

(Review)

Pollen Wall Development in *Cercidiphyllum*. A Review

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We have studied the microspore tetrad arrangement and pollen exine in both *Cercidiphyllum japonicum* Siebold & Zuccarini and *C. magnificum* (Nakai) Nakai with light, transmission and scanning electron microscopy. By means of differential staining of apertural and interapertural regions we show that the arrangement of apertures on microspore tetrads soon after meiosis is governed by Fischer's Law. Thus the apertures of *Cercidiphyllum* are perpendicular to the equator and, therefore, are correctly termed colpi, not sulci as has been postulated.

Both apertural and interapertural regions have exine templates, with the result that the tectum and its spinules on these regions develop similarly. While apertural and interapertural regions of the exine look the same in surface views in both light and scanning electron microscopes, they differ greatly in these regions when seen in section with a transmission electron microscope. Apertural regions lack bacules and footlayer and have a distinctive endexine.

There is every indication that length and width of the three apertures are equal during formation and development. They appear equal also in fresh mature pollen. After pollen grain dehydration portions of some apertures do not again expand or contract equally.

An unusual aspect of pollen exine development in *Cercidiphyllum* is the gradual, continuous growth of the exine, extending as it does from tetrad stages through microspore mitosis. In other taxa exines reach almost their definitive thickness soon after microspores are released from the callose envelope.

Key words : *Cercidiphyllum japonicum*, *C. magnificum*, pollen development, microspore tetrad arrangement, apertures

The family Cercidiphyllaceae has but one genus, *Cercidiphyllum* (the Katsura tree), with two closely related species, *C. japonicum* and *C. magnificum*, both from Japan. The pollen grains have three wide furrows with rounded ends. According to Erdtman (1952) they are 3-colp (oid) ate, signifying that he considered the furrows to be perpendicular to the equator. Kuprianova (1969), however, questioned this arrangement of furrows.

A combination of unusual features -- apertures of apparently unequal length with rounded ends has

had a lasting influence on the nomenclature of fossil pollen. The Early Jurassic-Cretaceous sporomorph originally called *Tricolpites troedssonii* but now known to be gymnospermous, was subsequently named *Eucommiidites* because it seemed to share these features with *Eucommia ulmoides* (Erdtman 1948). Erdtman (1948, 1952, 1963, 1969) reported that grains with colpi of unequal length with rounded ends occur in *Cercidiphyllum*, *Eucommia ulmoides* and *Euptelea pleiosperma*.

Endress (1986) called attention to *Cercidiphyllum* and *Euptelea* as anemophilous genera of the Trochodendrales. The exine surfaces of these genera of two species each, are remarkably similar, but the aperture margins differ. In *Euptelea* the margin is very abrupt (Endress 1986 : Figs. 35, 38, Walker and Doyle 1975 : Fig. 3 A, E, F) while that of *Cercidiphyllum* is gradual. This can be seen in Walker's (1976 a, b) and Endress' (1986) scanning electron micrographs (SEMs) and is also apparent in Rowley's (1992) transmission electron micrographs (TEMs).

Pollen of *Cercidiphyllum japonicum* was studied and described (LM unless marked SEM or TEM) by Agababian (1968, 1973), Harms (1916), Mitroiu (1963, 1970), Nakamura (1943), Praglowski (1975 : TEM), Rowley et al. (1979, 1980 : TEM), Rowley 1992 : TEM), Solereder (1899), Swamy and Bailey (1949), Takeoka (1965), Walker 1976a : TEM, SEM, 1976b : SEM), Chang (1983), Zavada and Dilcher (1986 : TEM, SEM). Erdtman (1952) studied *C. magnificum* as well as *C. japonicum*.

The microspore tetrad after meiosis

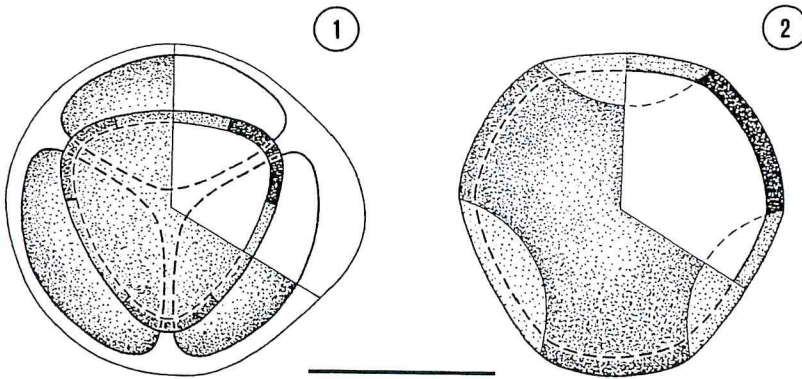
Congo red and maleic hydrazide stain contrasts the interapertural exine intensely but the exine of the furrows only moderately so that the distinction between them is readily apparent (Figs. 1 and 2). In polar view the optical section through the middle level of a microspore shows three intensely stained sectors alternating with three that are only faintly stained (Fig. 2). In the postmeiotic tetrad in Fig. 1 the upper microspore is shown in an equatorial plane. The equatorial outline looks like an equilateral triangle composed of six very distinct parts (Fig. 1).

Early microspores through pollen grain stages

Stages from early microspores to mature pollen grains are illustrated in Rowley (1992). Here we show the gradual transition from interapertural to apertural exine (Fig. 3) and the mature exine. The mature exine has an interapertural tectum with spinules and a thick footlayer appressed to, and often indistinguishable from, the endexine (Fig. 5). The height of the bacules is correlated with their distance from the aperture. The section in figure 4 is close to an aperture. There is no apertural footlayer and no bacules except near the aperture margin. The aperture margin shows the termination of the footlayer and reduction in bacular height.

Colpi and Fischer's Law.

In tetrads arranged as in Fig. 1, it can be seen that each furrow of the upper microspore lies above the center of one of the lower microspores. This indicates that the arrangement of the microspores is governed by Fischer's Law, which Erdtman (1952) described as the situation of apertures in 3-aperturate pollen grains where each aperture meets one of an adjacent grain at six points in the tetrad. Thus the apertures of *Cercidiphyllum* are perpendicular to the equator and, therefore, are colpi. They are not sulci as Kuprianova (1969) postulated.



Figs. 1 and 2. The postmeiotic tetrads and early free microspores for these figures were from buds of *C. magnificum* collected from trees at the University of Neuchâtel, Switzerland. The tetrads and early free microspores were extracted from fresh anthers with a fine needle and mounted in a mixture of Congo red and maleic hydrazide (Stainier, Huard and Bronckers 1967) which provides differential contrast.

Fig. 1. Postmeiotic tetrad. The inner limit of the early exine is represented on the upper level microspore only. The difference in intensity of staining between colpi and intercolpal region is shown in the optical section of the upper right sector of this microspore (see Fig. 2). Bar = 15 μ m.

Fig. 2. Microspore at the free microspore stage. The dashed line parallel with the outline of the microspore marks the inner limit of the early exine. The difference in intensity of the Congo red/maleic hydrazide staining between the colpi and intercolpal regions as seen in optical section is shown at the upper right sector. Bar = 15 μ m.

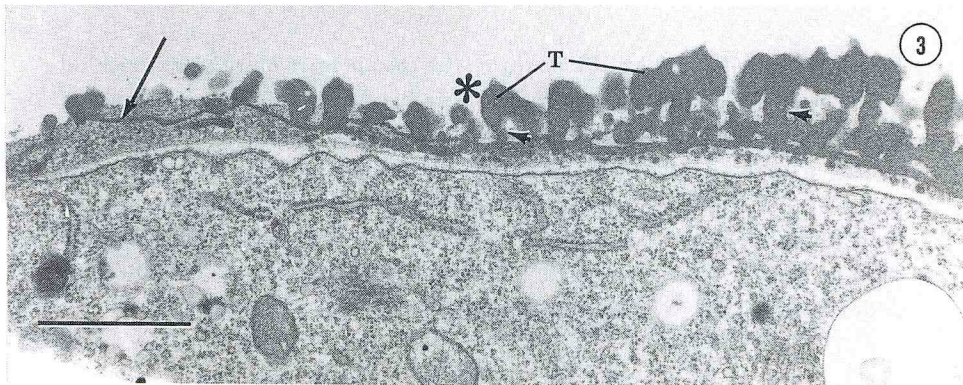


Fig. 3. The region of the aperture margin at an early pollen grain stage. The distinction in TEM sections between the apertural exine (left of the asterisk) and interapertural exine (T = tectum; arrowheads = bacules) is easily seen, but is less obvious in surface views (see SEM in Fig. 6). It can be seen that there is no footlayer or bacules on the apertural side of the aperture margin (asterisk). The endexine (arrow) connects with the apertural ectexine ("flecks of exine"). Stain: uranyl acetate followed by lead citrate. Bar = 1 μ m.

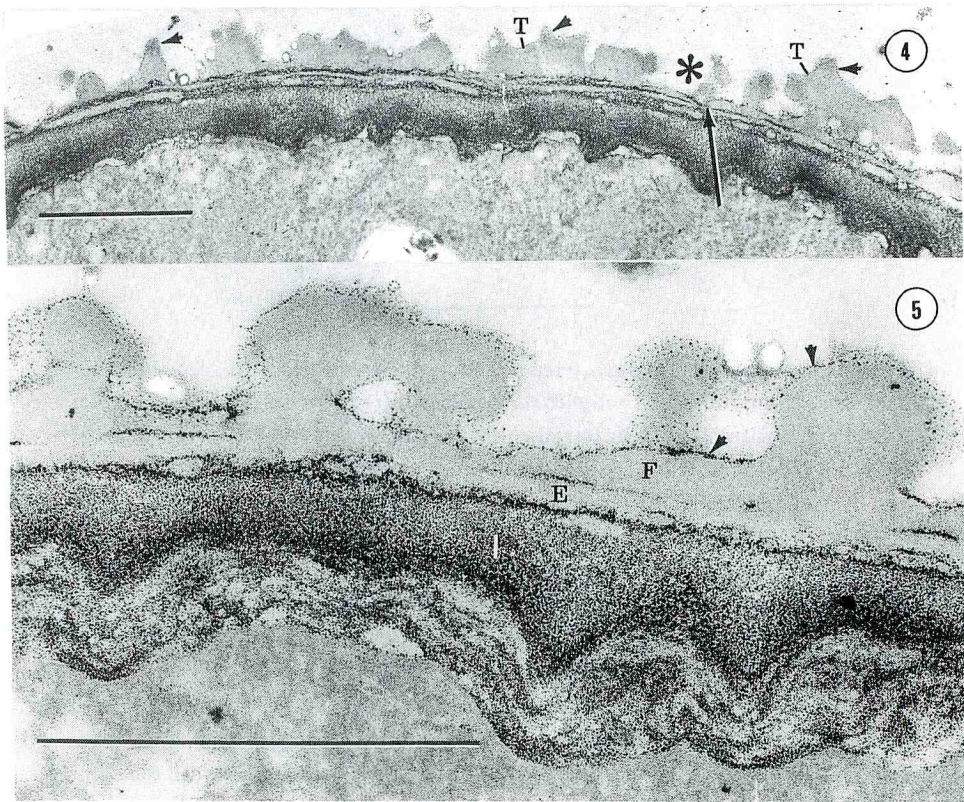


Fig. 4. Section of a mature pollen grain with the aperture margin (asterisk) at the far right. While the ectexine decreases in height over a lateral distance of three or more μm (as is seen in Fig. 3) there is an abrupt termination of the footlayer (arrow) and absence of bacules at the actual margin (asterisk). It is apparent that there is a similarity between tectum (T) and spinules (arrow-heads) of the aperture and interapertural exine. The dark contrast is due to the Thiéry (1967) reaction for carbohydrates (periodic acid oxidation to thiocarbohydrazide to silver proteinate. Bar = $1\ \mu\text{m}$.

Fig. 5. A mature pollen grain near the aperture margin. The bacules are short because of their proximity to the aperture margin. The ectexine footlayer (F) and the endexine (E) have the same contrast in this section and appear to be fused at several sites. The intine (I) and exine surface components (arrow-heads) are dark, indicating the presence of Thiéry-carbohydrates. Stain : same as Fig. 4. Bar = $1\ \mu\text{m}$.

Exine development

In our earlier works we described the later stages in development of the exine (Rowley et al. 1979, 1980), and many stages from the very early free microspore stage are illustrated in Rowley (1992).

A distinctive aspect of pollen exine development in *Cercidiphyllum* is its gradual growth from the tetrad stage to the microspore mitosis stage. In many other taxa exines reach almost their full thickness before or soon after microspores are released from callose envelopment (e. g., Larson and Lewis 1962, Leguminosae; Skvarla and Larson 1966, Poaceae; Angold 1967, Liliaceae; Heslop-Harrison 1968, Liliaceae; Dickinson 1970, Liliaceae; Sampson 1977, Monimiaceae; Horner and Pearson 1978, Compositae; Takahashi and Sohma 1980, Pyrolaceae; El-Ghazaly and Jensen 1986, Poaceae; Rowley and Rowley 1986, Ulmaceae; Suarez-Cervera and Seoane-Camba 1986, Labiatae; Blackmore and Barnes 1987, Compositae; Fernández and Rodríguez-García 1988, Oleaceae; Takahashi and Kouchi 1988, Malvaceae; Simpson 1989, Haemodoraceae; Rowley, J. R. and Flynn, J. J. 1990-1991, Monimiaceae; Rowley, Skvarla and Pettitt 1992, Eucommiaceae; Martinsson 1993, Callitrichaceae; Takahashi 1994, Hydrocharitaceae; Theilade and Theilade 1996, Zingiberaceae; Rowley and Dunbar 1996, Centrolepidaceae; Gabarayeva 1996, Magnoliaceae; Gabarayeva and El-Ghazaly 1997, Nymphaeaceae).

Exine development in *Cercidiphyllum* can be considered to be an example of heterochrony. As Raff (1996) notes, heterochrony has been the single most pervasive idea in evolutionary developmental biology.

The apertures

Cercidiphyllum apertures have what Wodehouse (1936a, b) referred to as "flecks of exine" covering germinal apertures. These occur in many families (see Wodehouse 1935): for example, Hamamelidaceae (*Liquidambar*), Juncaceae, (*Juncoides*), Naiadaceae (*Ruppia*), Oleaceae (*Fraxinus*), Plantaginaceae (*Plantago*), Platanaceae (*Platanus*), Salicaceae (*Salix*). Wodehouse (1935: p.321) described the germinal furrow of the *Juncoides campestre* grain in the tetrad as being not sharply defined but represented by a thin area of the exine distinguished from the rest of the grain by its elastic nature, resulting in a more open distribution of its granular flecks.

Wodehouse's description applies rather closely to conditions for seeing the edges of apertures in *Cercidiphyllum* pollen. It is extremely difficult to distinguish between interapertural and apertural exine in surface views even in scanning electron micrographs (see for example Walker 1974: Fig. 38, reproduced with permission as Fig. 6). With light microscopy or SEM the size limits of a *Cercidiphyllum* pollen grain aperture are appreciated largely because of the elastic nature of the apertural exine, as Wodehouse noted for *Juncoides*. The granular flecks on expanded apertures on *Cercidiphyllum* pollen have a more open distribution than they do on the interapertural region where similar spinules and tectal components are anchored to the ectexine. The expanded condition requires a hydrated protoplast, and it is the contracted condition of apertures that has given rise to reports of apertures in *Cercidiphyllum* pollen that are not always equilateral (e. g., Erdtman 1952: pp. 106-107, 1963: p. 30, 1969: p.86). In the expanded condition where the exine flecks are rather widely separated (see the TEM in Fig. 3) aperture margins should be readily distinguishable, even with the light microscope.

Our studies have failed to show evidence for aperture inequality in pollen of *Cercidiphyllum japonicum* during formation and development or in mature fresh pollen (Rowley, Rowley, Huynh and Qureishi 1979; Rowley, Rowley, Qureishi and Huynh 1980; Rowley 1992). This does not mean that

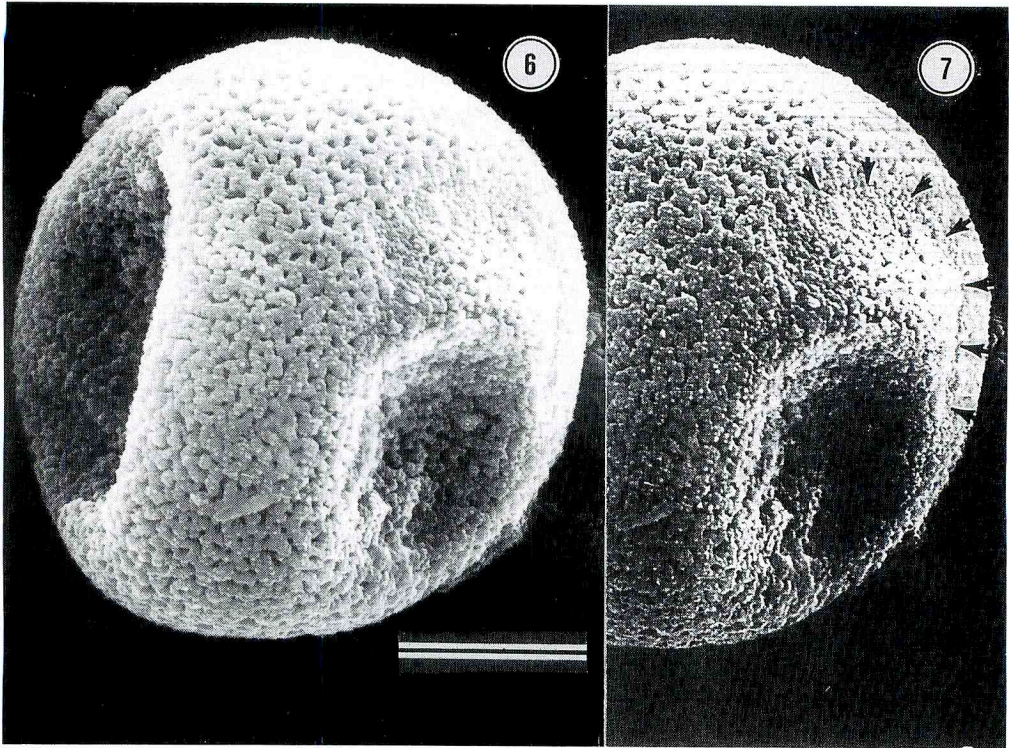


Fig. 6. James W. Walker's SEM of *C. iaponicum* showing two of the three large elipsoidal apertures. The aperture to the right appears to be shorter than the other one, but this is because only a portion of it is folded inward. The actual margin can be detected above the indented portion at a level comparable to the aperture on the left. This micrograph is from Walker (1976a : Pl. 12, A) and is used with permission of the author. Bar = 10 μ m.

Fig. 7. A photocopy of part of the SEM in Fig. 6. Only a lower portion of the "short" aperture is strongly folded inward. The aperture margin itself is outlined by a curved file of arrowheads. In Fig. 6, this margin can be seen to have a finer surface texture than the interapertural exine.

Erdtman and many others were wrong. Apertures appear unequal when grains are observed after they dehydrated (cf. sketches in Rowley 1992), especially so after the repeated dehydrations and rehydrations common in preparation for light or scanning electron microscopy.

A satisfactory demonstration of aperture equality is achieved with LM examination of fresh pollen taken at any stage from undehisced anthers and placed in an osmotically balanced buffer (we used phosphate buffer at pH 7 adjusted to 300 milliosmoles, mmol./kg). The contents of anthers can be quickly scored for general symmetry, since asymmetrical grains can be readily recognized with through focusing (rapid up and down focusing). Strongly asymmetrical grains can be easily spotted even when the microscopic field includes hundreds of grains. After *Cercidiphyllum* pollen grains have

been air dry and then are seen in rehydrating buffer, this same method of assay reveals something of the unusual construction of the apertural areas. The unequal expansion of their apertures on rehydration causes many grains to become strongly asymmetrical.

The aperture inequality feature of *Cercidiphyllum* pollen ought to be reconsidered as an ontogenetic character. An aperture appears to be short when only a portion of the aperture is folded inward.

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References

- Agababian, V. Ch. 1968. Palynomorphologie de quelques Angiospermes primitives II. Biol. Zh. Arménii. 21 (5) : 68-78.
- Agababian, V. Ch. 1973. Pollen of primitive angiosperms. Bot. Inst. Akad. Nauk Armenia, SSR. 169 pages, 28 Plates.
- Angold, R. E. 1967. The ontogeny and fine structure of the pollen grains of *Endymion non-scriptus*. Rev. Palaeobot. Palynol. 3 : 205-212.
- Blackmore, S. and Barnes, S. H. 1987. Pollen wall morphogenesis in *Tragopogon porrifolius* L. (Compositae : Lactuceae) and its taxonomic significance. Rev. Palaeobot. Palynol. 52 : 233-246.
- Chang, Yu-Lung. 1983. Cercidiphyllaceae In : Angiosperm flora of tropic and subtropic China. Inst. Bot. South China
- Dickinson, H. G. 1970. Ultrastructural aspects of primexine formation in the microspore tetrad of *Lilium longiflorum*. Citobiologie 1 : 437-449.
- El-Ghazaly, G. and Jensen, W. A. 1986. Studies of the development of wheat (*Triticum aestivum*) pollen I. Formation of the pollen wall and Ubisch bodies. Grana 25 : 1-29.
- Endress, P. K. 1986. Floral structure, systematics, and phylogeny in Trochodendrales. J. Missouri Bot. Gard. 73 : 297-324.
- Erdtman, G. 1948. Did dicotyledonous plants exist in early Jurassic times? Geol. Fören. Stockh. Förh. 70 : 265-271.
- Erdtman, G. 1952. Pollen morphology and plant taxonomy. Angiosperms. Almqvist and Wiksell. pp. 539.
- Erdtman, G. 1963. Palynology. In : Vistas in Botany 4 : 23-54, 4 text figs., 7 pl.
- Erdtman, G. 1969. Handbook of palynology. Munksgaard, Copenhagen. pp. 486.
- Fernández, M. C. and Rodríguez-García, M. I. 1988. Pollen wall development in *Olea europaea* L. New Phytol. 108 : 91-99.
- Gabarayeva, N. 1996. Sporoderm development in *Liriodendron chinense* (Magnoliaceae) : a probable role of the endoplasmic reticulum. Nord. J. Bot. 16 : 307-323.
- Gabarayeva, N. and El-Ghazaly, G. 1997. Sporoderm development in *Nymphaea mexicana* (Nymphaeaceae). Pl. Syst. Evol. 204 : 1-19.
- Harms, H. 1916. Über die Blütenverhältnisse und die systematische Stellung der Gattung *Cercidiphyllum* Sieb. et Zucc. Ber. dtsh. bot. Ges. 34.
- Heslop-Harrison, J. 1968. Wall development within the microspore tetrad of *Lilium longiflorum*. Can. J. Bot. 46 : 1185-1192.

- Horner, H. T., Jr. and Pearson, C. B. 1978. Pollen wall and aperture development in *Helianthus annuus* (Compositae : Heliantheae). Amer. J. Bot. 65 : 293-309.
- Kuprianova, L. A. 1969. On the evolution levels in the morphology of pollen grains and spores. Pollen et Spores 11 : 333-351.
- Larson, D. A. and Lewis, C. W. 1962. Pollen wall development in *Parkinsonia aculeata*. Grana palynol. 3 (3) : 21-29.
- Martinsson, K. 1993. The pollen of Swedish *Callitriche* (Callitrichaceae) - trends towards submergence. Grana 32 : 198-209.
- Mitroiu, N. 1963. Contribution à l'étude palynologique de certaines Polycarpées (Ranales). St. Cerc. Biol. Vég. 15 : 239-250.
- Mitroiu, N. 1970. Études morphopolliniques et des aspects embryologiques sur les "Polycarpiceae" et Helobiae, avec des considérations phylogénétiques. Lucr. Grad. bot. Bucuresti. 1969 : 3-243.
- Mollenhauer, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. Stain Technol. 39 : 111-115.
- Nakamura, J. 1943. Diagnostic characters of pollen grains. Sci. Sep. Tōhoku Univ. 4th ser., 17 : 4.
- Pragłowski, J. 1975. The pollen morphology of the *Trochodendraceae*, *Tetracentraceae*, *Cercidiphyllaceae* and *Eupteleaceae* with reference to taxonomy. Pollen et Spores 16 : 449-467.
- Raff, R. A. 1996. The shape of life. Genes, development and the evolution of animal form. University of Chicago Press, Chicago ; 520 pp.
- Rowley, J. R. 1992. Pollen of *Cercidiphyllum* (Cercidiphyllaceae). Botanicheski Zhurn. 77 (11) : 1-3, 2 plates.
- Rowley, J. R. and Dunbar, A. 1996. Pollen development in *Centrolepis aristata* (Centrolepidaceae). Grana 35 : 1-15.
- Rowley, J. R. and Flynn, J. J. 1990-1991. Tambourissa (Monimiaceae). Microspore development in the tetrad period. An. Sci. Naturel., Bot., Paris 13 série, : 125-147.
- Rowley, J. R. and Rowley, J. S. 1986. Ontogenetic development of microspores of *Ulmus* (Ulmaceae). In : Blackmore, S. and I. K. Ferguson (eds.), Pollen and Spores : Form and Function. Linnean Soc. Symposial Vol. 12 : 19-33.
- Rowley, J. R., Skvarla, J. J. and Pettitt, J. M. 1992. Pollen wall development in *Eucommia ulmoides* (Eucommiaceae). Rev. Palaeobot. Palynol. 70 : 297-323.
- Rowley, J. R., Rowley, J. S., Huynh, K. -L. and Qureishi, S. Z. 1979. Germinal aperture size in exines of *Cercidiphyllum* pollen. J. Ultrastruct. Res. 66 : 94-95.
- Rowley, J. R., Rowley, J. S., Qureishi, S. Z. and Huynh, K. -L. 1980. Sporoderm development in pollen of *Cercidiphyllum*. V-International Palynol. Conf., Cambridge, p. 345.
- Sampson, F. B. 1977. Pollen tetrads of *Hedycarya arborea* J. R. et G. Forst. (Monimiaceae). Grana 16 : 61-73.
- Simpson, M. G. 1989. Pollen wall development of *Xiphidium coeruleum* (Haemodoraceae) and its systematic implications. Ann. Bot. 64 : 257-269.
- Skvarla, J. J. and Larson, D. A. 1966. Fine structural studies of *Zea mays* pollen I : Cell membranes and exine ontogeny. Amer. J. Bot. 52 : 1112-1125.
- Solereeder, H. 1899. Zur Morphologie und Systematik der Gattung *Cercidiphyllum* Sieb. et Zucc. mit Berücksichtigung der Gattung *Eucommia* Oiliv., Ber. dtsh. bot. Ges. 17.
- Stainier, F., Huard, D. and Bronckers, F. 1967. Technique de coloration spécifique de l'exine des microspores jeunes groupées en tétrades. Pollen et Spores 9 : 367-370.
- Suarez-Cervera, M. and Seoane-Camba, J. A. 1986. Ontogénèse des grains de pollen de *Lavandula*

- dentata* L. évolution des cellules tapétales. Pollen et Spores 28 : 5-28.
- Swamy, B. G. L. and Bailey, I. W. J. 1949. The morphology and relationships of *Cercidiphyllum*. J. Arnold Arbor. 30 : 187-210.
- Takeoka, M. 1965. Electron microscope investigations on the surface structure of pollen membrane of trees. Sci. Rept. Kyoto Univ., Agr. 17 : 43-69.
- Takahashi, H. and Sohma, K. 1980. Pollen development in *Pyrola japonica* Klenze. Sci Rep. Tôhoku University, Ser. IV (Biol). 38 : 57-71.
- Takahashi, M. 1994. Pollen development in a submerged plant, *Ottelia alismoides* (L.) Pers. (Hydrocharitaceae). J. Plant Res. 107 : 161-164.
- Takahashi, M. and Kouchi, J. 1988. Ontogenetic development of spinous exine in *Hibiscus syriacus* (Malvaceae). Amer. J. Bot. 75 : 1549-1558.
- Theilade, I. and Theilade, J. 1996. Ontogeny of pollen grains of *Zingiber spectabile* (Zingiberaceae). Grana 35 : 162-170.
- Thiery, J. P. 1967. Mise en évidence des polysaccharides sur coupes fines au microscope électronique. J. Microscopie 6 : 987-1018.
- Walker, J. W. 1974. Aperture evolution in the pollen of primitive angiosperms. Amer. J. Bot. 61 : 1112-1137.
- Walker, J. W. 1976a. Evolutionary significance of the exine in the pollen of primitive angiosperms. Linn. Soc. Symp. 1 : 251-308.
- Walker, J. W. 1976b. Comparative pollen morphology and phylogeny of the Ranalean complex. In, Origin and early evolution of Angiosperms. (ed. Beck, C.B.) Columbia Univ. Press, N. Y. 241-299.
- Walker, J. W. and Doyle, J. A. 1975. The bases of Angiosperm phylogeny : palynology. Ann. Missouri Bot. Gard. 62 : 664-723.
- Wodehouse, R. P. 1935. Pollen grains. McGraw Hill Book Co. p. 574.
- Wodehouse, R. P. 1936a. Pollen grains in the identification and classification of plants. VII. The Ranunculaceae. Bull. Torrey Bot. Club. 63 : 495-514.
- Wodehouse, R. P. 1936b. Pollen grains in the identification and classification of plants. VII. The Alismataceae. Am. J. Bot. 23 : 535-539.
- Zavada, M. S. and Dilcher, D. L. 1986. Comparative pollen morphology and its relationship to phylogeny of pollen in the Hamamelidae. Ann. Missouri Bot. Gard. 73 : 348-381.
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