(Short Communication)

Identification of *Ulmus* and *Zelkova* Pollen

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To distinguish the pollen of Japanese species of the genera *Zelkova* and *Ulmus*, three criteria- size, number of apertures and shape- were observed. *U. parvifolia* tends to have slightly smaller pollen grains than other species of *Ulmus*, but it is difficult to separate *U. parvifolia* from the other *Ulmus* species by pollen grain size. *U. parvifolia* has 4-aperturate pollen grains and *U. davidiana* var. *japonica* has 4- or 5-aperturate, while most of pollen grains of *U. laciniata* have 5 apertures. The pollen grain of *Zelkova* is distinguishable from that of *Ulmus* even by light microscopy on the basis of grain shape and structure.

Key words : *Ulmus*, *Zelkova*, morphology, pollen analysis

Introduction

The family Ulmaceae in Japan is currently represented by 5 genera : *Aphananthe, Celtis, Trema, Ulmus* and *Zelkova* [1, 2). These are important genera for pollen analysis in Japan. However, in the two groups *Aphananthe / Celtis* and *Ulmus / Zelkova*, generic identification of these pollen grains is known to be difficult. In the latter group, *Zelkova* is represented by *Z. serrata* and *Ulmus* has 3 species. These species show a distinct geographical distribution and are adapted to specific habitats. *Z. serrata*, for example, one of the most important constituents of intermediate-temperate forest, is widely distributed throughout Japan except Hokkaido and is commonly found on gentle slopes near valleys and rivers with moist soil in the warm- and cool-temperate zones. *U. davidiana* var. *japonica*, which is distributed mainly in northeastern Japan, forms wetland forest on flat flood-plains of valley bottoms in the cool-temperate zone. *U. laciniata*, which is distributed throughtout Japan and grows in the same place as *U. davidiana* var. *japonica*, usually forms a mixed forest though *U. davidiana* var. *japonica* often forms a pure-forest. *U. parvifolia*, distributed in western Japan, is found on natural levees by rivers.

The pollen morphology of *Ulmus* and *Zelkova* in Japan has been described by several workers [3-7), and in more detail by Nirei [8). These studies have clarified the size, pore structure, wall thickness and surface exine sculpture of these genera using light microscopy (LM) and scanning electron microscopy (SEM). A few palynologists in Japan have felt able to distinguish each other genus using LM [9, 10), but this still remains difficult for many palynologists. Some palynologists in Europe think that *Ulmus* can be classified to species level using their pollen grains [11, 12]. If the fossil pollen of *Ulmus* and *Zelkova* could be separated from each other, and even to a specific rank, this would be valuable for clarifying the vegetational history and climatic changes that have occurred in Japan.

In the present study we attempted to find difference in generic or specific order between pollen grains of Japanese species of *Zelkova* and *Ulmus* on the basis of pollen morphology.

Materials and Methods

The pollen grains of 3 species of *Ulmus* and one species of *Zelkova* currently native to Japan were investigated. Pollen samples were secured from various sources, as described in Table 1.

The samples for LM observation were prepared as follows : (1) acetolysis for 10 min [9 : 1 acetic anhydride : conc. sulfuric acid], (2) washing with glacial acetic acid, (3) washing with water, (4) heating for a short time with 10% KOH, (5) washing with water, and (6) mounting in glycerin jelly with fuchsin stain. A Nikon Eclipse E400 microscope with a × 10 ocular and a × 100 objective was used for the measurements. One hundred measurements were obtained from each collection. The numbers of apertures on the pollen grains were counted, and micrographs were taken with a Zeiss Axioscope microscope equipped with a × 100 plano-fluorite Ph3 objective (N. A. 1.30).

For SEM observations, the acetolysed grains were stained with 2 % osmium tetroxide, and then transferred to a brass stub and coated with gold-palladium for several minutes. The photomicrographs were taken using a JEOL JSM-5300 SEM and a JSM-890 SEM.

Table 1. Specimens examined

Specimens examined	Collection No.	Locality	Collector	
U. davidiana var. *japonica*	2	Bot. Gard., Tohoku Univ., Sendai, Miyagi Pref.	Y. Morita	Apr. 9, 1981
	4	Bot. Gard., Tohoku Univ., Sendai, Miyagi Pref.	Y. Morita	Apr. 3, 1981
U. laciniata	1	Towada, Aomori Pref.	Y. Morita	May 24, 1978
	2	Kawatabi, Miyagi Pref.	Y. Morita	May 5, 1989
U. parvifolia	1	Katahira, Tohoku Univ., Sendai, Miyagi Pref.	Y. Morita	Sept. 18, 1979
	2	Hohraiji, Aichi Pref.	Y. Morita	Oct. ?, 1976
	3	Ise, Mie Pref.	Y. Morita	Sept. 23, 1991
	4	Hohraiji, Aichi Pref.	Y. Morita	Oct. ?, 1976
Z. serrata	5	Kyoto Pref. Univ., Kyoto	Y. Morita	Sept. 28, 1994
	1	Itsutsubashi, Sendai, Miyagi Pref.	Y. Morita	May 1, 1985
	3	Yamagata, Yamagata Pref.	Y. Hirabuki	May 3, 1981
	4	Kochi, Kochi Pref.	Y. Morita	May 3, 1977
	5	Katahira, Tohoku Univ., Sendai, Miyagi Pref.	Y. Morita	Apr. 22, 1979
	6	Mt. Tokura, Kakuda, Miyagi Pref.	Y. Morita	Apr. 30, 1981
	7	St. Aoba, Sendai, Miyagi Pref.	Y. Morita	May 1, 1991
	8	St. Aoba, Sendai, Miyagi Pref.	Y. Morita	Apr. 29, 1989
	9	Bot. Gard., Tohoku Univ., Sendai, Miyagi Pref.	Y. Morita	Apr. 28, 1978
	10	St. Aoba, Sendai, Miyagi Pref.	Y. Morita	May 5, 1978

Table 2. Pollen morphological data based on LM observation

	Collection No.	Equatorial diameter (μm) Max.	Min.	Mean	S.D.
U. davidiana var. *japonica*	2	41.75	32.25	35.79	1.588
	4	39.75	32.50	35.74	1.704
U. laciniata	1	45.25	33.75	38.24	2.027
	2	46.50	34.25	38.99	2.142
U. parvifolia	1	33.00	27.50	30.59	1.337
	3	37.00	28.00	32.74	1.859
	4	34.50	25.50	29.78	1.942
	5	35.25	26.25	31.21	1.812
Z. serrata	1	51.25	38.25	43.39	2.059
	3	48.50	38.00	43.48	2.109
	4	50.00	38.00	44.61	2.592
	5	50.00	40.25	44.44	2.013
	6	48.00	37.50	44.48	2.068
	7	49.75	39.00	43.18	1.967
	8	51.50	39.25	44.18	2.324
	9	48.00	37.75	42.23	2.295
	10	49.50	39.75	42.98	1.729

Results and Discussion

Variation in size

Equatorial size measurements of these pollen grains are shown in Table 2. The largest grain we observed was that of *Z. serrata* with a diameter of 51.50 μm, and the smallest was that of *U. parvifolia*, the sample collected showing a diameter of 25.50 μm. Fig. 1 shows the equatorial size range with the 95% confidence interval. *Z. serrata*, with pollen grains lager than those of *Ulmus*, tended to have a diameters of more than 40 μm. The size of *Ulmus* pollen grains varied widely. Although *U. parvifolia* had a tendency to have smaller pollen grains and *U. laciniata* to have slightly larger pollen grains than those of other *Ulmus* species, it was difficult to separate them from the other members.

The pollen sizes of these Japanese species have been reported previously [(3-8), (13)]. Although the chemical treatments and mounting media employed differed from ours, the previous results showed the same tendency as ours.

Variation in number of apertures

The variation in the number of apertures was plotted on a triangular diagram with 100% 4-, 5-, and 6-aperturate pollen grains at the corners and 0 % on the opposite sides (Fig. 2) according to Stockmarr's method [(12)]. As can be seen from the figure, most of the samples collected fell into either the 4-aperturate or the 5-aperturate category. *U. laciniata* differed from the other species of *Ulmus* in having grains with more than 5 apertures. Similar characteristics have been reported by Nakamura [(5)] and Xin et al. [(13)], but other workers such as Nirei [(8)] have not observed this. It does not see impossible to sort *U. laciniata* from other *Ulmus* species. Although *U. parvifolia* tends to have 4-aperturate

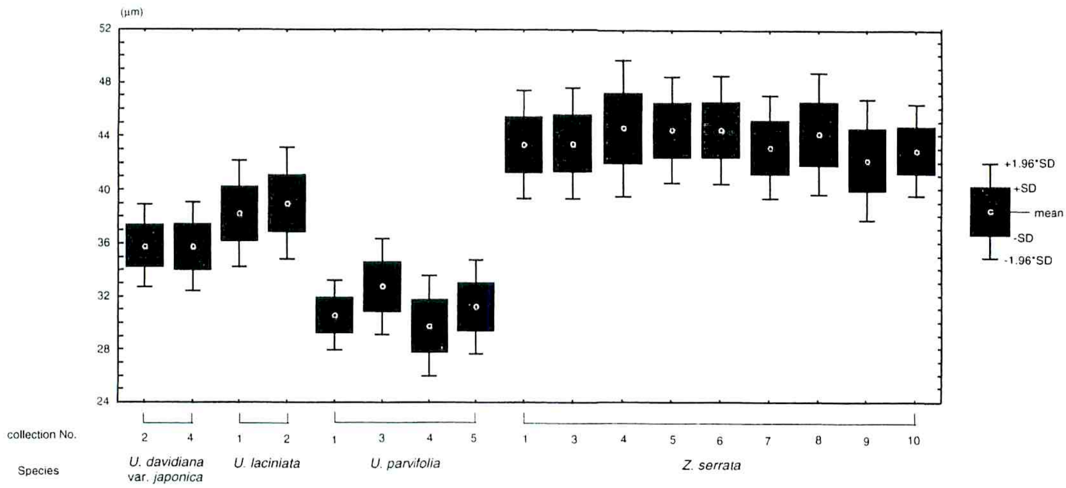


Fig. 1. 95% confidence interval of equatorial diameter.

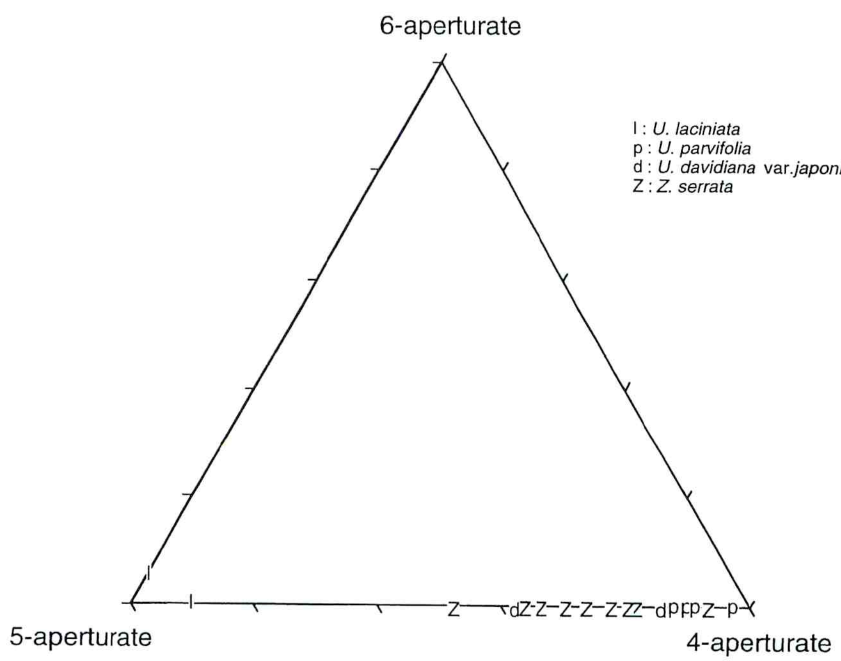
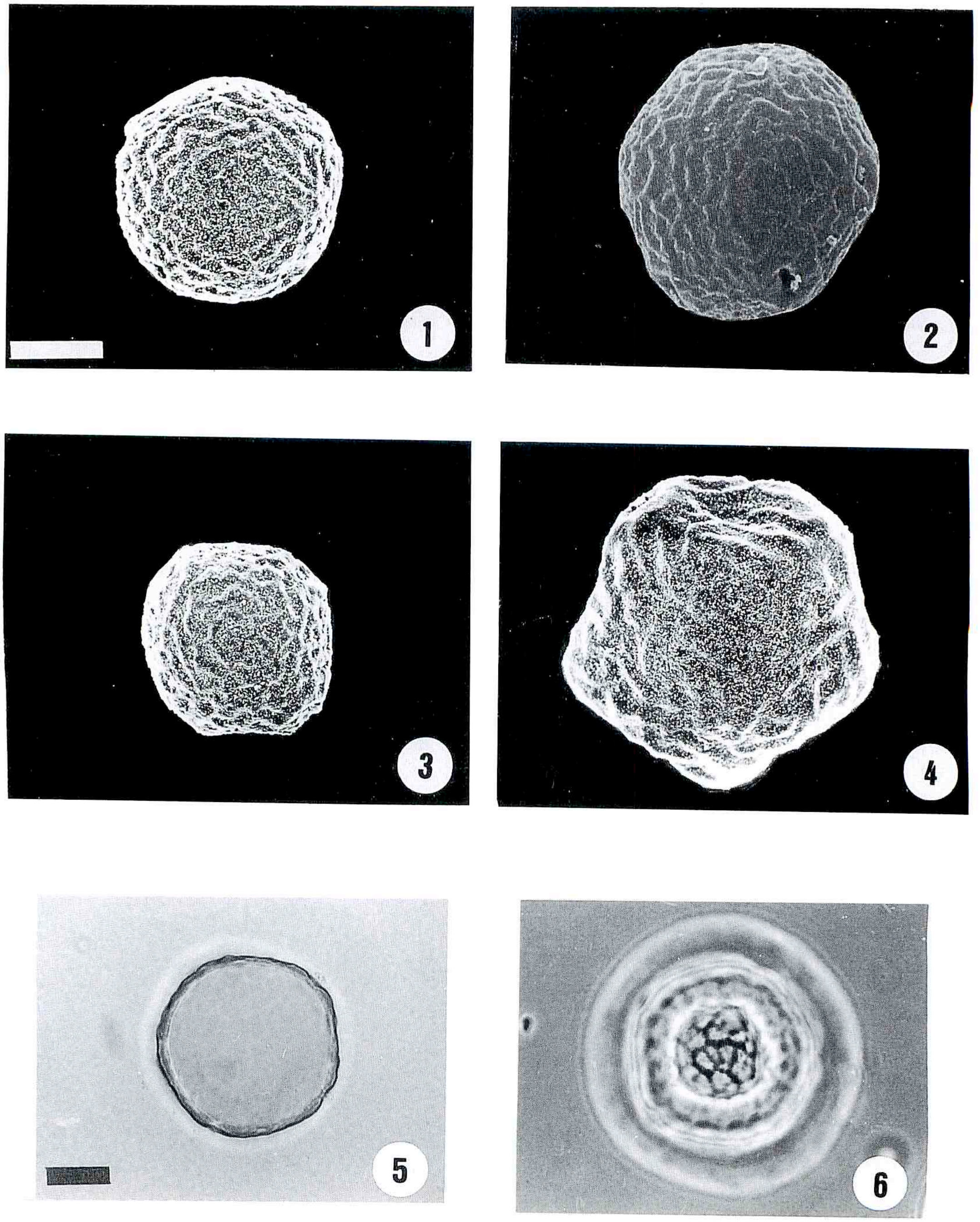


Fig. 2. Percentage distirbution by number of apertures.



Explanation of Plates

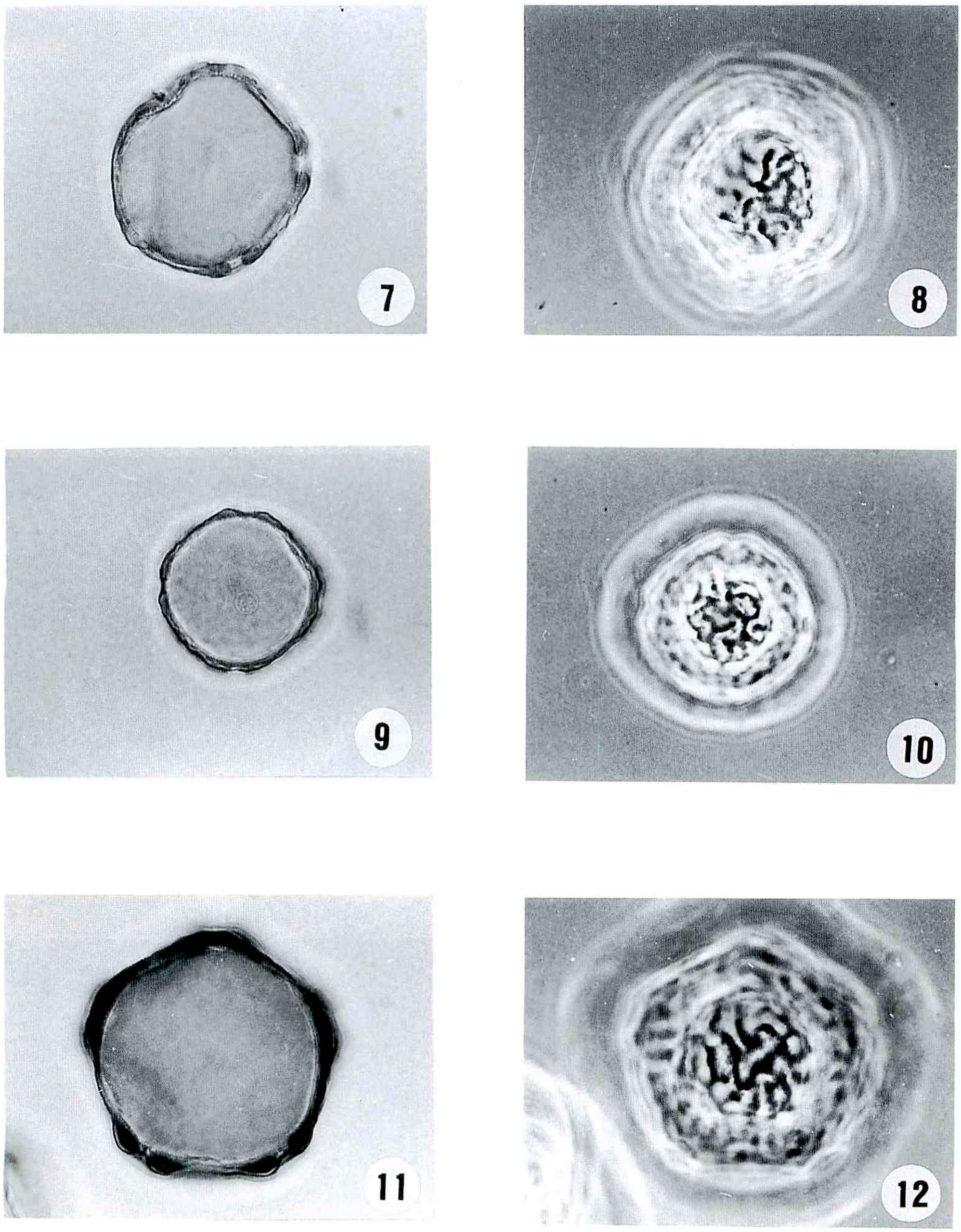
Scanning electron micrographs

1 *U. davidiana* var. *japonica* 2 *U. laciniata*

3 *U. parvifolia* 4 *Z. serrata*

Light micrographs

5 *U. davidiana* var. *japonica* 6 *ibid.* (phase contrast)



Explanation of Plates

Light micrographs

7 *U. laciniata* 8 *ibid.* (phase contrast)

9 *U. parvifolia* 10 *ibid.* (phase contrast)

11 *Z. serrata* 12 *ibid.* (phase contrast)

(solid line corresponds to 10μm)

pollen, it is difficult to distinguish from *U. davidiana* var. *japonica* and *Z. serrata* by this method due to the variability of aperture number in each species, producing a broad overlap.

General morphology

Ulmus and *Zelkova* pollen grains are rarely 6-aperturate and are mostly 4- and 5-aperturate, oval in equatorial view and angular in polar view. The exine has faint, undulating sculpturing that imparts a rugose or reticular appearance to the grain surface. The aperture usually bears an annulus.

The pollen grain of *Z. serrata* (Plate 4, 11, 12) has a thicker annulus, a thicker exine, coarser surface sculpture and is larger in size than that of *Ulmus* (Plate 1 – 3, 5 – 10). From these characteristics it can be said that *Zelkova* pollen can certainly be distinguished from *Ulmus* pollen by LM. Some workers in Japan have reported similar morphological characteristics [(4–8), (14, 15)].

Fossil pollen grains of *Ulmus* and *Zelkova* are usually found in Quaternary deposits in Japan. *Ulmus* / *Zelkova* began to expand its range during the early postglacial period on the Pacific Ocean side of Tohoku District, as deduced from the pollen records [(16)]. Our results could be of value for more clarifying the vegetational history in Japan.

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(* In Japanese ** In Chinese)

（短報）

ニレ属 - ケヤキ属花粉の同定について

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我が国のニレ属とケヤキ属はあわせて4種が知られている。これらは後氷期はじめには現在よりも多く存在したことが知られており、その生態的特性から森林変遷や気候変化を知る上で重要な樹種である。しかし、花粉分析では、両属の花粉形態が似ているため比較的区別が難しいとされ、両者をニレ属 - ケヤキ属として一括して表現する場合が多い。

そこで、これらの花粉が区別可能かどうか、4種計17標本について光学顕微鏡、走査電子顕微鏡で形態観察を行うとともに、各標本につき100個の粒径、発芽孔を計測した。その結果は以下のとおりである。

1）ケヤキ属は発芽孔周辺が肥厚すること、表面の脳皺状模様が荒いこと、粒径が大きいことから、光学顕微鏡でもニレ属と区別可能である。

2）オヒョウは他のニレ属よりも5孔型の花粉が多い傾向をしめす。

3）アキニレは小型の花粉が多い傾向があるものの、粒径からはニレ属内の細分は不可能である。