
Intraexinous Channels

A synapomorphy for Poaceae, defined and investigated

Jennifer J. Dixon - Iowa State University, Department of Ecology, Evolution and Organismal Biology

Abstract

The presence of intraexinous channels in the pollen wall has been proposed as a synapomorphy for the Poaceae since first identification in 1985. At the time of classification only three species of the Poaceae had been sampled with TEM methods and since then, little work has been done to determine if this character exists in all twelve subfamilies.

Though some TEM images are available from previous studies, none of them are focused on this specific character. The collection and preparation methods, if reported, differ in each publication; there is evidence that specimen age, collection source, area sectioned and processing method all impact the size, shape, and appearance of the exine in TEM images but the extent of the impact is unknown. Without this information, analysis of this character based on previously published work is uninformative.

Exine characteristics are distinctive and can vary within taxa, therefore with a thorough analysis of intraexinous channels within the Poaceae it will be possible to determine if their presence is a synapomorphy for the group and whether there is a relationship of channel characteristics within clades and to pollen arrangement type. Additionally the different patterns of these channels may allow more accurate classification of pollen from both modern and palaeobotanical sources.

This study proposes a TEM analysis of intraexinous channels within the Poaceae by sampling members of each subfamily using a single protocol. Channel characteristics from each subfamily will be described offering a deeper understanding of the evolutionary relationships and will determine not only if this character is a synapomorphy but if it can be used for classification purposes.

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Introduction

The monophyletic plant family Poaceae is defined by five morphological synapomorphies: caryopsis fruit type, embryo highly differentiated, embryo in a lateral position, and pollen lacking scrobiculi, and intraexinous channels in the pollen wall (Grass Phylogeny Working Group, 2001). All but the pollen characters are easily studied using basic visual observation and standard light microscopy, to view the ultrastructure of pollen walls one must use specialized techniques i.e. transmission or scanning electron microscopy.

For a putative synapomorphy, information on this character was rather difficult to find. Many papers refer to intraexinous channels as a synapomorphy for the Poaceae but little independent work was located. The earliest mention and most frequently cited source describes the character as "channels running parallel and right angles [to the pollen wall]" (Linder and Ferguson, 1985). The original image from Linder and Ferguson (Fig. 1) is a black and white micrograph identifying an intraexinous channel with an arrowhead showing fine channels running perpendicular and larger channels parallel to the pollen wall in the tectum (Fig. 2).

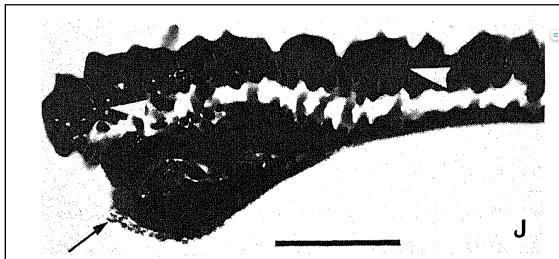


Figure 1: First TEM micrograph which specifically indicates intraexinous channels in *Poa pratensis*, shown at the arrowhead. (Linder and Ferguson, 1985)

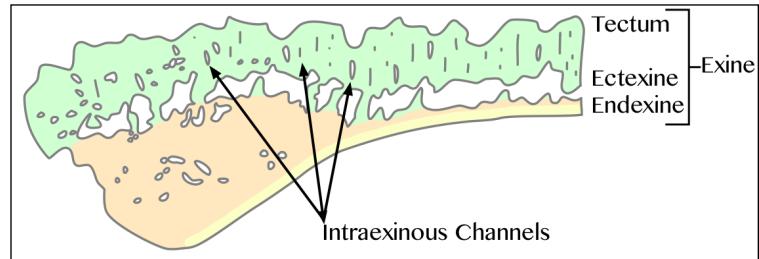


Figure 2: Modified graphic of the original micrograph. Each color highlights a particular layer of the exine. (Jen J. Dixon, created for this document)

Linder and Ferguson sampled two members of Poaceae (*Poa pratensis* and *Poa annua*) and noted that channels had been seen previously in seven other studies: Rowley (1960, 1964), Larson et al. (1962), Skvarla and Larson (1966), Christensen and Horner (1972), Christensen et al. (1974) and Heslop-Harrison (1979). These studies together offer images of intraexinous channels in *Poa pratensis*, *Zea mays*, and *Sorghum bicolor*, representing only two of the twelve Poaceae subfamilies. Further review of the literature provides just a few additional images of pollen walls in other Poaceae subfamilies (table 1), expanding coverage to three subfamilies and eight species.

Table 1: Literature with TEM or SEM images of Poaceae showing channels

Subfamily	Species	Literature
Pooideae	<i>Oryzopsis paradoxa</i>	Furness and Rudall (2003)
	<i>Elymus repens</i>	Skvarla et al. (2003)
	<i>Festuca elatior</i>	Skvarla et al. (2003)
	<i>Poa compressa</i>	Skvarla et al. (2003)
	<i>Poa annua</i>	Linder and Ferguson (1985); Skvarla et al. (2003)
	<i>Phleum pratense</i>	Grote et al. (1994)
	<i>Oryzopsis paradoxa</i>	Linder and Ferguson. (1985)
Panicoideae	<i>Zea mays</i>	Skvarla and Larson (1966); Skvarla et al. (2003)
	<i>Sorghum bicolor</i>	Christensen and Horner (1974)

The intraexinous channels visible in these early papers seem to possess different morphologies. In *Poa pratensis* (Fig. 1), the channels are running both parallel and perpendicular to the pollen wall, some reaching the tectum, others terminating within the ectexine. In *Zea mays* (Fig. 3), few of the channels reach from the endexine to the tectum and are arranged perpendicular to the pollen wall. In *Sorghum bicolor* (Fig. 4), many of the channels run the length of the tectum but do not encroach into the ectexine or endexine and appear both parallel and perpendicular to the pollen wall. Each of these members of Poaceae undoubtedly possess intraexinous channels and each species seems to have morphologically distinct channel characteristics.

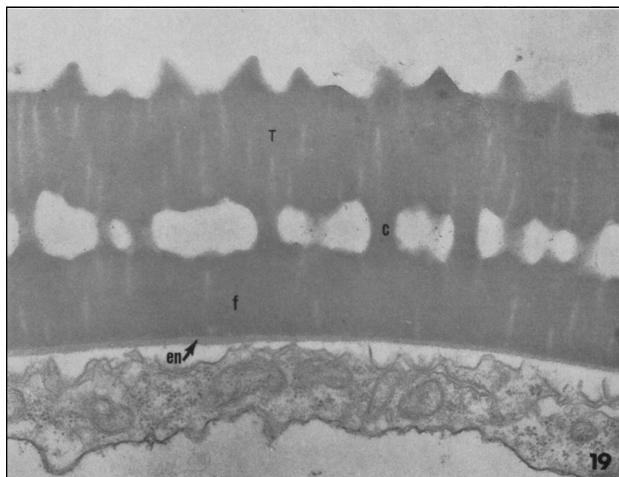


Figure 3: TEM of *Zea mays* showing parallel channels throughout the exine. (Skvarla and Larson, 1966)

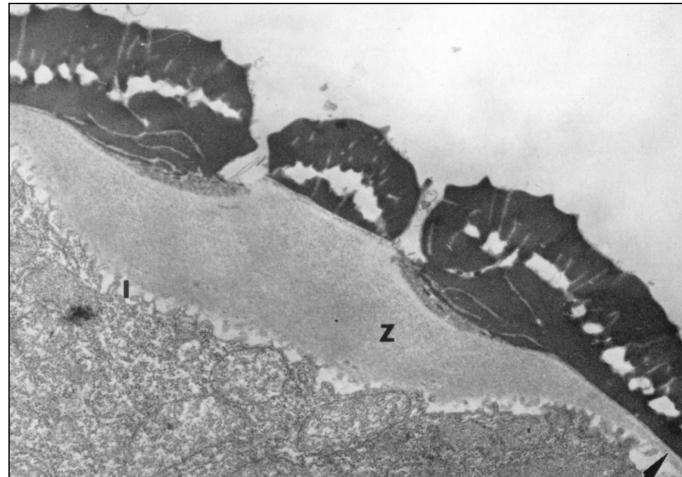


Figure 4: TEM of *Sorghum bicolor* showing variously arranged channels. (Christensen and Horner, 1974)

Pollen Development

The pollen grain is not a single cell but is the gametophyte generation of flowering plants which undergoes five developmental steps (Lersten, 2004; Heslop-Harrison, 1971):

1. Diploid microsporocytes develop within the theca of the mother plant.
2. Four haploid microspores develop through meiosis from each microsporocyte.
3. The microspores undergo mitosis creating a two celled or binucleate pollen grain.
4. Each pollen grain develops a thick sporopollenin wall.
5. Mature pollen is released from the theca.

The sporopollenin wall develops through secretions from the cells of the pollen and iterative depositions from the tapetum of the theca; secretion source(s) and molecular properties of depositions which make up the pollen wall vary by species. This means that intraexinous channels are developed in the pollen wall at different developmental stages depending on the species.

Pollen Arrangement

Two different types of pollen arrangement have been observed in the Poaceae, central and peripheral (Kirpes et al, 1996) yet a complete review of the family has not been done. What we do have tells us that some basal lineages have a central pollen arrangement while some later diverging species have a peripheral arrangement; there may be phylogenetic associations between pollen arrangement and exine structure but current literature does not hold enough information to offer this kind of insight.

Exine Structure and Impacts of Processing

No single study of has been done of the Poaceae to explore intraexinouse channels as a synapomorphy, and though many TEM images of Poaceae members are available in previously published literature, touching on various topics from pollen development to allergen research, it would be uninformative to use them as source material because various collection and preparation methods were used, each of which impacts pollen characters differently (Bashir et al, 2013). One example of these variances is found in the allergy research field (fig 5), though this study was not focused on intraexinous channels, their findings show that different treatments of pollen have distinct impacts on the appearance of intraexinous channels.

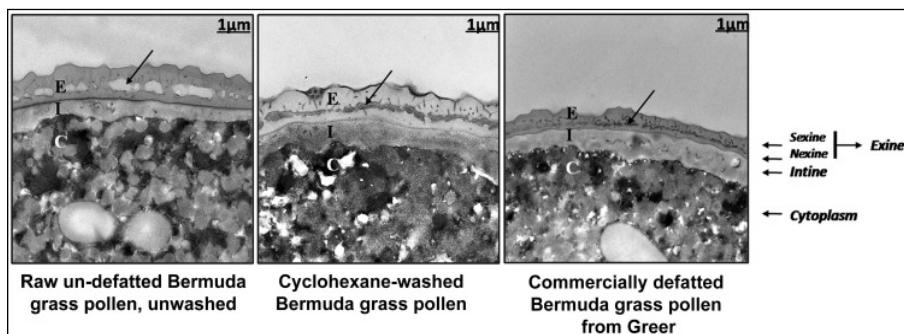


Figure 5: This series of images show various effects of different treatment methods on the pollen wall. Though this study dealt with treatments specific for allergen preparation for allergy treatment and immunology, it drives home the point that the treatment method alters the visibility of exine microstructure. (Bashir et al., 2013)

Most academic papers will state where pollen was sourced, often fresh squashed anthers or dried herbarium specimens, but most do not include details of the age of the pollen or how they were treated before TEM preparation. The chemical composition of layers deposited and the stage at which they are laid down create the unique patterns seen in TEM images, thus the age of a specimen could impact the presence or absence of intraexinous channels. Additionally, exposure to water has been shown in some species to cause rupture of the pollen wall and excretion of allergenic chemicals (Grote et al, 2001) which may be stored in these channels. Since the developmental stage of the pollen specimen and pre-TEM rehydration may impact the size, shape, and appearance of intraexinous channels, it is important that any family study use a single collection and preparation process for all specimens.

Exine Structure Variance

Exine structure can vary greatly within a single group of plants. Variances in ornamentation, exine layering, and thickness can be seen in Gnetales for example (Fig. 6). Not only do we see differences between species but there can be variance in structure based on location of sampling. In Nymphaeaceae for example, a distal pole section shows no columellae but in a proximal pole section thin columellae are evident (Yao et al, 2004).

In the few samples available within the Poaceae distinct differences in exine structure are evident but there is no literature which investigates this variances. Because none of the previously published works include details on where sections were taken from (although many available images are at or near the pore), we simply do not know if intraexinous channels are found throughout the pollen wall and how position relates to these characters.

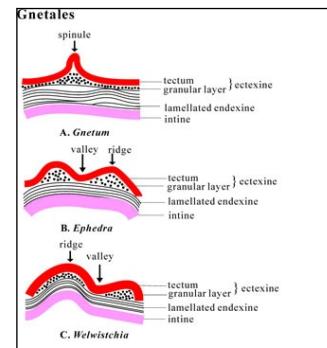


Figure 6: Pollen wall arrangements in Gnetales and ANITA group showing the variety of pollen characters which can appear in single groups. (Yao et al, 2004)

Artifacts of Processing

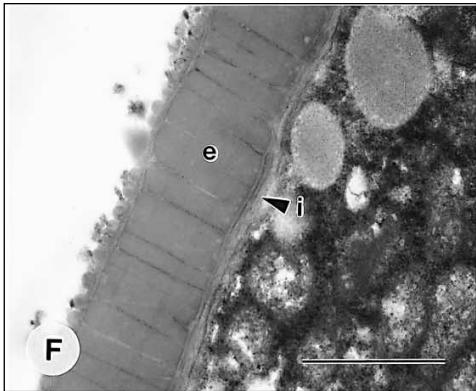
On initial observation, channels running in only one direction could be interpreted as an artifact of the sectioning process. Even glass blades can produce parallel "scratches" in the images however I have found it is possible to rule out artifact creation when the channels obviously terminate at a layer barrier like the endine. When these channels appear to travel beyond layer barriers it is possible to interpret the character as either an artifact of the process or damage to the section.

Channels in Other Species

Besides noting intraexinous channels in members of the Poaceae, Linder and Ferguson (1985) mention the resemblance of these channels to the "conspicuous small spherical intra-exinous cavities" found in Myristicaceae (Walker and Walker, 1979), yet no later papers address this observation. The channels in Myristicaceae appear to only run parallel to the pollen wall (Fig. 7), but it is unknown if this is characteristic of the family. To date, I have found what some may call intraexinous channels in TEM images in species of non-Poaceae monocots and the Magnoliids (Fig. 8).



Figure 7: This is the first TEM of Myristicaceae pollen showing what is referred to as "conspicuous small spherical intra-exinous cavities." There is no identification as to where the researchers saw these cavities specifically but the reader infers that they are the white "dots" in this image within the tectum. (Walker and Walker, 1979)



It is interesting to note that these other examples of channels in the exine of pollen also have distinct morphological characteristics which may be indicative of their species or family; the channels in Amarylidaceae for example appear to run only in a perpendicular pattern.

Figure 8: TEM of Amarylidaceae pollen. Here the parallel channels run the length of the exine, each parallel channel appearing to be composed of stacked perpendicular channels. (Fundress and Rudall, 2003)

Thesis Statement

It is my hypothesis that the appearance of intraexinous channels in the pollen wall is significantly impacted by the sampling and preparation methods used in TEM analysis due to the complex developmental process of pollen grains and their chemical composition making analysis of this character using previously published literature uninformative. Additionally, I will test the hypothesis that 1) the presence of intraexinous channels is a synapomorphy for the Poaceae, 2) a comprehensive analysis, sampling species from each Poaceae subfamily, will show pollen wall channel morphologies distinctive enough to allow identification and classification of modern and paleobotanical pollen specimens within a specific major clade, and 3) pollen arrangement within Poaceae has an evolutionary trend moving from central in the basal lineages to peripheral in the later diverging ones.

Implications of the Research

This study will expand the current body of knowledge by offering definitive answers on the impacts of processing on specimens which will allow previously published work to become informative for this and future studies. It will also determine if the presence of intraexinous channels in the pollen wall is indeed a synapomorphy for the Poaceae as well as provide detailed descriptions of the morphology of these channels in each subfamily allowing identification and analysis of existing specimens and both previous and future paleobotanical specimens. There are other less obvious implications of this research, such as the ability to determine a plant species or family via pollen wall sampling applicable to various fields, from law enforcement to allergen research.

Materials and Methods

Stage One: Protocol Development

All specimens will undergo the same processing method developed with the assistance of Harry Horner and Tracey Pepper (Microscopy and Nano-Imaging Facility, ISU). Methods gathered from previously published literature will be reviewed and methods producing the best images of intraexinous channels will serve as a starting point. A literature review of these materials will be conducted so the most current and effective methods are utilized.

A more complete "methods" section will be available once this review is complete which will include the chemicals and tools required for the study.

Stage Two: Collection Method Analysis

The first goal of this study will be to understand how age, drying, rehydration and preparation impact the resulting images. This will allow us to determine if previously published TEM images can be considered informative for this and future studies.

There are three main categories of specimens to analyze, fresh, recently dried, and dried aged. Fresh mature anthers will be gathered from a living plant grown in an ISU greenhouse, of a species for which we have a dried pressed herbarium specimen. Half of the fresh material will be squashed and processed, half will be dried and then processed, and voucher specimen will be collected and processed. Each specimen type will be processed using the same methods allowing us to identify how the appearance of intraexinous channels differs by collection methods.

Stage Three: TEM

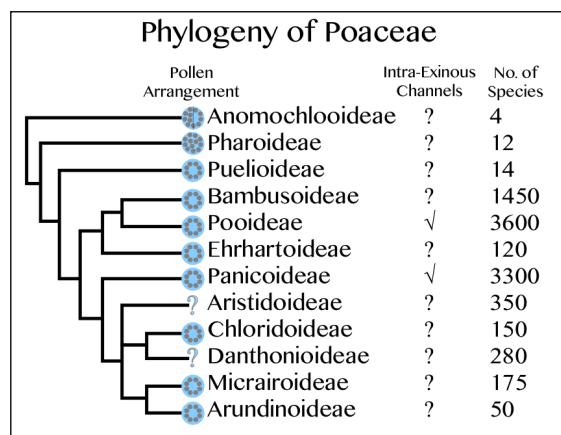
The results of Stage Two will determine how we will gather specimens for analysis, if dried vs. fresh vs. aged herbarium specimens do not significantly impact the appearance of the channels, mixed collection methods can be used. If collection method does impact the appearance of the channels we will select one method for all specimens. Representative species from each of the twelve subfamilies will be selected and specimens will be collected and processed to create a TEM image showing the pollen wall.

We will initially sample one species in subfamilies with less than 500 species and three species from the larger groups of Pooideae, Bambusoideae and Panicoideae; if we see only small differences in the intraexinous channels between each subfamily no further species need be sampled. However, if channel characteristics do vary between subfamilies additional sampling will be necessary to investigate the variation, How many additional specimens and of which species will be determined at that time.

It will be important to attempt imaging of sections from both proximal and distal positions of the pollen grain as this will offer insights into the developmental patterns and relationship of pollen wall ultrastructure and pollen arrangement type.

Outgroup specimens should also be processed and analyzed. It would be beneficial to sample specimens from families closely related to the Poaceae (Flagellariaceae, Joinvilleaceae, Ecdeiocoleaceae) as well as a dicot member. I believe that resampling a few of the families which have been shown to have channels described as "intraexinous" in previous literature, such as the Myristicaceae (Walker and Walker, 1985) and the Amarylidaceae (Fundress and Rudall, 2003) so distinction may be made between Poaceae and non-Poaceae intraexinous channels.

Each image will be analyzed for the presence or absence of intraexinous channels and if present, they will be described.



Optional Post-TEM Stage

TEM offers detailed 2-dimensional images allowing accurate analysis of intraexinous channel morphology. It would however be beneficial to observe some specimens with SEM techniques to produce 3-dimensional images. This may provide additional details as well as provide a valuable comparison on techniques.

Stage Four: Analysis

Once descriptions of the channels in each subfamily are recorded I will first optimize the character states along the phylogeny of the Graminoid Clade. Then further analysis will include a determination if individual pollen characteristics are distinctive enough to allow for classification within a subfamily and whether relationships between pollen arrangement and other pollen characters can be inferred based on channel characteristics.

Several papers will be submitted for publishing based on this research including:

- Review of processing impacts on pollen wall ultrastructure
- Suggested protocol for TEM pollen analysis
- A family wide analysis of intraexinous channels in the Poaceae
- A review of Paleobotanical publications of grasses pollen using channel morphology
- Additional papers depending on findings

Timetable

It is important to note that this timetable is an estimate.

- ❖ Stage One protocol development and process training will be completed by April 1, 2013.
- ❖ Stage Two is expected to take approximately one month allowing collection methods to be selected by June 1st.
- ❖ The timetable for Stage Three will be determined by the collection methods chosen. If herbarium samples can be used collection and preparation will be much simpler, if fresh specimens are required it may take several months to identify and collect specimens.
- ❖ Sampling and processing of specimens will take approximately three to four months.
- ❖ The Optional Stage of SEM processing will likely be done in tandem with Stage Three, if done.
- ❖ Stage Four analysis is expected to take approximately two to four months depending on the depth of analysis.

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