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Extraction and analysis of the tangential arrangement of cambial cells in Japanese hardwood species

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Abstract Arrangement of the cambium and marginal zone of xylem were illustrated by drawing the outlines of fusiform cells and tissue on serial tangential sections in *Zelkova serrata* and *Robinia pseudoacacia* with storied cambium and in *Albizia julibrissin* with non-storied cambium. The comparison of “cambium drawings” with “parenchyma drawings” revealed that the lengths and arrangement of parenchyma strands correspond closely with those of corresponding fusiform initials in all three species with storied or non-storied cambium. Based on this result, we deduced the correlation between length and variation of length and arrangement of the fusiform initials in 20 species of Japanese hardwoods using parenchyma drawings instead of cambium drawings. The species with a smaller variation of length tend to exhibit a storied arrangement regardless of the length of the fusiform initial. Thus, it is suggested that the variation of the length of the fusiform initial has more influence than the length itself on the development of the storied arrangement.

Key words Fusiform cambial cell · Parenchyma strand · Storied arrangement · Japanese hardwoods

Introduction

Xylem and phloem cells are derived from cambial initials, so the shapes and arrangements of those cells in a tangential plane are based on those of the cambial initials.¹ Bannan² traced the development of cambium on phloem and xylem serial tangential sections of *Thuja occidentalis*, whose tracheids mostly keep their original shapes during differenti-

ation. In hardwoods, however, elongation during differentiation from cambial initials to wood fibers and extreme expansion to vessel elements obscure their original shapes and arrangements.

Bailey³ suggested that the lengths of vessel elements indicate the lengths of fusiform initials, whereas some reports^{4–6} indicated that it is necessary to study further the relation between lengths of vessel elements and those of fusiform initials. On the other hand, axial parenchyma strands in many hardwoods are assumed not to have elongated and are thus supposed to be the same length as the fusiform initials from which they were derived.⁴ Especially in some hardwoods, parenchyma strands occupy the marginal zone of the annual ring, so this marginal zone could be considered to approximate the features of the cambial zone.^{7–10} Other investigators have cautioned that parenchyma strands might not accurately reflect the lengths of fusiform initials.¹¹ Therefore, it is necessary to examine the relation between the length of parenchyma strands and that of the fusiform initial by comparing the lengths of corresponding cells. For this purpose, cell shapes and arrangement were illustrated on serial tangential sections by drawing the outline of the fusiform cells and rays in the cambial zone and the parenchyma strands and rays in the xylem in three hardwood species. The “cambium drawings” and “parenchyma drawings” were then compared.

Takamatsu¹² directly observed the cambial zone of 150 species belonging to 50 families and classified them into four types. Takamatsu's report is important in that the cell shapes and their arrangements were observed directly in the cambium. It is uncertain, however, which part (e.g., stem or branch) of the trees were used, how old the trees were, how many cells were investigated, and how large were the areas surveyed.

In most cases, it is difficult to survey parenchyma strands in sufficient numbers from a sufficient area in a single microscopic field. To overcome this difficulty, we reconstructed parenchyma band layers including numerous parenchyma strands in a large area from serial tangential sections. The correlation between the length and the variation of length and the arrangement of the paren-

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chyma strands in 20 species of Japanese hardwoods using parenchyma drawings (cambium drawings for *Fagus crenata*) were also investigated in the present study. These species are of marginal or banded axial parenchyma except *Fagus crenata*. To evaluate the degree of development of the storied arrangement, Takamatsu's categories of the cambium type¹² were used, and the cell arrangement was analyzed using the "dot map" analysis method developed by Fujita et al.¹³ The variance of the lengths of parenchyma strands was expressed as the coefficient of variation (CV).

Materials and methods

Specimen preparation

Twenty species (23 samples) of Japanese hardwoods with marginal or banded axial parenchyma (except *Fagus crenata*) were used in this study, as shown in Tables 1 and 2.

Trees of *Zelkova serrata* (sample 7': diameter at breast height (DBH) 15 cm; 19 annual rings); *Robinia pseudoacacia* (sample 10': DBH 9 cm; 9 annual rings); *Albizia julibrissin* (sample 9': DBH 12 cm; 20 annual rings); and *Fagus crenata* (sample 5: DBH 11 cm; 15 annual rings) were selected as representative of each of Takamatsu's type and were felled in July 1998 in the Kamigamo Experimental Forest of Kyoto University, Kyoto, Japan. Fresh samples

Table 1. Changes in the length of parenchyma strand to fusiform cambial cell in three species

Sample no.	Species	N-cC (%)	Changes in length (%)			MD (mm)	Mean (mm)		Ratio (%) (MD/mean)	Counts	Type
			L	E	S		Cambium	Xylem			
7'	<i>Zelkova serrata</i>	2.1	52	7	41	0.03	0.22	0.21	11.4	108	II
10'	<i>Robinia pseudoacacia</i>	7.1	45	37	18	0.01	0.17	0.17	5.9	84	I
9'	<i>Albizia julibrissin</i>	2.8	68	6	26	0.02	0.35	0.34	5.7	72	III

N-cC, non-corresponding cell; L, parenchyma strand is longer than fusiform cambial cell; E, parenchyma strand is equivalent to fusiform cambial cell; S, parenchyma strand is shorter than fusiform cambial cell; MD, mean of the difference of the length between the fusiform cambial cell and the parenchyma strand; Mean, mean length of the fusiform cambial cell or parenchyma strand; Ratio, mean difference of the length/mean length of the fusiform cambial cell ratio; Counts, number of measured fusiform cambial cell or parenchyma strand; Type I, regularly stratified cambium; Type II, slightly stratified cambium; Type III, non-stratified cambium

Table 2. Length of xylem parenchyma strand in 20 species (23 samples) of Japanese hardwoods

Sample no.	Common name	Scientific name	Family name	Mean (mm)	SD	CV	Counts	Type
1	Doronoki	<i>Populus maximowiczii</i>	Salicaceae	0.67	0.16	0.23	237	IV
2	Onigurumi	<i>Juglans sieboldiana</i>	Juglandaceae	0.66	0.12	0.19	179	IV
3	Sawagurumi	<i>Pterocarya rhoifolia</i>	Juglandaceae	0.65	0.17	0.26	263	IV
4	Mizume	<i>Betula grossa</i>	Betulaceae	0.67	0.15	0.23	212	IV
5	Buna ^a	<i>Fagus crenata</i> ^a	Fagaceae	0.54	0.15	0.27	136	IV
6	Harunire	<i>Ulmus propinqua</i>	Ulmaceae	0.25	0.03	0.12	390	II
7	Keyaki	<i>Zelkova serrata</i>	Ulmaceae	0.22	0.06	0.25	346	II
7'	Keyaki	<i>Zelkova serrata</i>	Ulmaceae	0.21	0.03	0.15	108	II
8	Hōnoki	<i>Magnolia obovata</i>	Magnoliaceae	0.69	0.19	0.28	136	IV
9	Nemunoki	<i>Albizia julibrissin</i>	Leguminosae	0.35	0.05	0.15	237	III
9'	Nemunoki	<i>Albizia julibrissin</i>	Leguminosae	0.34	0.04	0.14	72	III
10	Harienu	<i>Robinia pseudoacacia</i>	Leguminosae	0.17	0.02	0.14	451	I
10'	Harienu	<i>Robinia pseudoacacia</i>	Leguminosae	0.17	0.02	0.13	84	I
11	Inuenju ^a	<i>Maackia amurensis</i> var. <i>burergeri</i> ^a	Leguminosae	0.15	0.02	0.11	380	I
12	Itayakaede	<i>Acer pictum</i>	Aceraceae	0.30	0.05	0.17	247	III
13	Tochinoki	<i>Aesculus turbinata</i>	Hippocastanaceae	0.52	0.07	0.13	185	I
14	Shinanoki	<i>Tilia japonica</i>	Tiliaceae	0.61	0.08	0.12	241	I
15	Aogiri	<i>Firmiana platanifolia</i>	Sterculiaceae	0.25	0.03	0.10	230	I
16	Kaki	<i>Diospyros kaki</i>	Ebenaceae	0.39	0.03	0.08	106	I
17	Yachidamo	<i>Fraxinus mandshurica</i>	Oleaceae	0.26	0.03	0.12	380	II
18	Aodamo	<i>Fraxinus sieboldiana</i>	Oleaceae	0.38	0.07	0.17	415	III
19	Shioji	<i>Fraxinus commemoralis</i>	Oleaceae	0.32	0.03	0.11	170	II
20	Kiri ^a	<i>Paulownia tomentosa</i> ^a	Bignoniaceae	0.28	0.06	0.22	384	III

Among the samples, 5, 7', 9', and 10' were sampled from leaving trees, and others were sampled from a wood collection; Mean, mean length of xylem parenchyma strands; SD, standard deviation; CV, coefficient of variation; Counts, number of measured xylem parenchyma strands; Type I, regularly stratified cambium; Type II, slightly stratified cambium; Type III, non-stratified cambium; Type IV, irregular cambium

^aIn the case of *Fagus crenata*, a cambial tangential section was investigated because of lack of marginal or banded axial parenchyma. In the case of *Maackia amurensis* var. *burergeri*, narrow vessel elements at the end of the annual ring in clusters were investigated. In the case of *Paulownia tomentosa*, banded axial parenchyma was investigated

were obtained at breast height and immediately fixed with 3% glutaraldehyde in 1/15M phosphate buffer at pH 7.2. Small blocks (tangential faces of about 4×3 mm and radial lengths of about 10 mm) containing cambium and the adjacent phloem and xylem that included at least one annual ring were taken from the samples and were embedded in the softest epoxy resin following the Luft method.¹⁴ Each series of tangential sections, starting from the phloem to the outermost annual ring in the xylem, was cut from the embedded blocks with an ordinary sliding microtome. These sections ($20\mu\text{m}$ thick) were stained with safranin and mounted with Canada balsam after washing and drying.

For other samples, wood blocks containing one or two annual rings were prepared from the wood collection (collected from large trees) stored in our laboratory. These blocks with tangential faces of about 15×8 mm and radial lengths of about 15 mm were boiled in water for 2–12 h to soften the woods. Then serial tangential sections including marginal parenchyma or banded parenchyma were cut with a sliding microtome. These sections ($20\mu\text{m}$ thick) were stained with safranin and mounted with Canada balsam after passing through a graded alcohol series.

Drawing preparation and data analysis

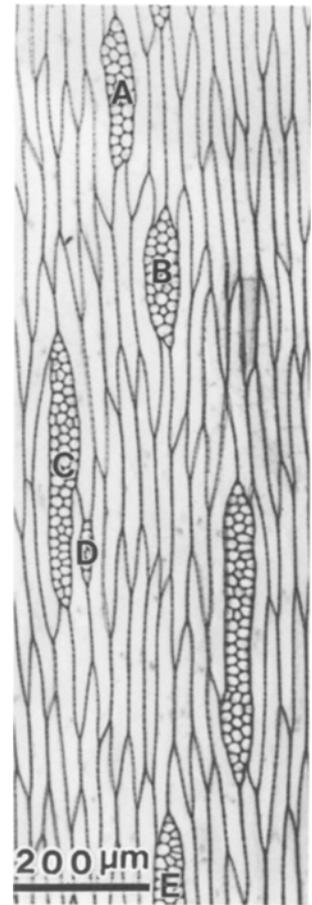
Although cells in the cambial zone can be observed on the serial tangential sections, it is difficult to distinguish the initials from their most recent derivatives. Thus the term “cambial cells” instead of cambial initials is used in this paper.

Among the serial tangential sections, those including numerous fusiform cambial cells or parenchyma strands were selected by microscopic examination of the 23 samples. Photographs were taken continuously in each series with a $10 \times$ objective and $2.5 \times$ ocular lens, and the films were enlarged onto photographic papers (Fig. 1). These photographs were used for tracing, as described below.

Outlines of each fusiform cambial cell and ray were traced on tracing paper in the cambial zone, as were those of each parenchyma strand and ray in the xylem. When the number of fusiform cambial cells or parenchyma strands were not sufficient to investigate or the ends of those were difficult to identify on a single section, the tracing was compensated from adjacent sections. In this way, a drawing that included sufficient fusiform cambial cells or parenchyma strands in a large area was prepared for each of the 23 samples (Fig. 2).

For the three species of fresh samples, the length of each fusiform cambial cell and corresponding parenchyma strand were measured on the respective drawings. Then the difference of the length between them and the mean of the difference were calculated. Additionally, the mean length of the fusiform cambial cell and parenchyma strand as well as the mean difference of the length/mean length of the fusiform cambial cell ratio were calculated (Table 1). In the case of *Fagus crenata*, only the lengths of fusiform cambial cells were measured on the cambium drawings because of lack of marginal or banded axial parenchyma.

Fig. 1. Tangential section of *Albizia julibrissin* cambial zone with non-storied arrangement. A–E, rays (traced in Fig. 2)



On the other hand, the length of each parenchyma strand was measured from the respective parenchyma drawings, and then the mean length, standard deviation, and coefficient of variation in length were calculated (Table 2). The coefficient of variation was defined by the following formula.

$$CV = SD/\bar{x}$$

where CV is the coefficient of variation; SD is the standard deviation; and \bar{x} is the mean length.

Classification of cell arrangement

Because it is difficult to distinguish between types III and IV described by Takamatsu,¹² the “dot map” developed by Fujita et al.¹³ was introduced to classify the cell arrangement. Small dots were placed at the center of all parenchyma strands (fusiform cambial cells in *Fagus crenata*) of each drawing, and so the dot map was produced. Based on the visual judgment of the dot map, which reflects the information only of cell arrangement,¹³ the cell arrangements in 20 species were classified into the following four types, referring to Takamatsu’s categories.¹²

Type I, regularly stratified cambium: The cells are arranged quite regularly in horizontal layers (Fig. 3a).

Type II, slightly stratified cambium: This is the transitional form from type I to type III. Although the cell arrange-

Fig. 2. Cambium and parenchyma drawings of *Albizia julibrissin*. A–J, rays; 1–74, fusiform cambial cells in the cambium and the corresponding parenchyma strands in the xylem; *, non-corresponding cells

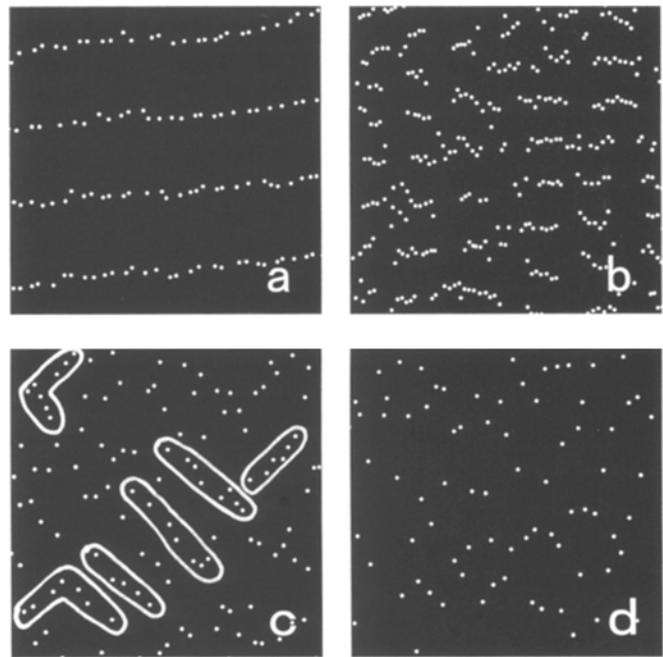
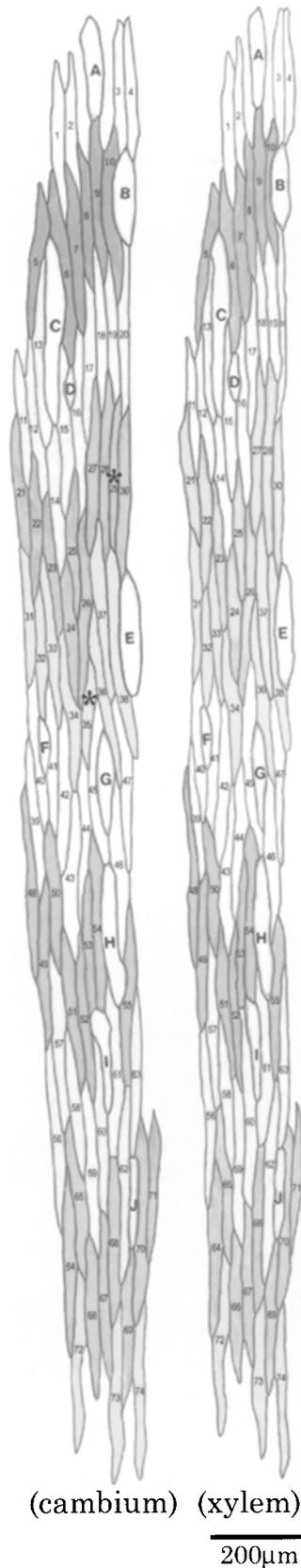


Fig. 3. “Dot maps” of parenchyma strands. **a** *Diospyros kaki* showing type I. **b** *Zelkova serrata* showing type II. **c** *Fraxinus Sieboldiana* showing type III. Parenchyma strands are arranged obliquely or in zigzags in small groups. **d** *Populus Maximowiczii* showing type IV. Refer to Table 2 for types I–IV

ment is not quite regular like type I is, the cells still appear to be in horizontal rows (Fig. 3b).

Type III, non-stratified cambium: Horizontal arrangement of the cells is no longer perceptible, and the cells are arranged obliquely or in zigzag formation in small groups (Fig. 3c).

Type IV, irregular cambium: The arrangement of the cells has no regularity; that is, the cells are randomly arranged (Fig. 3d).

Results and discussion

Relation between cambium and parenchyma drawings

Every ray and fusiform cambial cell in the cambium drawings were lettered and numbered, for the three species (*Zelkova serrata*, *Robinia pseudoacacia*, *Albizia julibrissin*), respectively. Corresponding rays in the parenchyma drawings were lettered using the same letters, and corresponding parenchyma strands were identified and numbered using the same Arabic numerals. Cells in the cambium drawings that did not correspond to any parenchyma strands in the parenchyma drawings (i.e., non-corresponding cells) were marked by asterisks (Fig. 2). After measuring the lengths of these corresponding cells and strands, the lengths between the corresponding cells and strands were compared. The cell arrangements were also compared.

As shown in Table 1, although there is almost no change in mean length between fusiform cambial cells and paren-

chyma strands, three differences were detected during the length comparison of the corresponding cells and strands in all three species. Although some parenchyma strands tended to be longer than their corresponding fusiform cambial cells, some were the same length, and some tended to be shorter. This indicates that elongation and shrinkage occur during the differentiation from fusiform initials to parenchyma strands. It is important for the present study to determine whether the change in length that occurred during differentiation is significant. Each length difference of corresponding cells was obtained by subtracting the parenchyma strand length from the length of the fusiform cambial cell. The mean difference (MD) was calculated by dividing the sum of the absolute values of each length difference by the total number of measured fusiform cambial cells, and the MD/mean length of the fusiform cambial cell ratio was calculated. It was 11.4% for *Zelkova serrata*, 5.9% for *Robinia pseudoacacia*, and 5.7% for *Albizia julibrissin*. As far as the object of the present study is concerned, such a difference is possible. On the other hand, the percentage of non-corresponding cells was 2.1% in *Zelkova serrata*, 7.1% in *Robinia pseudoacacia*, and 2.8% in *Albizia julibrissin*. Although there were 2%–7% non-corresponding cells, the cell arrangements in the xylem showed patterns essentially similar to those in the cambium in all three species. This result suggests that the parenchyma drawings nearly coincide with the cambium drawings regardless of the development of the storied arrangement, although there are some differences in length and some non-corresponding cells. Thus it is possible to deduce the correlation between the length and variation of length and arrangement of the fusiform initials using the parenchyma drawings instead of the cambium drawings for the tree species with marginal or banded axial parenchyma.

Cell arrangement

To evaluate the degree of development of the storied arrangement, parenchyma drawings (cambium drawings for *Fagus crenata*) of 20 species were investigated and classified into four types based on Takamatsu's categories and visual judgment of the dot maps (Table 2). As a result,

six species were classified as type I, four species as type II, four species as type III, and six species as type IV.

Our results did not fully coincide with those of Takamatsu. For example, *Diospyros kaki* was classified as type I in the present study but type II in Takamatsu's investigation; *Ulmus propinqua* and *Fraxinus mandshurica* were classified as type II in the former but type III in the latter; *Paulownia tomentosa* was classified as type III in the former but type II in the latter; and *Populus Maximowiczii* was classified as type IV in the former but type III in the latter.

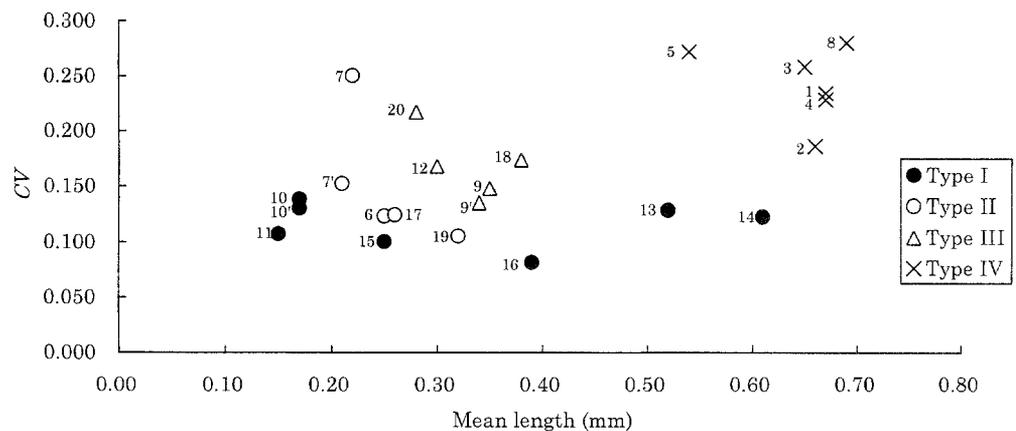
These differences might be due to the following factors: (1) The size of the area and the numbers of cells surveyed. It is uncertain how large was the area examined and how many cells were surveyed in Takamatsu's investigation; a large area with numerous cells were surveyed in the present study. For a precise judgment of the cell arrangement, it is necessary to survey a large area with many cells. (2) Differences in the age of the tree at the point of sampling. For example, it is reported that a storied arrangement is visually discernible from approximately the 25th annual ring in *Aesculus turbinata*,¹⁵ whereas *Firmiana platanifolia* has a conspicuous storied arrangement when it is quite young according to our previous study. Therefore, the age of the sample is an important factor that directly influences the cell arrangement. The age of the trees sampled in Takamatsu's investigation is uncertain.

Correlation between length and variation of length and arrangement of the parenchyma strand

As shown in Fig. 4, the species classified as type IV (irregular cambium) had a longer mean length. Most of the species classified as type I (regularly stratified cambium) or type II (slightly stratified cambium) had a relatively short mean length. However, two species with typical storied arrangement – *Aesculus turbinata* (sample 13) and *Tilia japonica* (sample 14) – had a longer mean length. This indicates that the species with longer mean length can also have a storied arrangement.

The species categorized as type I or type II had a smaller CV, except sample 7 (*Zelkova serrata*, sampled from the

Fig. 4. Correlation between the length and the variation of length and arrangement of the parenchyma strands in 23 samples of Japanese hardwoods. The species belonging to types I or II have a smaller coefficient of variation of length (CV) except sample 7; the species belonging to type IV have a larger CV; the species belonging to type III also tend to have a larger CV. Arabic numerals, number of samples. Refer to Table 2 for types I–IV



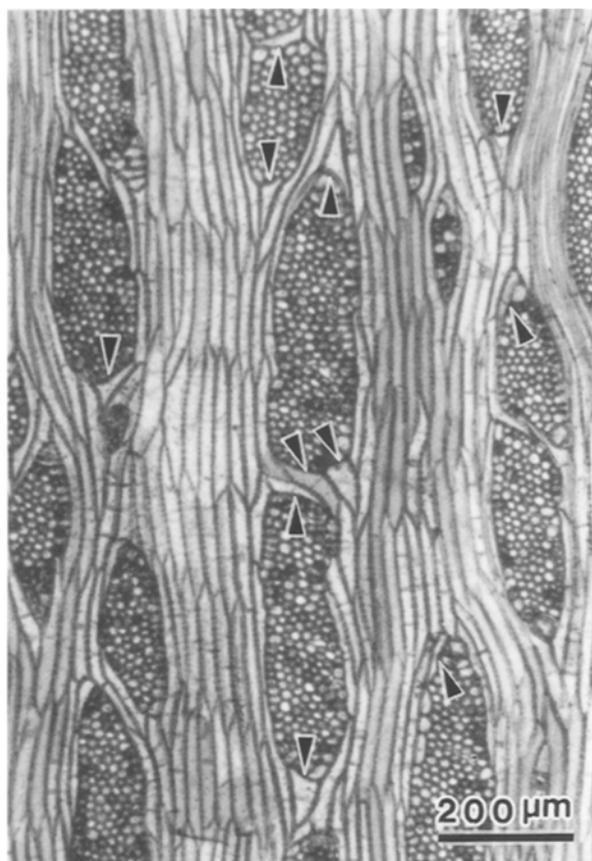


Fig. 5. Tangential section of a *Zelkova serrata* (sample 7) marginal zone of the annual ring with storied arrangement. There are a large number of broad rays and many extremely short parenchyma strands (arrowheads) around these broad rays

wood collection); and the species in the type IV group had a larger CV. In addition, the species in the type III group (non-stratified cambium) also tended to have a larger CV. Here sample 7 is an exception, as it still shows a storied arrangement despite its large CV. Figure 5 is a parenchyma tangential section of sample 7, and there are a large number of broad rays and many extremely short parenchyma strands around these broad rays. This may be a major factor that leads to larger variation of length. However, these short parenchyma strands are located almost in the upper or bottom margin of the rays, so they have little effect on the storied arrangement of the usual parenchyma strands. A similar phenomenon was observed in sample 7' (*Zelkova serrata*, sampled from the leaving tree), but there were fewer extremely short parenchyma strands than in sample 7,

so the CV of sample 7' (0.15) is smaller than that of sample 7 (0.25).

It is known that trees with a storied arrangement are of relatively short fusiform initials with little variation in length.^{1,7} A similar trend has been found in *Ulmus propinqua* (sample 6), *Robinia pseudoacacia* (10, 10'), *Maackia amurensis* var. *burergeri* (11), *Firmiana platanifolia* (15), and *Fraxinus mandshurica* (17). On the other hand, a different situation has been found in *Aesculus turbinata* (13) and *Tilia japonica* (14); that is, both species exhibited a storied arrangement despite their longer parenchyma strands. However, both had little variation in length. Based on these results and analysis, it can be concluded that the variation in the length of the fusiform initial has more influence than the length itself on the development of the storied cambium.

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