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# An Observation on Perforation Plate Differentiation in *Fagus crenata* BL., using Scanning Electron Microscopy\*

By

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走査型電子顕微鏡によるブナせん孔板の  
分化についての観察\*

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## Preface

In a previous paper of the authors<sup>9)</sup>, it was reported that warts on the vessel wall were scarcely present in the early stage and remarkably in the late stage within an annual growth increment of Japanese beech wood (*Fagus crenata* BL.), and that it seemed to be able to distinguish between the earlywood and the latewood in the annual ring of this species on the basis of the warts occurrence, putting a narrow transitional zone between them. It was also reported that simple and multiple perforation plates occurred, respectively, in the vessels belonging to the earlywood and the latewood which were defined based upon the warts occurrence stated above. In that paper, however, there was no description about season when the transitional zone, or the latewood began to form in a living

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tree. Generally speaking, the authors presented no informations concerning seasonal process of development of the annual ring of this species.

In this paper, an observation on differentiation of perforation plate of vessels at various seasonal periods of Japanese beech wood is described. The authors are specially interested in clarifying the season transitional from the earlywood formation to the latewood in the present study.

### Materials and methods

Wood samples containing the cambium and the outermost growth rings were periodically collected, at intervals of three weeks or shorter when required from June to Oct. in 1974, at the breast height of a living tree of *Fagus crenata* Bl. (Diameter B. H.: 40 cm, Height: 22 m) grown near Hakodate in the southern Hokkaido. After the removal, the wood samples were immediately placed in fixative FAA.

Solvent exchange drying using alcohol and acetone, freeze drying and critical point drying were tried to obtain the best scanning image. Because satisfactory result was obtained in the trial, the critical point drying was preferentially used for specimen preparation of this study. Procedure of this method used is as follows:

- 1) Small blocks (cubes about 4 mm per side) containing the cambium and the outermost annual rings were cut from the fixed wood samples.
- 2) The blocks were rinsed with running water and dehydrated through a graded series of ethanol.
- 3) They were then passed through absolute ethanol and isoamyl acetate (1:1), and immersed in two changes of 100% isoamyl acetate for each 30 min.
- 4) Finally, they were dried by the critical point drying method using liquid carbon dioxide as a transitional fluid.

The longitudinal radial and tangential surfaces to be observed were obtained from the dried blocks by cutting with a razor blade, or by splitting. The longitudinal tangential surface was cut slightly obliquely to radial direction so as to expose vessels as many as possible in various stages of differentiation. Specimens were finished in the form of ca. 4 mm × 4 mm × 1 mm and stuck on brass standard stubs for this electron microscope with electric conductive paint. The surface to be observed was coated with carbon and gold. Observations were made with a JSM-2 scanning electron microscope at 15 kV.

### Results

#### 1. Differentiation process of the perforation plate

##### 1) Simple perforation plate

Photo 1 depicts an early stage in the differentiation of (simple) perforation plate. It is judged from this photo that primary wall formation in both the end

wall and lateral wall of adjoining vessel elements is almost complete. The smoothed surface texture of the partition (labelled P in the photo), composed of the primary walls of the adjoining vessel elements and the intervening intercellular layer in the area to be perforated<sup>1,6)</sup>, is found to have a continuity from that of the lateral wall. Photo 2 shows a simple perforation plate of a differentiating vessel element which is in the formation stage of  $S_1$  layer. Judgment of the  $S_1$  layer, as well as  $S_2$  and  $S_3$  layers of particular vessel element was generally made on the basis of microfibrillar orientation of its lateral wall. The  $S_1$  layer is being laid down on the primary wall except the area to become the (simple) perforation and pit membranes in this photo. Perforation rim (R in the photo) which begins to form is clearly shown. Photo 3 shows simple perforation plate of a vessel element in the early stage of  $S_2$  formation. Photos 4 and 5 show the wall surface of a vessel element in the formation stage of  $S_3$  layer, but illustrating different places with each other. Photo 4 reveals the cytoplasm attached to the surfaces of both the partition of simple perforation and the lateral wall. Dispersed, filamentous organelles are found on the partition, but well oriented ones are on the lateral wall as seen at the bottom of the photo which is continuous to the top of photo 5, as marked with  $\triangle$  in both photographs. The  $S_3$  layer being deposited is clearly seen at the left in the photo 5. In this photo, both the microfibrils on the wall surface ( $S_3$ ) at the left and the filamentous cytoplasmic organelles at the right are oriented almost at a right angle to the vessel axis and thus both run in parallel with each other. Approximate coincidence of orientation of the filamentous organelles with that of the microfibrils newly deposited on the existing cell wall material was often found in the vessel elements in respective stages of the formation of  $S_1$  and  $S_2$  layers, as well as the  $S_3$  layer as shown here (cf. also photo 20). Photos 6 and 7 show partition in the vessel in which deposition of  $S_3$  layer is almost complete. Partition in this stage of vessel wall formation was similar in thickness and in surface appearance to those of preceded stages of it, i. e., in the formation of the  $S_1$  and  $S_2$  layers, at least in the present SEM observation. In other words, the partition does not reveal changes in thickness and in surface appearance throughout the deposition of the secondary wall on the lateral wall in a differentiating vessel elements.

Photo 8 depicts a stage of degradation of the partition, followed by its enlarged view in photo 9. The partition (P in the photos) are fallen down on the surface of the vessel wall revealing a fine loose structure in its appearance. An increase in thickness of the partition is found in its whole area as a result of becoming loose structure. Although the stage of partition degradation shown in photos 8 and 9 was of the earliest detected in the present observation, it must be rather advanced stage of the degradation, judging from the obvious distinction between the photos 7 and 8. The partition in the initial stage of its degradation was, however, not able to detect in spite of careful observation. Even location of the partitions shown in photos 8 and 9 was very difficult. Photos 10 and 11 show more advanced stage of the degradation compared with that in the former

two photographs. In photo 10, numerous residual fragments of the degraded partition scatter on the vessel wall. They are granular, and fibrillar in appearance as shown in photo 11. It can be seen in this photo that a part of the partition (arrowed in the photo) consisting of only fibrillar fragments remains in periphery of the perforation rim. Examples in such stage of the partition degradation are also shown in photos 12 and 13. In these photos, an increasing loose structure of the partition in a centripetal direction, having a randomly dispersed texture of the microfibrils, can be seen. Two layers which are in different progress in the degradation are found in photo 13. These are originated from the primary walls of the adjoining vessel elements, resp.

Photos 14, 15 and 16 show nearly final stage of the degradation. Disintegration of microfibrils of the partition is more proceeded than that shown in photos 12 and 13. Microfibrils of both two primary walls in the partition still remain in the case of photo 16, while those of one primary wall are still found but those of the other almost disappear in photo 15. After the subsequent degradation proceeds, microfibrils existed in places in the periphery of the perforation rim and numerous residual fragments of the degraded partition scattered on the vessel wall, simultaneously disappear. Finally, vessels with complete simple perforation plate as shown in photos 17 and 18 are formed.

## 2) Multiple perforation plate

Differentiation of the multiple perforation plate proceeds in essentially the same manner as that of the simple perforation described above. However, it is different from the latter case that partition of the multiple perforation plate remains intact until the deposition of the warty layer on the vessel wall is almost complete.

Photo 19 shows a multiple perforation plate in the adjoining vessel elements in the formation stage of  $S_1$  layer, illustrating bars (B in the photo) of the plate which begin to form by localized deposition of the  $S_1$ . Photo 20 shows a multiple perforation plate in the formation stage of  $S_3$  layer. As in photos 4 and 5, filamentous cytoplasmic organelles can be also observed in this photo. The orientation of these organelles is almost parallel to the axis of the bars. Photo 21 shows a multiple perforation plate of the vessel elements in which deposition of warty layer is almost complete on the whole surface of lateral wall as well as bars, but still remaining intact partition (arrowed in the photo) between the bars. Photo 22 shows portion of a multiple perforation plate just after the partition entirely disappeared. Only a few of the microfibrils (arrowed in the photo) constituting the partition remain in the periphery of the perforations. In photo 23, small ridges (arrowed in the photo) of the intercellular layer between borders of the perforation can be seen, although all the residual fragments of the partition entirely disappear. Membranous substance, probably residue of a degenerated plasma membrane, as shown at the right of this photo was often observed on the wall surface in mature vessels during the present observation. In the mature multiple perforation plate as shown in photo 24, the membranes (arrowed in the

photo) remain in the pit-like pores located in the margin of the plate<sup>5)</sup>.

## 2. Structure of perforation plate related to the season formed

It has already found that the secondary wall of vessel elements in beech wood is distinguished into the typical three layers<sup>3,4,9)</sup> and that warts are scarcely present in the early stage of an annual increment but remarkably in the late stage<sup>9)</sup>. Morphology of the perforation plate of the differentiating vessels was observed with samples collected from June to Oct., regarding the formation stages of the four layers, i. e.,  $S_1$ ,  $S_2$ ,  $S_3$  and warty layers.

Results obtained are summarized in Table 1.

Table 1. Seasonal development of perforation plate

Date of sampling	Type of perforation plate in differentiating vessel element in the formation stage of:				In mature vessel	
	$S_1$ layer	$S_2$ layer	$S_3$ layer	Warty layer	Type of perforation plate	Occurrence of warts
June 11	Simple	Simple	Simple	*	**	**
July 2	Simple	Simple	Simple	*	Simple	Absent
July 23	Multiple	Simple Multiple	Simple Multiple	*	Simple	Absent
Aug. 13	Multiple	Multiple	Multiple	Multiple	Simple Multiple	Absent Present
Sept. 3	*	*	Multiple	Multiple	Simple Multiple	Absent Present
Sept. 24	*	*	*	Multiple	Simple Multiple	Absent Present
Oct. 15	*	*	*	*	Simple Multiple	Absent Present

\* No differentiating vessel elements in the formation stage of the layer indicated in column were found.

\*\* No mature vessel elements were found.

In the sample collected on June 11, there were no mature vessels. All the perforation plates of differentiating vessels in the formation stage from  $S_1$  to  $S_3$  layers which were actually observed were simple, as illustrated in the Table. In the sample collected on July 2, mature vessels were first observed. Differentiating and mature perforation plates were all simple. In the sample collected on July 23, all the perforation plates of vessels in the formation stage of  $S_1$  layer were multiple, while in those of the vessels in the formation stages of  $S_2$  and  $S_3$  layers both simple and multiple were found respectively. In the stage of  $S_3$  layer formation, however, perforation plates of transitional type between the simple and multiple were often found. A typical example of such perforation plates is shown in photo 25. In the sample collected from July 2 to 23, mature vessels had all simple perforation plate and no warty layer. Warty layer formation was not yet found to begin, even in the sample of July 23.

In the sample collected on Aug. 13, all the perforation plates of differentiating vessels in the formation stage from the  $S_1$  to warty layers were multiple

(photo 20). Multiple perforation plate in the mature vessel was first found in this sample. Considering the observation of the perforation plate in the sample collected July 23, it becomes obvious that simple, multiple and transitional between the two should be found in the vessels matured during period from July 23 to Aug. 13. A representative of the multiple perforation plates found during this season is shown in photo 26. Such a plate was a sort of scalariform perforation plate with thin irregular bars. Warts were found in all the vessels with multiple perforation plate matured during this season. They were not remarkable compared with those of vessels matured after this season.

In the sample collected on Sept. 3, no differentiating vessels in the formation stages of  $S_1$  and  $S_2$  layers were found. Perforation plates of differentiating vessels in the formation stages of the  $S_3$  and warty layers were always multiple (photo 21). In the sample collected on Sept. 24, no differentiating vessels in the formation stage from  $S_1$  to  $S_3$  layers were found. All the differentiating perforation plates in the formation stage of warty layer were multiple.

In the sample collected on Oct. 15 no differentiating vessels were found. It is evident that all the vessels matured during Aug. 13 to Oct. 15 should have multiple perforation plate, according to the observation of the differentiating perforation plate in each sample collected from Aug. 13 to Sept. 24. It was also found that warts were always remarkable in the development on the wall surface of vessels matured from Aug. 13 to Oct. 15. Photo 27 shows a typical example of the multiple perforation plate matured during Aug. 13 to Sept. 3. Bars of this plate are forked irregularly and covered remarkably with the warty layer as well as the lateral wall of the vessel. Photos 23 and 28 show portion of a typical multiple perforation plate, a sort of foraminifera, matured after Sept. 3. Perforations of these plates are considerably smaller in size than those of the plate shown in photo 27, and also the development of warts on the plate is the most prominent.

### Discussion and conclusion

Differentiation of the perforation plate of vessel has attracted attention of many investigators. According to their works<sup>1,6,12</sup>, the primary walls and the intervening intercellular layer in the region of the perforation remain intact until the deposition of the secondary wall of vessel is almost complete. This fact was also confirmed in beech wood in the present study.

Although some observations concerning the degradation process of the partition have been reported recently, they have not revealed entirely the same result. SASSEN<sup>11</sup>) has investigated the breakdown process of the perforation plates in the central vessel of *Hordeum* and concluded that end wall first swells, it is then detached in its entirety from the lateral wall, after which the breakdown process is completed, and that this breakdown is made by enzymes present in the intercellular layer and primary walls of the end wall after the death of the cell protoplast. As a result of the SEM study of perforation plate development in *Knightsia*, MEYLAN and BUTTERFIELD<sup>6</sup>) have described that at a stage very late

in the process of cell differentiation, and possibly close to the time of death of the protoplast, the perforation partition begins to take on a more granular appearance as if the entire structure has been subjected to some enzymatic action, and that portion of this granular material then disappear, leaving numerous small holes in the partition. According to the electron microscopic study of the scalariform perforation plate in *Liriodendron* by THOMAS and BONNER<sup>12)</sup>, plate membrane removal begins with matrix degradation and almost simultaneous removal of the microfibril component after bar formation. They have also indicated a series of microphotographs, showing a decrease in thickness and a destruction of the membrane layers as development progresses. These results have revealed more or less different process of partition degradation, while it has in agreement been proposed by many researchers<sup>1,5-8,11-13)</sup> that the partition is degraded by the enzymatic action.

So far as the present authors detected, the stage shown in photos 8 and 9 is of the earliest of the partition degradation. Because of the obvious distinction between photos 7 and 8, however, this stage is considered to be rather advanced stage of the partition degradation. If matrix substance is first and microfibril component is then removed<sup>7,8)</sup>, even if removal of the both materials, more or less, overlaps, the partition consisting of only the microfibril component must be found. But such partitions were not detected in the present study, with the exception near the periphery of perforation rim where microfibrils fragments remain until late. As pointed out by THOMAS and BONNER<sup>12)</sup>, therefore, it is rather reasonable to assume that removal of the matrix and microfibril of the partition is made almost simultaneously, but being late in microfibril component in the periphery of the perforation rim, as shown in photos 11, 12 and 13. The microfibrils remained there are then probably slowly removed, because the stage as shown in photo 10-16 was often and easily observed. It was confirmed in the present study that residual fragments of the degraded partition scattered on the surface of vessel wall until removal of microfibril component remaining in the periphery of the rim was almost finished by the enzymatic action. This fact, thus, suggests that transpiration stream in the vessel does not effectively begin in this stage. Finally all the fragments on the periphery of the rim and on the surface of vessel wall disappear by subsequent transpiration stream as the vessel becomes functional for water conduction. In short, it is concluded that degradation of the partition is essentially not made by the mechanical force, such as transpiration stream, but by the enzymatic action.

Investigation into the relationship of cytoplasmic organelles in differentiating vessels to the partition is of very importance to elucidate the mechanism of the partition degradation, although detailed observations were not carried out in the present study. Thus, further study of this problem should be required. Filamentous cytoplasmic organelles in differentiating vessels as shown in photos 5 and 20 were often found in the present study. Filamentous organelles of the differentiating vessels during the secondary wall formation apart from its outermost part



were always oriented parallel to the direction of the microfibrils of the most recently deposited wall material. Based on shape and location of the filamentous organelles observed, they must be concerned with microtubules although diameter of the former does not coincide with that of the latter<sup>2)</sup>. Although the disagreement in both diameter is considered to be caused by the difference of fixation, drying method and so on, it must be further investigated to determine nature of the filamentous organelles.

PARHAM and BAIRD<sup>10)</sup> have investigated the occurrence and distribution of the warty layer in hardwoods having both scalariform and simple perforation plates, and concluded that warts are generally found in the more primitive-type vessel elements and the more advanced vessel types rarely display a warty layer. This evidence described by them<sup>10)</sup> is clearly accepted in the vessel of *Fagus crenata* BL. in the present study. Their evidence is also exactly applicable to vessels having multiple perforation plates in this species. The occurrence of warts in vessel elements becomes more remarkable, as the multiple perforation plates of them are more primitive. Especially, it is interesting that the occurrence of warts in vessel elements is also closely related to the season of the vessels formation during one growth season (and, therefore, the position of the vessel within an annual ring).

From the observation of the seasonal development of the perforation plate and warts shown in Table 1, following matters are noticeable. Mature vessel elements with both typical multiple perforation plate and warts were first found in the sample collected on Aug. 13, although those with simple and transitional perforation plates without warty layer were also found in it. According to this, therefore, it can be said that the demarcation between the earlywood and the latewood used by the authors is not unreasonable. From the detail illustrating in Table 1, it is found that the transitional or the typical latewood of this species begins to form in the middle of July, and they begin to appear as mature ones early in Aug., in southern Hokkaido where the samples were taken.

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## 要 約

ブナ (*Fagus crenata* BL.) の道管のせん孔板の分化過程およびせん孔板の季節的発達経過を走査型電子顕微鏡により観察した。

供試木は、函館営林局函館営林署西岐担当区内に正常に生育していたブナ生立木1本であり、その胸高直径は40 cm、樹高22 mであった。供試木の胸高付近より形成層を含む小ブロックを、1974年6月11日から10月15日まで原則として3週間ごとに採取した。採取後、試料を直ちにFAAで固定した。水洗後、試料を主として臨界点乾燥法により乾燥し、検鏡用試料の観察表面に炭素一金の二重蒸着を施し、走査型電子顕微鏡(JSM-2型)で観察した。

得られた結果を要約すると、次の通りである。

1) せん孔板の partition (隣接している道管要素の1次壁とその間に介在している細胞間層よりなる) は、その道管の細胞壁が殆んど完成するまで存在している。即ち、単せん孔の partition は、その道管の S<sub>2</sub> 層が、また、多孔せん孔の partition は、その道管のいぼ状層が殆んど完成するまで存在している。

2) partition の崩壊は、まず partition 全領域の非晶物質とせん孔縁周縁部を除く部分のセルロース・ミクロフィブリルの殆んど同時の分解により始まる。これらの分解は、とくにその初期において極めて急速に行われ、酵素によるものと考えられる。その後、せん孔縁周縁部

の partition のセルロース・ミクロフィブリルは比較的ゆっくり酵素により分解される。最後にせん孔縁上に残っているごく僅かのミクロフィブリルおよび道管壁上に散在している崩壊した partition の残渣は、蒸散の流れにより除去されると考えられる。

3) せん孔板の季節的発達経過の観察結果は、表 1 に示されている。表 1 に示されるそれぞれの採取試料についての分化段階にある道管および成熟した道管のせん孔板の形態といぼ状突起の出現の観察結果をもとにして、1 生長期間での成熟した道管における単せん孔から多孔せん孔へ移行する時期といぼ状突起の出現する時期は一致し、その時期は 7 月下旬から 8 月上旬の間であることが認められた。以上の結果から、ブナ道管のせん孔板の形態およびいぼ状突起の出現をもとにして、ブナ材の早・晩材を区別することは可能であり、かつ合理的と考えられる。

### Explanation of photographs

**Note:** The vessel axis is vertical in all photographs (↑). All the photographs except photo 1 were taken from the specimens prepared through the crytical point drying method.

Date of sampling is entered in the end of each explanation.

**Key to labeling:** B=Bar, C=Cytoplasm, P=Partition, R=Perforation rim,

S<sub>1</sub>=Outer layer of the secondary wall,

S<sub>2</sub>=Middle layer of the secondary wall,

S<sub>3</sub>=Inner layer of the secondary wall.

- Photo 1.** Portion of (simple) perforation of a differentiating vessel in the formation stage of primary wall. This specimen was prepared through the freeze drying method. July 2.
- Photo 2.** Portion of simple perforation of a differentiating vessel in the formation stage of S<sub>1</sub> layer. July 2.
- Photo 3.** Portion of simple perforation of a differentiating vessel in the early stage of S<sub>2</sub> layer formation. July 12.
- Photo 4.** A surface view of portion of a differentiating vessel in the formation stage of S<sub>3</sub> layer, showing cytoplasm attached to wall surface of the partition of simple perforation and the S<sub>3</sub> layer. July 23.
- Photo 5.** Wall surface of a differentiating vessel in the formation stage of S<sub>3</sub> layer. Portion of the wall surface shown in this photo is situated just beneath the vessel wall in photo 4. Mark △ in photos 4 and 5 indicates the same place of the vessel observed. July 23.
- Photo 6.** A differentiating simple perforation between two adjoining vessel elements exposed by a tangential cut. Although deposition of S<sub>3</sub> layer is almost complete, the partition is still intact. July 23.
- Photo 7.** Portion of simple perforation of a vessel in which the deposition of S<sub>3</sub> layer is almost complete. July 23.
- Photo 8.** More or less advanced stage of the partition degradation of simple perforation showing loose structure of it. July 12.
- Photo 9.** An enlarged view of the partition in photo 8. July 12.
- Photo 10.** An advanced stage of the partition degradation of simple perforation. Note numerous residual fragments of the degraded partition scattered on the wall surface of the vessel. July 12.
- Photo 11.** A part of photo 10. Arrows indicate the partition remaining in the periphery of the rim. July 12.
- Photo 12.** Portion of the partition remaining in the periphery of the rim of a simple perforation, showing dispersed microfibrils. July 12.
- Photo 13.** Portion of the partition remaining in the periphery of the rim of a simple perforation, showing two separate primary walls of adjoining vessel elements. July 12.

- Photo 14.** A more advanced stage of the partition degradation of a simple perforation. July 12.
- Photo 15.** An enlarged view of a part of photo 14. July 12.
- Photo 16.** Portion of a degraded partition, showing a more advanced stage of the partition degradation of a simple perforation. July 12.
- Photo 17.** A mature simple perforation. No residual fragments of the degraded partition can be seen in the wall surface of vessel. (Compare with photo 10). July 23.
- Photo 18.** An enlarged view of a part of photo 17. July 23.
- Photo 19.** Portion of multiple perforation plate of a differentiating vessel in the formation stage of  $S_1$  layer. July 23.
- Photo 20.** A surface view of portion of multiple perforation plate of a differentiating vessel in the formation stage of  $S_3$  layer, showing filamentous cytoplasmic organelles attached to the inner surface of the plate. Aug. 13.
- Photo 21.** Portion of a differentiating multiple perforation plate. Although the deposition of warty layer is almost complete, the partition (arrowed in this photo) is still intact. Sept. 3.
- Photo 22.** Portion of a mature multiple perforation plate. Arrows indicate microfibrils of the partition. Sept. 3.
- Photo 23.** Portion of a mature multiple perforation plate. Arrows indicate the small ridges of the intercellular layer between borders of the perforations. Sept. 24.
- Photo 24.** Portion of a mature multiple perforation plate. Arrows indicate the membranes existing in the pit-like pores of the lower end of a multiple perforation plate. Aug. 23.
- Photo 25.** Portion of a perforation plate of the transitional type between simple and multiple perforation plates of a differentiating vessel in the formation stage of  $S_3$  layer found in sample collected on July 23.
- Photo 26.** A typical multiple perforation plate matured from July 23 to Aug. 13.
- Photo 27.** A typical multiple perforation plate matured from Aug. 13 to Sept. 3.
- Photo 28.** Portion of a typical multiple perforation plate matured after Sept. 3.



















