POLLEN WALL STRUCTURE IN *ANIGOZANTHOS VIRIDIS* (HAEMODORACEAE)

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ABSTRACT

The exine of *Anigozanthos viridis* Endl. pollen is divided into two zones by a plane which has been referred to as a "commissural line"; in our results we refer to it as a *junction plane* – a more apt description. Following young stages there is a pronounced reduction in thickness of the inner exine, probably due to stretching of the exine without the addition of new material as development progresses.

We describe the fibrillar zone under the exine in microspores as oncus-like. Since this onciform zone precedes microspore mitosis and intine formation the fibrillar zone is divided into two layers in mature pollen an onciform part and the intine proper – it is not a two layered intine.

KEY WORDS: pollen, exine, "commissural line", *junction plane*, *Anigozanthos*, *Haemodoraceae*.

INTRODUCTION

In his circumscription of tribes of the *Haemodoraceae* Simpson (1983) reviewed differing opinions concerning family definition and variations. He concluded that Erdtman's (1971) and Radulescu's (1973) pollen studies using light microscopy have probably been more useful in assessing taxonomic relationships in the family so far than studies in the fields of anatomy, biochemistry, cytogenetics, and embryology.

Erdtman (1971) recognized three pollen types within the family. All five genera of the tribe Conostylideae (*Anigozanthos*, *Blancoa*, *Conostylis*, *Phlebocarya* and *Tribonanthes*) show a characteristic LO pattern. At low focus dark islands with faint dark dots (bacules) appear separated by brighter channels (Erdtman 1971). Although these genera have isopolar, subisopolar, or apolar pollen with 2-, 3-, or 8-porate apertures Erdtman concluded that, in spite of variation in aperture number, they belong together by virtue of their common LO-pattern. Thus our illustrations of stages in the development of one species of *Anigozanthos* may perhaps have relevance for the tribe Conostylideae as a whole.

MATERIALS AND METHODS

Anigozanthos viridis Endl. buds and flowers were collected and fixed at Kings Park and Botanic Garden, Perth, Western Australia. The fixative was 4% paraformaldehyde in 0.1M cacodylate buffer (pH 6.9, room temperature, 60 days). The anthers were transferred to 2% aqueous osmium tetroxide for 2 hours. They were then dehydrated in a graded ethanol series to 100% ethanol and transferred to 100% acetone. One anther from a mature bud was removed from the acetone to be acetolysed (see below). The rest of the anthers were infiltrated and

embedded in Epon-Araldite (Mollenhauer 1964; mixture no. 2). At the beginning of polymerisation of the epoxy the capsules were heated to 80°C and placed under vacuum for 10 minutes (Skvarla 1966).

The anther to be acetolysed was placed in the acetolysis mixture (Erdtman 1960), heated to 100°C and held at that temperature for four min. The isolated exines thus obtained were washed, then returned to 100% acetone and infiltrated with Epon-Araldite. The exines were centrifuged into a small pellet in a conical embedding capsule and the epoxy polymerized as above.

Following sectioning the epoxy embedded material was stained for general contrasting using aqueous uranyl acetate (UA; 10 min) followed by lead citrate (Pb; 4 min; UA-Pb). For detection of carbohydrates sections on gold grids were immersed in 1% periodic acid (10 min) then immersed in 1% phosphotungstic acid in 10% chromic acid (pH < 1.3, 10 min) Roland et al. (1972). Sections were examined with a Zeiss EM-10a transmission electron microscope.

RESULTS

We have two well defined stages and two others from aborted microspores which probably died early in microspore development. Micrographs of exines of aborted microspores from two stages of development are shown in Figs 1-5. Microspores undergoing vacuolation are shown in Figs 6-10. Pollen grains from mature anthers are shown in Figs 11-13; these anthers were undehiscent, but came from open flowers. At all stages there is a junction plane between parts of the exine which will be referred to herein as inner and outer parts of the exine.

Figs 1-2. Exines of aborted microspores recovered from mature anthers. Exine components are not separated. The junction plane (arrows) separates differently contrasted outer and inner parts of the exine. There is an indication of spinules (arrowheads). Fig. 1 shows the interapertural part of the microspore and Fig. 2 an apertural. The inner part and small globules have taken up more stain than the outer exine. Section stain: UA-Pb. Bars: 1 μ m.

Figs 3-5. Exines like those in Figs 1-2 were aborted during the early microspore period and recovered from mature anthers. These exines in Figs 3-5 are from microspores older than those above, e.g., the components are separated to a greater extent than those in Figs 1-2. This exine was from an acetolysed anther and it is evident that the small globules under the exine are acetolysis resistant as are strands (arrowheads) between globules. Two spinules are marked with arrows. Figs 3 and 5 are interapertural while the exine in Fig. 4 is apertural. Fig. 5 is section stained and the inner and outer exine are more similar in contrast than section stained figures 1-2. Fig. 5 shows a unit structure with substructural loops (arrowheads) that are about 30 nm in diameter. Treatment: acetolysis. Section stain: Figs 3-4 = none; Fig. 5 = UA-Pb. Bars: 1 μ m.

Figs 6-10. Microspores in the vacuolated stage of development. The outer and inner parts of the exine and the onciform layer are contrasted. In the cytoplasm only the carbohydrate rich components (e.g., starch grains) are strongly contrasted. Stain: Periodic acid oxidation followed by phosphotungstic acid at pH ca 1.3. Fig. 6. The thickened onciform layer and lateral separation of exine components at the apertures is evident at both top and bottom of the figure. Two of the starch grains within plastids in the cytoplasm are marked "S". The tapetal materials in the locular fluid are marked with arrows. Magnification Bar: 10 μ m. Fig. 7. Detail of aperture margin in Fig. 6. The onciform layer (O) is relatively thick in the aperture and its margin. Organized structures of tapetal origin are marked by arrows. Magnification scale: 1 μ m. Fig. 8. Oblique section of infolded exine with the aperture at the right. The infolded exine in the center of the figure is sectioned very obliquely. This oblique section accents the irregularity of the inner exine and shows that there are small globules (arrowheads) at the inner surface of the exine, as in Figs 1-5. Magnification scale: 1 μ m. Fig. 9. Two of the regulate exine components with inner exine parts (arrowheads) protruding into the onciform layer (O). A starch grain is marked "S". Magnification scale: 1 μ m. Fig. 10. The junction plane (arrow) is apparent between the regulate outer exine and the inner exine. The inner exine protrudes into or is interbedded with the onciform layer (O). Organized tapetal materials are visible in the locule near the exine. Magnification scale: 1 μ m.

Figs 11-13. Late stage pollen grains with the lightly contrasted onciform zone next to the exine and dark intine on the plasma membrane and cytoplasm. Stain: UA-Pb. Magnification scales: 1 μ m. Fig. 11. Section from an interapertural region. The irregularity of the excine surface is emphasized by the holes and notches (arrowheads) seen in this slightly oblique view. This oblique section emphasizes the distinct nature of the inner exine (arrows) more than is apparent in Figs 12 and 13. Fig. 12. The aperture is covered during this late pollen grain stage by small darkly contrasted globules. Fig. 13. Section near an apertura margin. The left side of the figure is part of that margin and shows a thickened intine and onciform zone. Small, generally stalked, globules (arrowhead) are common on the surface of the pollen wall.

Fig. 14. Mature pollen grains had tapetal products (pollenkitt) alongside the exine in addition to the very dark globules (arrowhead) like those shown in Fig. 12. The inner exine (arrows) that is so prominent in early stages is barely discernible here and in Fig. 13.

Early stages as seen in aborted microspores

The exines in Figs 1-2 were recovered from unacetolysed mature anthers. When these exines were stained by UA and Pb there was a distinct contrast difference between the outer and inner portions of the exine (Figs 1-2). In these figures one can see many small globules under the inner exine. The exines in Figs 3-5 are from the acetolysed anther. Figs 3-4 were not section stained, and therefore contrast is mainly due to osmium added during fixation. The exine surface is contrasted in Figs 3-4 as are the junction plane, inner exine and associated globules. After section staining (UA-Pb) the inner and outer exine are similar in density to electrons (Fig. 5).

Spinules are evident in these young stages in both unacetolysed and acetolysed exines. In transverse section they are circular with a central dark site (Fig. 4). In Fig. 5 there is a unit structure that is about the diameter of spinules seen in transverse section (Fig. 4); this unit structure shows loops that are about 30 nm in diameter. The small globules under the inner exine in all these figures are to some extent aligned. In the acetolysed material in Fig. 3 there is a connecting strand between globules.

Vacuolate stage of microspore development

The increased lateral separation of outer parts of the exine (Figs 6-10) is most especially apparent in apertures (Figs 6-7; compare with stages in Figs 2 and 4). The inner part of the exine is less in evidence (Figs 6-10) than earlier. Small dark globules as seen in the central part of Fig. 8 where the invaginated exine is sectioned obliquely, are similar in location and size to globules associated with the inner exine in Figs 1-5. There is a fibrillar onciform layer under the exine (Figs 6-10).

The presence of structures from tapetal cells in the loculus (Figs 6-8, 10) indicates that tapetal cell degeneration resulted in release into the loculus of organelles and products of protoplasmic activity.

Mature pollen

Three of our TEMs at fairly high magnification have been published in Erdtman (1969: Plates 110 and 111); these micrographs show examples of an aperture, aperture margin and the interapertural exine. Fig. 11 is a more extensive part of the exine shown in Erdtman's (1969) Plate 110. It is used here to show the holes in the exine not emphasized in other published TEMs of *Anigozanthos* pollen. Figs 11 and 12 are from micrographs near an aperture margin. The sporopollenin-like globules, prominent in Figs 1-5, 11, 12 and in our plates from Erdtman 1969, occur below the exine in early stages and above it in late pollen grain stages. The inner exine is much less prominent than during early stages (e.g., Figs 1-5 and 10).

The micrograph in Fig. 14 shows remnants of tapetal products in the loculus near the exine of a pollen grain.

DISCUSSION

It was widely believed that once an exine template was established specific thickening took place even on aborted microspores (e.g., Tischler 1908). In a study of a taxon having a high rate of pollen sterility Rowley and Flynn (1969) saw exines that looked immature mixed with mature pollen grains in mature anthers. They concluded that when death occurs early in development the exines do not become characteristic for the species. Kronstedt-Robards and Rowley (1989) in work on pollen development in *Strelitzia* exines found that aborted grains seen in mature anthers were structurally the same as

the proexine in early microspore stages. They concluded that the exine on sterile grains gives what may be a rare view of a stabilized immature exine.

Currently, we consider it to be very unlikely that changes in exine development occur after death of microspores or pollen grains. Considering the time and expense required for studies of microspore development it could be expedient for us to gain information about early development from aborted grains in association with mature pollen.

The aborted exines of *Anigozanthos* in Figs 1-5 represent, in our view, very young stages in exine formation. The exine components in Figs 1 and 2 are connected rather than being separated into rugulate elements as they are later. The junction plane lies between the inner part of the exine which can be strongly contrasted and the outer part which takes up less of the stains used. The acetolysed anther also contained exines of aborted microspores. These (Figs 3 and 4) are considered to represent a later, though still very young, stage of microspore development. They are adjudged to be later because of the separation between exine components, especially in the apertures (Fig. 4). The exine parts, junction plane and spherical structures are resistant to acetolysis, and we may surmise that they are sporopolleninous.

In the later vacuolate stage of microspore development the relatively small spherical structures, so prominent in Figs 1-5, are much less evident in transverse sections (Figs 6, 7, 9 and 10). They can be seen, however, still to be numerous in the oblique section in Fig. 8 by virtue of the the plane of section.

In mature pollen there is very little exine below the junction plane although there are many spherical structures above and apparently attached to it and to the exine (Figs 12 and 13; Erdtman 1969: Figs 111 and 112). We interpret the loss of exine below the junction plane to indicate that the inner material is not replenished. It is stretched out and compacted during exine enlargement so that components are isolated and appear to be relatively small. It seems that pollen of monocots does not have the robust endexine common to pollen of dicots or gymnosperms. Early in microspore development, however, some kind of comparable zone exists at the inner portion of the exine in monocot pollen. There are reports of endexine-like zones in monocot pollen by Rowley 1964; Skvarla and Larson 1966; Rowley and Dunbar 1967; El-Ghazaly and Jensen 1986, 1987; Martinsson 1993.

The inner exine in *Anigozanthos* shows several features that are much like endexine. In pollen that has not been acetolysed or dried and then rehydrated, in extraction of material that accepts stains, the endexine takes up more stain than the ectexine – as is the case in Figs 1 and 2. After acetolysis the endexine contrasts less than the ectexine indicating that the endexine had lost more stain-accepting materials than the ectexine. In Fig. 5 the difference in contrast before and after acetolysis is greatest for the inner exine. In pollen of dicots the endexine is "separated" from the ectexine by a "white line" which Rowley (1987-88, 1995) called a *junction plane*. Rowley's idea is that the white line called a "commissural line" or junction plane results from loops in the tubules of the exine units. The radially oriented units of the inner exine join with radially oriented units of the outer exine. The junction plane is like the knots in a Berber carpet where the loops in the subunits of one unit join with subunits of several other units making a very strong union of the many tufts. We consider the inner exine in *Anigozanthos* to be an endexine equivalent. El-Ghazaly and Jensen (1986, 1987) referred to the endexine-like layer in *Triticum* pollen as an elementary endexine.

We refer to the fibrillar layer seen during the stage of microspore vacuolation as oncus-like rather than considering it to be an intine. The intine forms after microspore mitosis during the pollen grain stage and then there is a two-layered fibrillar zone (Figs 11-13); the outer part is onciform and has been in place long before the mitosis.

The pollen of five of the ten species of *Anigozanthos* has been described; two species, *A. manglesii* D. Don. (with drawings) and *A. humilis* Lindl. in Erdtman (1971), and three of our transmission electron micrographs (TEMs) of *A. viridis* were included in Erdtman's 1969 book. Zavada (1983) published TEMs and scanning electron micrographs (SEMs) of *A. flavus* D.C. ex Red. Simpson (1983) shows SEMs and TEMs of fixed and acetolysed pollen of *A. flavus* D. C.

Simpson (1983) illustrates examples of the genera of the family with many details in excellent TEMs and SEMs of each species covered. His descriptions agree with those of Radulescu (1973), Erdtman (1971, 1969), and Zavada (1983). The use of electron microscopy and intensive coverage, however, enabled him to describe them more fully. Our observations on *A. viridis* agree with Simpson's descriptions for pollen of *A. flavus*.

As Simpson (1989) wrote, pollen of species of the Haemodoraceae has a unique wall structure – the pollen is 2 or (rarely) 3-porate, hemispheric, isopolar, and rugulate. The exine is 2-layered with a *junction plane* separating the outer layer from the inner papillate zone.

The nature and function of the junction plane is far from clear as is the extent of its occurrence with regard to species. Simpson (1987) illustrates examples in the *Pontederiaceae* as well as from the *Haemodoraceae*. Rowley and Dunbar (in press) show that *Centrolepis aristata* microspores have a junction plane with an inner exine below it. Both the junction plane and the inner exine are indistinct or have been lost in mature pollen.

The locular space contained structures indicating that the degeneration of tapetal cells was probably due to breakdown of the plasma membrane and release of organelles and products of protoplasmic activity into the loculus where still relatively intact structures come to lie adjacent to the exine.

We draw a tentative conclusion that the inner zone in the *Anigozanthos* exine exhibits features similar to those of endexines in, e.g., dicot groups.

The presence of spinules in early microspore exines indicates to us that the exine of *A. viridis* is likely to be composed of unit structures like those, for example, scanned by atomic force microscopy in exines of *Nuphar* (Rowley et al. 1995).

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STRUKTURA ŚCIANY PYŁKU *ANIGOZANTHOS VIRIDIS* (HAEMODORACEAE)

STRESZCZENIE

Egzyna pyłku *Anigozanthos viridis* Endl. jest podzielona na dwie strefy przez płaszczyznę, nazywaną dotychczas "commissural line" – my nazywamy ją bardziej trafnie "junction plane". Śledząc młode stadia wiadać, że wewnętrzna egzyna staje się cieńsza – prawdopodobnie wskutek rozciągania się egzyny, podczas gdy nowy materiał nie jest wbudowywany.

Opisujemy fibrylną strefę pod egzyną mikrospor jako podobną do oncus. Fibrylna strefa pojawia się w mikrosporze przed mitozą i utworzeniem intyny. W dojrzałym pyłku fibrylna strefa jest podzielona na dwie warstwy: warstwę podobną do oncus i intynę właściwą – nie ma jednak dwuwarstwowej intyny.

SŁOWA KLUCZOWE: pyłek, egzyna, "commissural line", "junction plane", *Anigozanthos*, *Haemodoraceae*.