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An evo-devo perspective on no Ordovician land plants

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ABSTRACT

Molecular phylogenetic studies of land plant (embryophyte) origins have begun to tease apart those evolutionary contributions derived from prior algal genes and those *de novo* genes that evolved during a charophyte–embryophyte transition. Applying the concept of genomic *assembly* in plant evolution to the fossil record leads to a paradigm shift in the interpretation of the Ordovician record of land plants. Traditional phylogenetic thinking requires fossil species taxa to occupy nodes on a phylogeny. An evo-devo approach can view character evolution separately from species taxa, freeing up fossil spores and tissue fragments to become clues to underlying developmental pathways or gene regulatory networks. This results in a re-assessment of what is meant by the presence of land plants in the Ordovician landscape. The new model helps to reconcile discrepancies between molecular time-trees and the "missing" record of fossil plants during the Ordovician Period.

Introduction

In recent years, with the application of phylogenomic techniques to the study of land plant origins, a consensus is now emerging on the Zygnematales as the most closely related group to the Embryophyta (Wodniok et al. 2011; Zhong et al. 2013; Wickett et al. 2014), although on the basis of classical morphological and developmental characters, the Coleochaetales possess synapomorphies today that seem more likely to parallel what we anticipate the last common algal ancestor would have possessed. These include zygospores with resistant walls that contain sporopollenin-like compounds (Delwiche et al. 1989). *Zygnema* and related species also produce zygospores that are known in the fossil record (Hall and McCourt 2017). Thus, it appears that the perennation function provided by a resistant cell wall was transferred in evolution from the algal zygote to the spore wall. Evidence for such a sporopollenin transfer hypothesis (Graham 1984; Hemsley 1994) has been supported by studies of spore development (sporogenesis) in bryophytes (Brown and Lemmon 2011; Renzaglia et al. 2023). Bower (1908), on the basis of comparative developmental morphology of living bryophytes, also saw the evolutionary origin of the plant spore as the initial stage in his interpolational, or antithetic, hypothesis for the origin of the plant sporophyte.

The interpolational hypothesis provides a framework with which to explore an evo-devo model of land plant origins. It begins with the origin of the spore, which is followed by the vegetative plant sporophyte. There is a sequence of origins of developmental pathways which were subsequently incorporated into an evolving genomic complex, out of which came the initial embryophyte. The term "developmental pathways" is vague, but it meant to include both Character Identity Networks (ChINs, Wagner 2014) and structural fingerprints (Tomescu and Rothwell 2022). Studies in the phylogenomics of extant plants and charophyte algae show that these components of the embryophyte genome (the developmental toolkit) that distinguish the embryophytes from their ancestors are a combination of re-purposed (or co-opted) prior algal genes and *de novo* genes that are unique to the embryophytes (Floyd and Bowman 2007; Harris et al. 2022).

Integrating the Ordovician fossil record within an evo-devo model

Basically, an evo-devo model of the algal-plant transition considers genomic assembly as the underlying evolutionary process by which the transition occurred. The extent to which fossils can be tied to particular ChINs can, then, help to construct an

evolutionary timeline of character addition. This frees us to consider the fossil record in terms of the evolution of land plant characters, rather than as species taxa. And, in this way, fossils can be utilized to predict the order in which novel features were added to the evolving embryophytic genome. However, such characters, or features, are limited to a large extent to cells and tissues that are composed of recalcitrant biopolymers that facilitate their preservation in the sedimentary record. These include cryptospores *s.l.*, trilete spores, tracheary elements (TEs) and cuticles. A simple sketch of such a character-based time line is shown in Fig. 1.

The Cambrian cryptospore polyads *Adinosporus* and *Agamachetes*, which persist into the Lower Ordovician, represent the earliest Ordovician occurrences of an evolving subaerial charophytic algal complex (Strother and Foster 2021). These spores occur in packets of enclosed spores that retain their developmental pattern of cell division in the topology of surrounding, resistant walls (Strother and Taylor 2018), patterns that are comparable to reduction division seen in charophyte algae, including *Coleochaete* (Haig 2010, 2015), but not in land plants (Brown et al. 2010, 2015). *Adinosporus* probably represents an early stage in the transfer of sporopollenin from the zygote wall to the spore wall, in which nuclear division is uncoupled from cytokinesis and in which resistant-walled spores are formed, rather than flagellated zoospores. This later feature is assumed to have occurred in response to perennation in subaerial habitats.

Cryptospore dyads that are indistinguishable from *Dyadospora murusattenuata* Strother and Traverse have their first occurrence in the Tremadocian of Australia (Strother and Foster 2021). *D. murusattenuata* and other species of *Dyadospora* persist throughout the entirety of the Ordovician as they occur in the Katian–Hirnantian of Iran (Ghavidel-Syooki 2016; Ghavidel-Syooki and Piri-Kangarshahi 2021). The morphology of simple dyads makes it treacherous to assume much about their systematic affinities. They occur in the planar, orthogonal spore thalli of *Grododowon*, which clearly demonstrate an algal derivation, but permanent dyads are also known to occur in the sporangia of true land plants as late as the Lochkovian (Wellman et al. 1998; Habgood 2000; Morris et al. 2012).

Tightly adherent tetrahedral tetrads have long been considered the precursors to the trilete spore (Gray and Boucot 1977; Wellman and Gray 2000) and there is little disagreement as to their affinity with the land plants. Both *Tetrahedraletes* and *Cryptotetras* first occur in the Darriwilian and this benchmark is often cited as the fossil basis for the timing of land plant origins. However, more loosely adherent
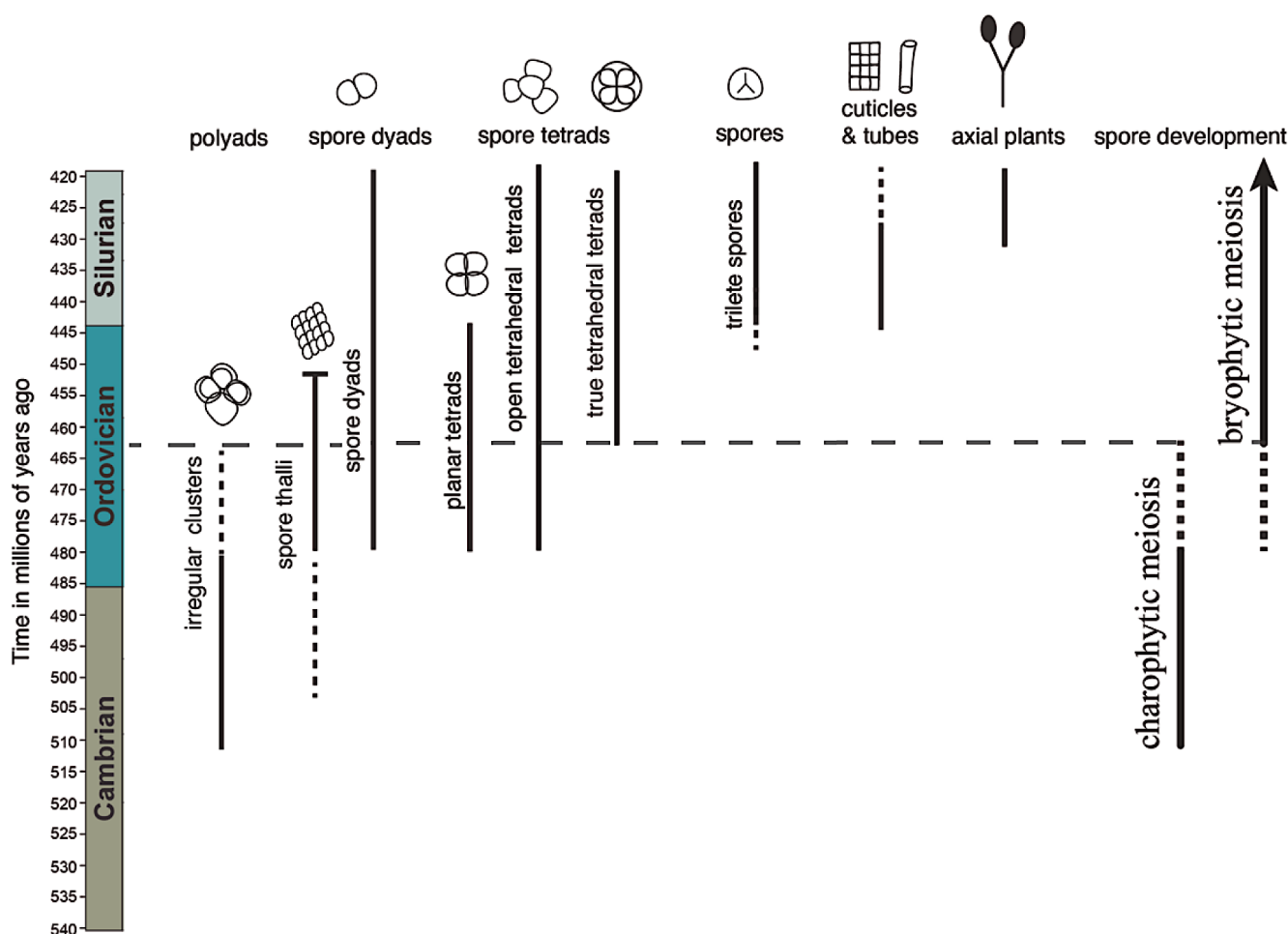

Fig. 1. Fossil record of microfossils related to land plant origins seen as character distributions. The horizontal dashed line represents the conventional origin of land plants based on the first occurrence of tetrahedral cryptospore tetrads. "Spore development" tracks the transition from charophytic spore formation to embryophyte sporogenesis, as recorded in cryptospore polyad topology (see Strother and Taylor 2018).

tetrahedral forms classified as *Rimosotetras* Burgess are morphologically very similar to *Tetrahedraletes*, and these are known throughout the entirety of the Ordovician. In a phylogenetic way of thinking, the presence of Ordovician cryptospores that are known to occur in Lochkovian land plant sporangia acts as a proxy for the existence of embryophytes in the Ordovician. However, as pointed out more recently (Strother and Taylor 2018; Strother and Foster 2021), in an evo-devo scenario, isometric spore tetrads, strictly speaking, are proxy evidence for the canalization of an embryophytic form of meiosis (sporogenesis) – not of the existence of the full developmental toolkit that is the genomic basis of embryophytes *per se*. It is entirely possible, even likely, that the first true plant spores/cryptospores were produced in a sporangium that was not attached to an axial sporophyte. This provides a rational basis for why axial plant stems are missing from the subsequent Ordovician rock record. Intriguingly, the single example of an Ordovician (Katian) sporangium (Wellman et al. 2003) shows precisely that – spore masses attached to partial coverings of amorphous organic matter.

Beginning in the Hirnantian, palynological assemblages of non-marine provenance contain scraps of cuticle and a range of tubular organic macerals (*Laevitubulus* and *Porcatitubulus*) (Burgess and Edwards 1991), which have long been considered to be remains of the problematic group Nematophyta Strother (1993). Nematophytes from Lochkovian strata are now considered to be possible lichens (Edwards et al. 2018), and a fungal ecology (Hueber 2001) and physiology (Taylor and Wellman 2009) for these organisms has been proposed. The ties to fungi are very tenuous, however, particularly when it comes to dispersed tubular macerals – none of which possesses any tangible fungal synapomorphies. Niklas and Smocovitis (1983) considered macerated nematophytic thalli of Llandovery age to be conducting cells of a pre-vascular embryophyte, and a recent FTIR analysis of *Nematoplexus* from the Rhynie Chert (Loron et al. 2023) indicated that the organic composition of this Devonian nematophyte reflected a land plant over fungal affinity. An evo-devo model of sporophyte assembly would predict that such isolated tubular macerals are some form of "proto" tracheary elements – apoptotic fluid-conducting cells. The genomic basis for this cell type was later incorporated into the embryophyte genome, where it formed the basis of conducting cells in the developing sporophyte axis.

Conclusions

The origin of land plants is one of only four cases of the *de novo* origin of complex multicellularity that was achieved through embryonic development (Sebé-Pedrós et al. 2017). As such, it represents an evolutionary transition that was much more complex, and fundamentally different, than any other node in the phylogeny of the Streptophyta, because this transition includes the evolution of development in plants. Fossils which can be traced back to their developmental origin can provide clues about the nature and timing of this evolutionary transition, and the Ordovician is an important

time segment in this regard. If our assumptions about the charophycean affinity of the Cambrian and Early Ordovician cryptospore record are correct (Strother and Foster 2021), then the arrival of tetrahedral cryptospores during the Middle Ordovician is not a record of land plant origins, but is instead an indication of the canalization of meiosis in plant embryogenesis. The reason we do not find plant axes in any Ordovician strata is because the shoot apical meristem (SAM), which is responsible for building a plant stem, did not evolve until the Silurian. This does not, however, preclude the possibility of a thalloid flora at a bryophyte (liverwort) grade of morphological complexity living in a subset of terrestrial settings during the latter half of the Ordovician. The overall similarity, based on wall ultrastructure and morphology, between Ordovician cryptospore dyads and tetrads with similar Silurian taxa supports such a conclusion. However, other likely features of plant development found as fossils, such as dispersed cuticles and proto-tracheary elements, do not appear until very near the end of the Ordovician. This would seem to indicate that the genomic assembly of the embryophytes continued well into the Silurian, culminating in the evolution of the apical meristem and subsequent axial growth by the Wenlock. The Ordovician is a time of cryptospore evolution, but the plant thalli that bore these spores have yet to be recovered in Ordovician strata.

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