicative divisions (Zagórska-Marek 1975; Kojs et al. 2003, 2004; Evert 2006). Therefore, the fusiform initials of storeyed cambia become rearranged much more frequently (Kojs et al. 2004). Resultantly, the non-storeyed cambia produce a coarse-grained xylem which is more splittable than the finely-grained xylem produced by the storeyed cambia, in spite of a mechanism of ephemeral tilt changing of the xylem derivatives of the non-storeyed cambium (Włoch et al. 2002).

The most confusing character of the non-storeyed cambia is certainly a huge excess of formation of the fusiform initials and subsequent mass lost of newly formed ones (Bannan 1960; Philipson et al. 1971; Larson 1994; Iqbal 1995). Up to ~100% of newly formed fusiform initials are reported to be lost in some specimens (Evert 2006).

The net loss of the fusiform initials is so great that it evoked ideas about its utility for cambium functioning. The mass loss of these cells was attributed to a way of selecting more vigorous initials that are longer (Bannan & Bayly 1956; Philipson et al. 1971; Larson 1994; Evert 2006), have more contacts with the rays (Bannan & Bayly 1956; Philipson et al. 1971; Larson 1994; Evert 2006) and are capable to intensively elongate intrusively (Mauseth 1988) to push out their weaker counterparts from the initial position in the cambial zone (Włoch et al. 2013). The mass loss of newly formed fusiform initials was also thought to be a mode of maintaining efficient length of these cells (Bannan & Bayly 1956; Evert 2006) or accelerating cell tilt

Table 1. Comparative characteristics of two cambia and their xylem derivatives.

Cambium type	Intracellular communications	Path length the phragmoplast to run during the additive divisions	Intrusive elongation	Formation of the secondary plasmodesmata and pit fields	Derivative tracheary elements	Xylem texture	Loss of newly formed fusiform initials
Non-storeyed cambium	long	long	long-scaled	numerous	mostly narrow to very narrow	coarse-grained	up to 100%
Storeyed cambium	short	short	nearly absent	none	wide to very wide	finely-grained	nearly absent

changing to make the xylem finer-grained and stronger (Kojs et al. 2003). The mass loss of newly formed fusiform initials was considered a way of cambium purification from the genetic load and damaged cells (Iqbal 1995). High redundancy of the formation of fusiform initials was believed to be simply a way of relaxing the shearing strains in the cambium which are caused by increasing secondary xylem (Włoch et al. 2013).

All of the above considerations seem untenable, because selection of vigorous fusiform initials without genetic load, frequent cell tilt changing, maintaining of the efficient cell length and relaxing shearing strains are certainly equally significant for storeyed cambium functioning, but loss of its newly formed fusiform initials is minute (Larson 1994) or absent (Butterfield 1972; Włoch et al. 2013).

Eventually, the non-storeyed cambia appear less efficient than the storeyed ones regarding their functioning and the characteristic of the xylem they produce (Table 1). Therefore, the homoplasic origin and widespread occurrence of the advanced storeyed cambium should be expected. However, the storeyed cambia are surprisingly too rare. Some evolutionary constraint(s) on the transformation of non-storeyed cambium into the storeyed one should consequently be assumed.

Evolutionary constraint(s): Carlquist's approach

Carlquist seems to be the first who analyzed constraints in cambium evolution. He assumed intracambial and extracambial factors which made difficult the evolution of the non-storeyed cambium into the storeyed one.

Intracambial factor is the nature of the multiplicative divisions of the fusiform initials of the storeyed cambia. Carlquist considered these cell divisions to be strictly radial (Fig. 2B, b_1). Accordingly, "the insertion of a cell plate into a narrow cell tip may not be readily achieved" (Carlquist 2001: 301). As the cell tips of the fusiform initials of non-storeyed cambia are typically much narrower than those of the fusiform initials of storeyed cambia, the multiplicative divisions would tend to be oblique and result in the non-storeyed cambia. That is why the latter are difficult to evolve into the storeyed ones. However, the multiplicative divisions of the fusiform initials of storeyed cambia are really oblique (Beijer 1927; Larson 1994; Iqbal 1995) and the cell plates do not have to insert into very narrow cell tips of the fusiform initials (Fig. 2B, b).

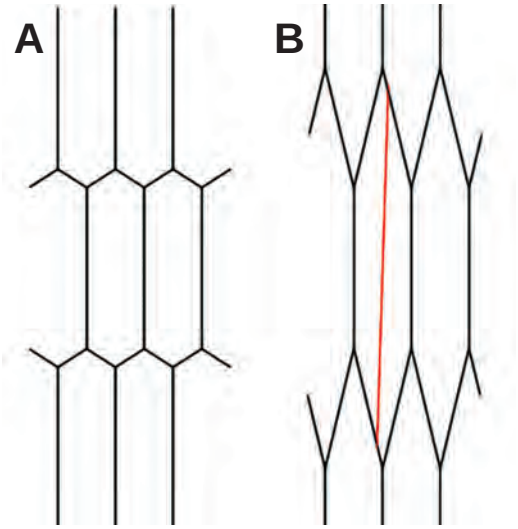

Figure 4. Real (A) and imaginable (B) border line between the fusiform initials of neighbouring storeys. red line – cell wall of multiplicative division.

The extracambial factor is the incompatibility of the storeyed cambia with the vesselless xylem, because such cambia would cause too small contiguous cell walls between the tracheids of adjacent storeys. Such cell walls would result in too high resistance to water transportation between these tracheids (Carlquist 2001; also Kedrov 2013). Indeed, the very short fusiform initials of the known storeyed cambia would be capable of generating only minimally overlapped tracheids of adjacent storeys.

However, long storeyed-arranged fusiform initials can be imaged which seem to significantly overlap with those of the adjacent storeys (Fig. 4B). Besides, the tracheids are reported to be capable of slight elongating after their origin (Larson 1994). If only the tracheids grew further on, they would be sufficiently overlapped even when originated from the short storeyed fusiform initials. Both imaginable options do not realize in nature, but their constraint(s) is/are unexplored so far.

Carlquist's (l.c.) conclusions thus seem either incorrect or incomplete, respectively.

Evolutionary constraint(s): nature of the fusiform initials

In modern biology, the causes of every phenomenon are usually sought in the genotype and in molecular mechanisms of its realization in the phenotype. Accordingly, the evolutionary constraints are now usually explained by the loss of genes or by the pleiotropic effect of gen or by too many genes determining the trait under consideration (Hoffman 2014; Hansen 2015).

Genetics of the cambium is under rapid progress now, but only in terms of comparison with the genetics of shoot/root apical meristem and procambium to cambium transition (Baucher et al. 2007; Elo et al. 2009; Du & Groover 2010; Spicer & Groover 2010; Yordanov & Busov 2011; Miyashima et al. 2013; Jouannet et al. 2015; Greb & Lohmann 2016; etc.), while comparative genetics of the non-storeyed and storeyed cambia is still unexplored. Therefore, direct assessment of the genetic causes of constraint(s) of the evolution of non-storeyed cambia into storeyed ones is impossible. However, the variety of modes the cambium develops from

the procambium and variety of cell divisions of cambial fusiform initials make feasible indirect assessment of the presumptive genetic constraint(s) of cambium evolution.

The non-storeyed cambium originates from either non-storeyed or storeyed procambium (in different species) and so is the storeyed cambium (Larson 1994). Therefore, both cambia are identical in their genesis.

The additive divisions of the fusiform initials are also identical in both cambia (Larson 1994; Iqbal 1995). They are mostly strictly longitudinal and result in a pair of equally long cells (Fig. 3A,B, b). One of them continues as the fusiform initial, whereas another becomes either xylem or phloem mother cell, respectively. Rarer, the additive divisions are tangential oblique to give rise to paired cells of unequal length (Fig. 3A,B, c). There is the shorter one that usually continues as the fusiform initial (Kojs et al. 2003). Such additive divisions thus result in shortening of the fusiform initials which can be an indication of oncoming loss of these initials (Larson 1994; Evert 2006) or their preparation to transform into ray rudiment (ray initial *sensu* Schmid 1976) (Larson 1994; Evert 2006). Such a cell shortening could be a mode to recover length of the fusiform initials of storeyed cambia after their intrusive elongation.

The newly formed fusiform initials of the non-storeyed cambia elongate intrusively to equal the length of their parent cells (Philipson & Ward 1965; Philipson et al. 1971; Larson 1994; Iqbal 1995; Carlquist 2001; Barlow et al. 2002) (Fig. 2A). The newly formed fusiform initials of the storeyed cambia are often thought to be unable elongating (Iqbal 1995; Barlow et al. 2002), but they do really elongate intrusively, though slightly (Fig. 2B), to restore their wedge-shaped tips (von Höhnel 1884a; Beijer 1927; Larson 1994; Evert 2006).

The pseudotransverse multiplicative divisions of the fusiform initials of non-storeyed cambia are traditionally contrasted with the radial multiplicative divisions of the fusiform initials of storeyed cambia (Bailey 1923; Philipson et al. 1971; Butterfield 1972; Olsson & Little 2000; Carlquist 2001; Evert 2006; Krishnamurthy et al. 2015). However, the multiplicative divisions of the latter are actually hardly oblique (Fig. 2B, b) to multiply the fusiform initials (Beijer 1927; Larson 1994) or even fairly oblique (pseudotransverse) to make them shorter or restore their storeyed arrangement (Zagórska-Marek 1984).

The fusiform initials of the non-storeyed cambia actually divide radially (Fig. 5A, b) to give rise to the ray initials via the partial lateral segmentation (Larson 1994). Such divisions are highly unequal and they are called 'transformative'. The longer daughter cell retains the fusiform initial, while the much shorter one becomes the ray rudiment which undergoes serial transverse divisions (Fig. 5A, c) to result in a strand of ray initials (ray cell initials according to Schmid 1976). The fusiform initials of non-storeyed cambia more often generate the ray rudiments by means of partial tip segmentation (Larson 1994). The short ray rudiment results from highly unequal pseudotransverse division (Fig. 5A, d) and it gives rise to a strand of ray initials by serial transverse divisions (Fig. 5A, e), whereas the longer cell elongates intrusively to equal the mother cell length and remains the fusiform initial. These unequal pseudotransverse divisions are also termed transformative.

In the storeyed cambia, the whole fusiform initials transform into the ray rudiment and undergo complete segmentation by serial transverse divisions (Larson 1994) (Fig. 5B). These serial divisions are termed 'transformative'.

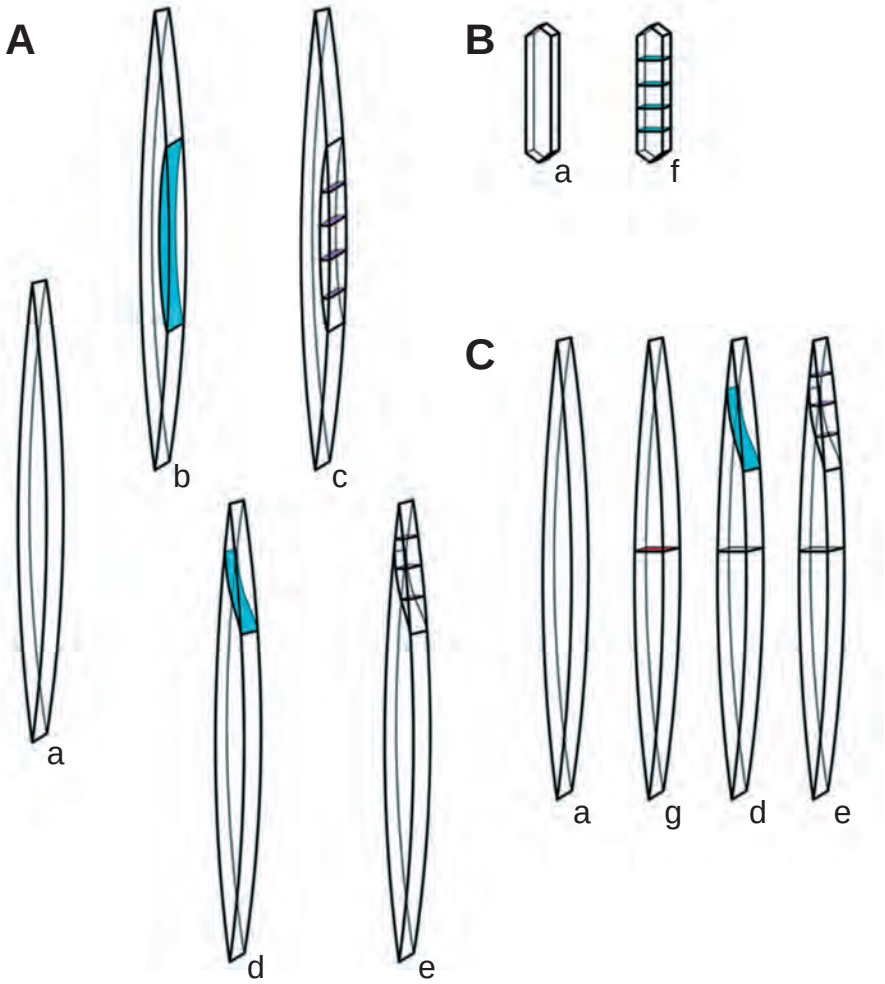

Figure 5. Transformative and preparatory divisions of fusiform initials. A – transformative divisions in non-storeyed cambium; B – transformative divisions in storeyed cambium; C – preparatory division followed by the transformative divisions in non-storeyed cambium. a – fusiform initial; a–b–c – partial lateral segmentation; a–d–e – partial tip segmentation; a–f – total segmentation; g – preparatory division; g–d–e – partial tip segmentation.

There are also almost equal single transverse divisions of the fusiform initials of storeyed cambia which result in adding a new stratum of the fusiform initials (Butterfield 1972). That is why they were termed 'multiplicative' (Butterfield l.c.).

Single, almost equal, genuine transverse divisions of the fusiform initials take place in the non-storeyed cambia (Fig. 5C, g). Such divisions are preparatory, as they antecedent the transformative division of one of the twins (and loss of another one due to its short length) (Larson 1994) (Fig. 5C). The cambia of both types thus have the same sets of fusiform initial divisions, although these divisions partly differ prospectively (Table 2).

Eventually, the fusiform initials of both-typed cambia do not differ in the processes of their genesis from the procambium, cell divisions and intrusive elongation. It is logical to assume that these identical processes are based on some identical intracellular mechanisms, the latter being determined in turn by the identical gene systems. Therefore, the molecular genetic constraints are unlikely to have affected evolution of the non-storeyed cambia into the storeyed ones. Hence,

Table 2. Sets of division types of fusiform initials in two cambia.

Cambium type	Tangential longitudinal	Tangential oblique	Transverse	Pseudo-transverse	Radial
Non-storeyed cambium	additive	additive	preparatory	multiplicative and transformative	transformative
Storeyed cambium	additive	additive	transformative	multiplicative (usually termed radial)	?

the cambial evolutionary constraint(s) should be searched for in the structure of the xylem the cambium produces. (The phloem structure is unsuitable for this searching, because correlation between the phloem structure and cambium structure is still highly underexplored).

Evolutionary constraint(s): structure of vesselless xylem

The vascular cambium is usually assumed to have arisen in the vesselless seed plants (Spicer & Groover 2010). It had to produce the (secondary) xylem whose tracheids performed the conductive and supporting functions combined. The conductivity of the vesselless xylem was determined by the intratracheidal capillary resistance and the intertracheidal resistance of pit membranes (Fig. 6A), the latter being nearly twice predominant (Sperry et al. 2006). Accordingly, the minimal numbers of sites of intertracheidal water transportation per unit xylem length (Fig. 6B,C) or, in other words, the maximally long tracheids are necessary for efficient water conducting.

The tracheids of maximal length could be attainable either by their origin from equally long fusiform initials of cambium (Fig. 7A) or by intrusive elongation of developing tracheids which have originated from the short fusiform initials (Fig. 7B). The second mode seems more resource-consuming as every tracheid would have to elongate intrusively.

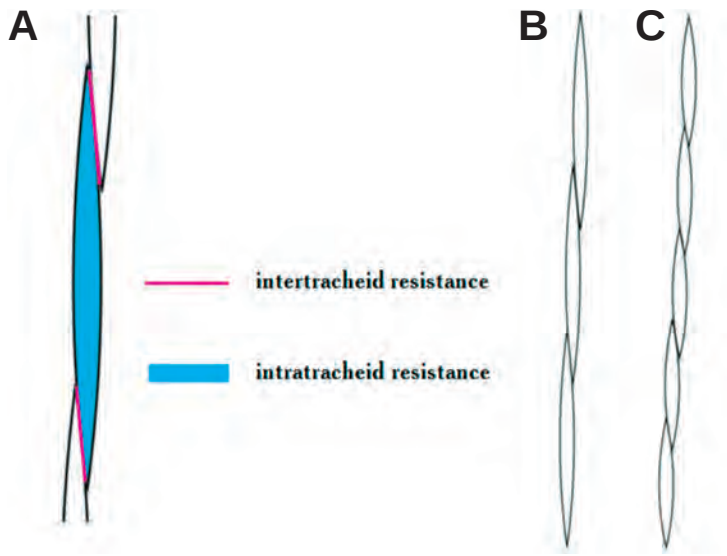

Figure 6. Principal water-transport resistance in vesselless xylem (A) and inverse relation between inter- and intratracheid resistance (B,C). red – intertracheid resistance; blue – intratracheid resistance.

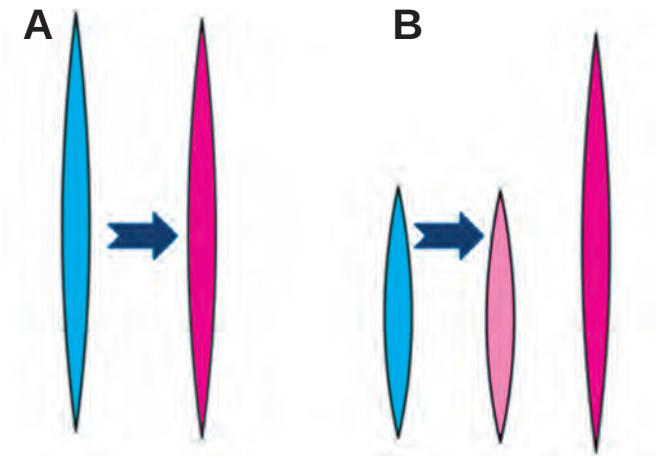

Figure 7. Origin of long tracheid from equally long (A) and short (B) fusiform initial. blue – fusiform initial; light magenta – tracheid mother cell; magenta – tracheid.

Besides, the intertracheidal resistance depends on the number of contiguous pits the consecutive tracheids intercommunicate through (Kedrov 1984; Carlquist 2001). The less are the consecutive tracheids overlapped, the less is the number of their contiguous pits, the more is the local intertracheidal resistance of the xylem (Kedrov 1984; Carlquist 2001). The more are the consecutive tracheids overlapped, the more numerous are the contiguous pits and the less is the local intertracheidal resistance of the xylem. However, the more are the tracheids overlapped, the more numerous are the sites of intertracheidal water transportation per unit xylem length, and the more is the total xylem resistance. Only some optimal overlapping of the tracheids would maintain maximum efficiency of water transportation by the vesselless xylem (Kedrov 2012) (Fig. 8).

The seed plants seem to have no mechanisms to regulate strictly the intrusive elongation of their cells and the extent the cell are overlapped. Accordingly, the optimal overlapping of the intrusively elongating cells is attainable only by chance. Furthermore, the optimal overlapping, if even been attained by certain tracheids, would never be transmitted to other elongating tracheids, because only the cambial initials are capable to transmit their relative arrangement to (some) their xylem derivatives (Larson 1994).

Finally, the intrusively elongating fusiform initials of cambium easily form secondary plasmodesmata and secondary pit fields to communicate with their new neighbouring cambial initials (Larson 1994). On the contrary, the intrusively elongating cells which have begun differentiating into elements of permanent tissue has great difficulty to form secondary pit fields (Klinken 1914) or they usually form no new pit fields at all (von Höhnel 1884b; Gorshkova 2009). Resultantly, the intrusively elongating tracheids, if they were, would have no pits in their expanding tips. Such tracheids would be useless for conducting water.

Consequently, the intrusively elongating tracheids are incompatible with the conductive functioning of the vesselless xylem. That is why the necessary long tracheids have to originate from the equally long fusiform initials of the cambium.

The long fusiform initials could be sufficiently overlapped irrespective of their storeyed or non-storeyed arrangement (Fig. 1A; 4B). The storeyed arrangement of the fusiform initials is steadily

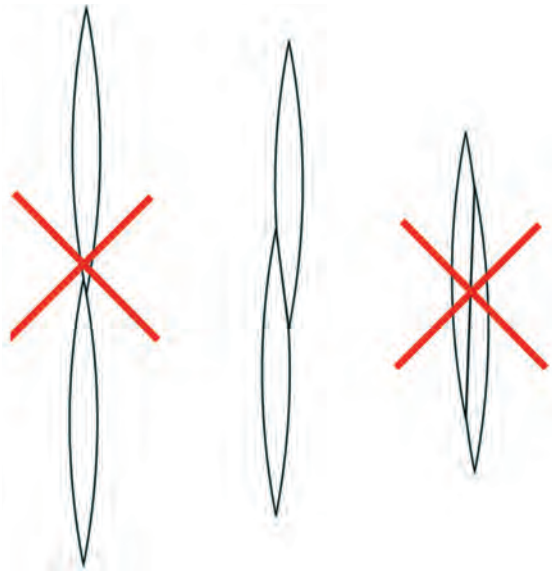

Figure 8. Optimal (in the centre) and avoidable (left and right) relative arrangements of the tracheids.

maintained only by their (nearly) radial multiplicative divisions which result in one-leveled cells (Fig. 4B). These cells would have to elongate intrusively between the cells of adjacent cambium stratum to become overlapped with all their neighbours. If so, the fusiform initials would become longer after every multiplicative division, i.e. their elongating would be unlimited. This is certainly impossible because the nucleocytoplasmic ratio is limited (Strasburger 1893; also Umen 2005). Otherwise, the specific number of completely overlapped fusiform initials would constantly increase with the secondary thickening thereby reducing the conductivity of the xylem they produce. Consequently, the storeyed cambia are incompatible with the vesselless xylem.

Long fusiform initials of the non-storeyed cambia remain significantly overlapped after any multiplicative division and they efficiently transmit their relative position to the descendant tracheids (Fig. 1B). The latter do not accordingly consume resources for intrusively elongating to become overlapped. The optimally overlapped fusiform initials can form potentially unlimited number of the optimally overlapped tracheids. However, every multiplicative division of the fusiform initials followed by their intrusive elongating disturbs the pattern of their arrangement. Only very few newly formed fusiform initials intrusively elongate to the extent to attain accidentally optimal or at least appropriate overlapping with adjacent fusiform initials. The vast majority of newly formed fusiform initials are failed and they are eliminated from the cambium so as not to produce inefficiently functioning tracheids. The non-storeyed cambia are capable of producing functional vesselless xylem albeit at the cost of mass loss of their newly formed fusiform initials.

Thus, the vesselless xylem structure is a constraint on evolving storeyed cambia.

Evolutionary constraint(s): structure of vessel-bearing xylem

Emerging of the perforations highly reduced the resistance to the water transport between the vessel elements and made possible the evolutionary shortening of the latter. Such a shortening enabled the vessel elements to increase their diameter without breakage of the nucleocytoplasmic ratio. The wider vessel elements are much more conductive in accordance with the Poiseuille's

law. Functional advantage of the wider vessels caused the evolutionary trend toward widening and shortening of the vessel elements. As the length of the vessel elements nearly equals the length of their mother fusiform initials, their evolutionary shortening had inevitably caused an evolutionary trend towards the shortening of the fusiform initials of cambium. The shorter fusiform initials should indeed be considered more advanced (Bailey 1923).

The origin of the vessels was *a sine qua non* precondition for evolutionary shortening of the fusiform initials and subsequent changing of the non-storeyed cambium into the storeyed one, yet it has been insufficient.

The vessels are sometimes up to several meters long (Zimmermann & Jeje 1981; Evert et al. 1990), but they are always shorter than the distance the xylem transports water. Therefore, there is always water transfer between successive vessels in the xylem. In most heteroxylic plants, the vessels are isolated from each other, or they are in contact but have no pits in contiguous cell walls of their elements, or the pits are too small and scanty to maintain efficient intervessel water flow (Kedrov 1968). Such vessels are communicated through the surrounding vascular and/or fibre tracheids. These tracheids should not be able to elongate, otherwise they would develop long unpitted end part that are useless for transporting water. At the same time, they have to be sufficiently overlapped to fulfill the supporting function. Only rather long non-storeyed tracheids meet these requirements. Only rather long fusiform initials of non-storeyed cambium can produce such tracheids. That is why, most heteroxylic plants have non-storeyed cambium of rather long fusiform initials, which are albeit shorter than those in the homoxylic plants.

Tracheid-mediated communication between the vessels in most heteroxylic plants limits the evolutionary shortening of cambial fusiform initials and prevents the non-storeyed cambium from changing into the storeyed one.

Constraint elimination

Occurrence of efficient direct communication between the vessels made the tracheid intermediation unnecessary. The tracheids became able to evolve into the libriform fibres which specialized exclusively in supporting function. Their functioning was less dependent on the degree of their overlapping than the functioning of tracheids, and paucity of the pits even improved their strength. Thereby, the evolutionary constraint of the intrusive elongation of the libriform fibres disappeared. The length of the libriform fibres was no longer determined by the length of the fusiform initials of cambium.

The attainment of xylem coherency was transferred from the overlapping of long cambial fusiform initials to the individual developing libriform fibres which could become up to 9-fold longer than the fusiform initials they had originated from (Chattaway 1936). Accordingly, neither long fusiform initials of the cambium nor their non-storeyed arrangement became necessary. Both the evolutionary shortening of the fusiform initials and their non-storeyed-to-storeyed rearrangement became resultantly attainable.

Conclusion

There is a close conformity between the structure of vascular cambium and the structure of xylem it produces. In ontogeny, many characters of xylem depend on the cambium structure and functioning (Carlquist 2001; Jura et al. 2005). Contrariwise, the cambium evolution has

been highly dependent on the xylem evolution. The primitive non-storeyed cambium with long narrow fusiform initials fits well to generate the efficient vesselless xylem. The extreme loss of the newly formed fusiform initials in this cambium and its other disadvantages, as well as the high resistance of the narrow tracheids it produced, caused an evolutionary trend toward shortening of the fusiform initials. However, this evolutionary trend has been blocked in homoxylic plants, because its realization would inevitably decrease the conductivity of xylem.

The occurrence of vessels made possible some shortening of the fusiform initials, but not their storeyed arrangement. The reason is that the most vessel-bearing xylems retain the tracheid-mediated intervessel water transportation. The maximal shortening of the fusiform initials and their storeyed arrangement became possible after emergence of efficient direct intervessel communications coupled with the appearance of libriform fibres, which were narrowly specialized in the supporting function and they were not involved in conducting water. It was the lack of efficient direct intervessel communications that was the evolutionary constraint that precluded the origin of advanced storeyed cambium in the most heteroxylic plants.

Occurrence of the direct intervessel communications can rightfully be considered a key aromorphosis (Iordansky 1990) or a key innovation (Liem 1974), which not only raised the organization of xylem (Braun 1970; Kedrov 2012), but also made possible the emergence of the advanced storeyed cambium in seed plants.

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