Molecular self-assembly: Hypothesized for “hair” of Macroneuropteris scheuchzeri (Pennsylvanian-age seed-fern)

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1. Introduction

The current concept of Macroneuropteris scheuchzeri (Cleal et al., 1990; Hoffmann, 1827) includes four character states on the abaxial pinnule, i.e., extracuticular deposits (this study), hair (trichome in botanical Greek), files (unicellular transparent structures), and papillae which are comparatively small and curved. Of these features, the trichomes very densely populate the abaxial lamina (see Barthel, 1961; Cleal and Zodrow, 1989, and others). In contrast to these features of which extracuticular deposits are clearly observable under a loupe or even by the naked eye as they are up to 5 mm long, Hoffmann did not mention the presence of any abaxial features in his diagnosis of the species. However, the presence of extracuticular deposits, using the name hair, has been taken as an important taxonomic character state since 1847 to distinguish the identity of M. scheuchzeri from among similar larger-leaved Pennsylvanian foliage (literature survey: Bunbury, 1847 to Stull et al., 2012). A notable exception is Leo Lesquereux who instead I regard as extracuticular deposits very millimeter-long hair-like structures on Macroneuropteris scheuchzeri (Cleal et al., 1990, 2013).

This paper focuses on the hypothesis that the long, hair-like structures of M. scheuchzeri are really extracuticular deposits resulting from the process of dynamic molecular self-assembly (summary: Koch and Ensikat, 2008). A review of the taxonomy/systematics of M. scheuchzeri is accordingly recommended.

2. Materials and methods

The Carboniferous seed-fern M. scheuchzeri bears polymorphic pinnate foliage that is seen above and below a basal frond dichotomy; individual pinnule lengths range from 3 mm to 120 mm (summaries: Cleal and Zodrow, 1989; Laveine, 1997; Laveine and Belhis, 2007; Zodrow, 2003, and many others).

The compression specimens for this study were collected by the author from the roof shale of the Lloyd Cove Seam, which is known in the literature for its rich content in plant fossils that are well-preserved (Fig. 1). For the experimental work, only compressions freed from the rock matrix were used from which extracuticular deposits were collected in a Petri dish. Trichomes were obtained from a cuticle of a macerated compression (cf. Cleal and Zodrow, 1989).

Spectrochemical analyses of extracuticular deposits and trichomes were performed using FTIR (Fourier transform infrared spectrometry) and the KBr-pellet technique. Interpretive details, particularly of IR (infrared) spectra of M. scheuchzeri, supported by carbon 13 nuclear magnetic resonance studies, are found in Lyons et al. (1995), or D’Angelo et al. (2010, 2013).
3. Results

3.1. Physical aspects of extracuticular deposits

The confusion in the literature between hair [trichomes] and extracuticular deposits stems mainly from lack of observing the abaxial surface of \textit{M. scheuchzeri} compressions after being freed from the entombing rock matrix, though previous exceptions are noted (Barthel, 1961; Cleal and Zodrow, 1989; Gothan, 1916). To untangle the confusion necessitates (1) examining freshly unearthed compressions immediately after collecting, (2) examining the HF solution used for freeing the compressions from the rock matrix, (3) real-time studying of the compressions during Schulze’s (1855) maceration process, and (4) examining the ammonium hydroxide solution that produces the cuticle. Summarizing my experimental results, extracuticular deposits (1) tend to drop-off in storage because of dehydration of the exposed compression (not of the extracuticular deposits), (2) were found loose in the plastic dishes, (3) had slowly solubilized on the compression in ca 3–5 h, and (4) intact trichomes were found in Petri dishes, correlating with structural holes found subsequently in the corresponding abaxial cuticles.

Microscopic observations include that trichomes (ca. 300 µm long) are not ordinarily visible on compressions, freed or still attached to the rock matrix, but molds of extracuticular deposits are marked (Fig. 2A), and extracuticular deposits flatly overlie the abaxial venation at an acute angle in a more or less consistent parallel arrangement with the midvein (Fig. 2B). The distribution pattern, as correctly drawn by Bunbury in 1847, follows a trend, i.e., it is biogenetically non-random. Extracuticular deposits are straight in shape, doubly-pointed, black, solid, and fracture easily (Fig. 2C to E). Microscopic examination of the abaxial surfaces of compressions at ×250 magnification, and critical observation and photography of the compressions during maceration, show no evidence for organic attachment of the extracuticular deposits. Most importantly, they are acellular (Fig. 3).

Fig. 4A, B shows IR spectra of individual extracuticular deposit and trichome, respectively. In particular, the former is relatively aliphatic-rich, as indicated by the larger CH$_2$/CH$_3$ ratio of 3.0, which at the same time implies comparatively longer and straight hydrocarbon chains with relatively few side branchings. This ratio is computed after deconvolution in the 3000–2800 cm$^{-1}$ aliphatic stretching region (see Zodrow and Mastalerz, 2001, Figs. 6 or 7). The Al/Ox ratio of aliphatics to oxygen-containing compounds [(3000–2800/1800–1500) cm$^{-1}$ band] is comparatively very small at 0.32, which suggests a significant joint contribution of oxygen-containing groups and aromatic carbon. The peak at ~3400 cm$^{-1}$ is due to hydroxyl absorbance, at 1727 cm$^{-1}$ (C=O) ester, at 1634 cm$^{-1}$ (C=O) ketones, at 1385 cm$^{-1}$ (symmetric COO$^-$), and at 1029 cm$^{-1}$ (C–O–C) ether, or Si–O stretch in silicates (see Chen et al., 2012). The inescapable conclusion is that extracuticular deposits are the last physiological event in the development of the \textit{M. scheuchzeri} pinnule, representing an excreted biochemical deposit.

In contrast, the trichomatous IR spectrum is relatively poor in terms of functional groups, confirmed by a second spectrum. In particular,
absent are the aliphatic stretching bands (C–H) that are necessary for calculating the two ratios mentioned (D’Angelo et al., 2010; D’Angelo et al., 2013).

4. Discussion

4.1. Comparison of IR-spectra: extracuticular deposit vs compression

The combination of long and straight aliphatic chains with increasing contents of oxygenated/aromatic carbon groups does not fit the usual IR signature for medulosalean tree-fern compressions (compare with Lyons et al., 1995; D’Angelo et al., 2010, 2013, and others). In medulosalean compressions, the Al/Ox ratio is generally much larger than 0.32, but the CH2/CH3 ratio may be comparable. In comparison with vitrain from the Lloyd Cove Seam (sample location of the M. scheuchzeri specimens in this study), the Al/Ox ratio is similar, but the CH2/CH3 ratio is not, being unity or less for the vitrain (D’Angelo et al., 2010, Table 5, analyses #17 and #19). In the original chemical study of M. scheuchzeri, Lyons et al. (1995) concluded that the compression (understood without the cuticle) underwent chemical changes similar to those of the vitrain in the Lloyd Cove Seam. However, the IR spectrum of the extracuticular deposit is different from that of the compression (understood without the cuticle) by having more aliphatic C–H groups, relative to carboxyl/carbonyl groups, and much reduced aromatic bands in the out-of-plane region. I mention, however, that...
the CH₂/CH₃ and Al/Ox ratios for compressed trichomes of *Alethopteris pseudograndinioides* correlate with the compressions to which they were attached (unpublished research notes, 2013). This IR result suggests that the chemical signature of the mother tissue is carried into the epicuticular feature which is not the case for *M. scheuchzeri*, but supports the argument for a biochemical deposit. Moreover, the biological reason and the significance why the spectrochemistry of the trichome-mother tissue of *M. scheuchzeri* is non-convergent remain unknown for lack of experimental data.

### 4.2. Molecular self-assembly and plant surfaces

Self-assembly, more specifically molecular self-assembly, is defined “as a process by which molecules adopt a defined arrangement without guidance or management from an outside source” (Wikipedia, the free encyclopedia). As a field of study, the concept originated with organic chemistry, and is applicable at all scales to a host of natural phenomena, including polymeric morphologies (Barrett et al., 2011). As such, it is not just confined to nano or biomacromolecular scales. Self-assembly is not synonymous with formation because the process involves “… pre-existing components … controlled by proper design of the components” (summary, Whitesides and Grzybowski, 2002, p. 2418). Moreover, Mandelbrot’s fractal geometry (Mandelbrot, 1983) is an example where, instead of molecules, geometric entities (fractal dimensions) adopt a defined arrangement by self-similarity, with application to fractal taxonomy of Carboniferous spore-bearing ferns (Heggie and Zodrow, 1994). It is outside the scope of this paper to argue for synthesis of self-assembly and self-similarity.

The role of waxes in the make-up of extant cuticles has been known for a long time (summary: Holloway, 1994), and Koch and Ensikat (2008) review the process of molecular self-assembly for epicuticular-wax crystallization on the cuticular substrate. It is these microscopic wax crystals that impart to extant [and fossil] cuticles alike the hydrophobic nature with well-known physiological properties that include regulatory water management (Szafranek et al., 2008). Wax crystallization is influenced by temperature, solvent and cuticular substrate, and self-assembly is carried out in solution at or near an interface, where non-covalent interactions among molecules are the driving forces, among other things. As Koch and Ensikat (2008) explained it, the process takes place by diffusion “through the cuticle via lipoidic pathways”, and they stressed that all true aliphatic waxes had X-ray diffraction patterns, i.e., are crystalline (see Merk et al., 1997). But unanswered questions remain about the influence of the cuticle as substrate on spatial distribution and pattern building of the epicuticular waxes. The existence of fossil-wax crystals is inferred from the micron-sized platelet-like particles on the cuticle of the Carboniferous seed-fern *A. pseudograndinioides* (Fig. 5), and thus the possibility exists that the hair-like structures may be of similar origin, but on a macroscale. Significant in this respect was the discovery by Gao (1992), using transmission-electron microscopy, that cuticles of *M. scheuchzeri* from the Sydney Coalfield showed microchannels that I now compare with the lipidic pathways. Consequently, I hypothesize a process of molecular self-assembly and propose the name extracuticular deposit, based on the experimental results of this study. The name “extracuticular deposit” reflects the origin and at the same time the emergent nature as cuticular sensu Koch and Ensikat (2008).

### 4.3. Systematic review

The reported experimental results raise three arguments for reviewing the systematics of the current concept of *M. scheuchzeri*,

![Fig. 4. Macroneuropteris scheuchzeri. IR spectra. (A) Extracuticular deposit. (B) Trichome from a cuticle.](image-url)

![Fig. 5. Alethopteris pseudograndinioides, cuticle. SEM image. The small elongate features are interpreted as platelets, wax crystals (Stoyko et al., 2013).](image-url)
which are outside the scope of this study, but summarized briefly. The first is if the position of *Macroneuropteris* Cleal et al., 1990 is tenable within the neopterid taxa, given the presence of the character state of extracuticular deposits that are not known from any other member in the medullosan seed-fenn group (Zodrow, 2013; D’Angelo and Zodrow, manuscript in progress)? The consequence of an untenable position is a destabilized nomenclature for a widely-known Carboniferous species. The second argument involves Hoffmann’s types if they still can be the basis for *M. scheuchzeri*, i.e. is evolving science-technology a factor in nomenclatural change? The last argument is if a reexamination of the neopteroid type specimens known to show these long hair-like structures shouldn’t be the first step in a systematic review?

5. Conclusions

- The theory of dynamic molecular self-assembly is introduced into palaeobotony.
- ‘Hair’, the long-time recognized 1–5 mm long diagnostic feature on the abaxial surface of *M. scheuchzeri*, is a misnomer. Instead, extracuticular deposit is proposed as being an appropriate name.
- Extracuticular deposits, built-up of long-chain aliphatics, do not compare morphologically or spectrochemically with trichomes nor with organic matter taken from the pinnule lamina of compressed *M. scheuchzeri* itself.
- The existence of extracuticular deposits paves the way to an answer of the abaxial surface of *M. scheuchzeri*.

A review of the neopteroid systematics is recommended.

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References