57-8

BIOMARKERS IN PALEOZOIC CRINOIDS (BORDEN GROUP, MISSISSIPPIAN): IMPLICATIONS FOR PHYLOGENY

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Forty species of crinoids were uncovered in LeGrand, Iowa and fossils from this site became famous for their characteristic and peculiar species-specific coloration. Similar trends in color occur in the Borden Group (Mississippian) in Indiana, as well as other locations across North America and Europe, but the reasons behind these trends and the applications of this information have not been investigated. Inorganic pigments are usually the cause of unusual coloration in fossils, but in this case the species specific coloration is due to organic molecules, and is not a relic of taphonomic processes. Biomarker molecules are preserved and have been extracted from these Mississippian crinoids.

Fossil echinoderms, specifically crinoids, possess chromophoric organic molecules, fringelites, that resist diagenetic bleaching, are chemically stable over geologic time, and have occurrences and abundances that are species specific. When hue, chroma and saturation are determined for the colors of these fossils by comparison to a Munsel color chart, discriminant function analysis indicates that based on coloration, these crinoids sort into their morphologically determined Classes. Therefore, these fringelites and other related molecules, anthraquinones, are candidates to function as a proxy for phylogenetic reconstruction.
In this study, spectral data were defined for several extracts for several species of crinoids. Identifiable basal and radial plates were tested for biomarker molecules, and spectral data of the organic molecules present in the crinoids were obtained by UV-Vis, Fluorescence, HPLC (High Performance Liquid Chromatography), and GC/MS (Gas Chromatography / Mass Spectrometry). Preliminary results obtained by UV-Vis spectroscopy and Fluorometry indicate the presence of fringelites and anthraquinone: both are pigment molecules present in modern crinoids. Complex quinone molecules in the extracts are analyzed for their utility as trackable characters for phylogenetic analysis and are compared to the phylogeny of crinoids based on their morphology.

The goal for this research is to produce a biomarker index based on the presence and abundance of organic molecules in Paleozoic crinoids, and to use information from the preserved organic molecules as a proxy for phylogenetic reconstruction.

Localities

Lower Mississippian Borden Group (Edwardsville Formation) will be used in this analysis. The environment preserved by this formation is an interchannel mudstone delta platform.

The two localities are approximately 20 miles apart.

Boy Scout Camp, near Bloomington, Indiana

Etter Farm, Martinsville, Indiana.

The Jurassic of Switzerland near Basle was used to reproduce part of the early work in fringelite molecules performed by Max Blumer. The environment preserved in this area is described at a seabed with a soft, muddy bottom.

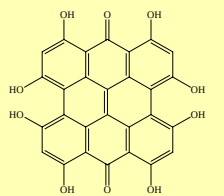
Basle, Switzerland
Fossil crinoid stem found in the Jura of the pre-Alpine region of Basle, Switzerland.

"Why is it that each of the forty species of crinoids found at LeGrand [Iowa] is colored in a characteristic way peculiar to that species alone? Are these colors indicative of the colors they wore in life? It is hoped that someday these mysteries, and others far more important, will be solved with clues that have not yet come to light, or have so far been overlooked". (1962)

- Species-specific coloration is also found in Crawfordsville, Indiana and was noted in the Borden Formation by N. Gary Lane (1986).
- Better equipment for chemical quantitative analysis is available now than when these molecules were first isolated and described.
- Biomarker molecules presumably responsible for this coloration can be extracted from these Mississippian crinoids.
- Work in the study of fringelites has not yet taken the natural next step to test for phylogenetic relationships through characteristic structure and concentrations of fringelites in crinoid fossils.

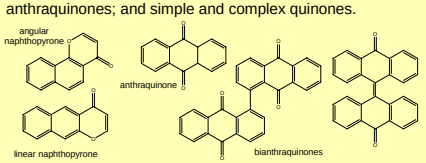
Extraction of Organic Molecules from Crinoids

•Extraction of organic material from crinoids began with a grinding of the fossil material into a powder.
•Hydrochloric acid was added dropwise until no reaction was apparent. The entire sample was centrifuged, then the liquid solution (which was generally yellow to orange) was decanted from the remaining solids.
•Next, a 4:1 Acetone: Methanol solution was added to allow organic or aqueous molecules to go into solution. This too was centrifuged, and the solution (this time red to yellow-green) was decanted.
•Finally, a solution of methanol was added, centrifuged, and decanted.
•Spectral analysis indicated by the presence of fringelites in the *Apiocrinus* sample that the molecules of interest were in the Acetone:Methanol solution, and further analysis was carried out on extracts in this solvent.

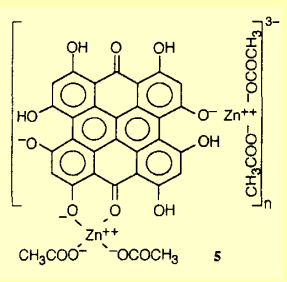


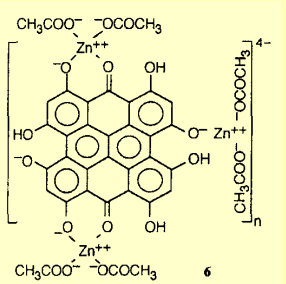
Fringelite D

The fringelites are molecules known to occur in European fossil crinoids. Naturally occurring pigments in modern crinoids include molecules in several classes of compounds, including: linear and angular naphthopyrones, anthraquinones; and simple and complex quinones.

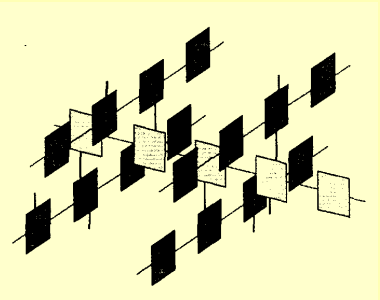


Diagenetic Stability Through Time…

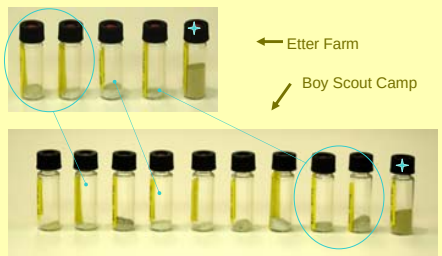




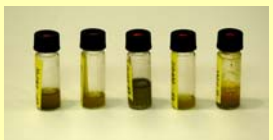
Above, the *bay* salt and *peri* chelate formation of fringelites.



Schematic structural aspects of the polymer-like fringelite complex lattice in the mineral matrix of fossils; squares denote Fringelites D, dashed lines connecting them denote *bay* phenolate bonds to alkaline earth metal or transition metal ions (above, Zn), and solid lines denote link of the thus chain forming fringelites *via* coordination to a transition metal ion. (from Falk and Mayr, 1997)



Etter Farm Samples after dissolution in acid (HCl)



Extraction or organic molecules form crinoid fossils (unfortunately) requires the grinding and dissolving of calcite to release organic material.

	Camerata			Cladida		Disparida	Articulata	
	Platycrinites	*Paradichocrinus*	*Gilbertsocrinus*	*Cyathocrinites*	*Barycrinus*	*Halysiocrinus*	*Apiocrinus*	Unknown
Boy Scout Camp								
Etter Farm								
Basel, Switzerland								

Table shows plates and parts chosen for analysis by species and location. Black and white inset images are representative photos for the genus.

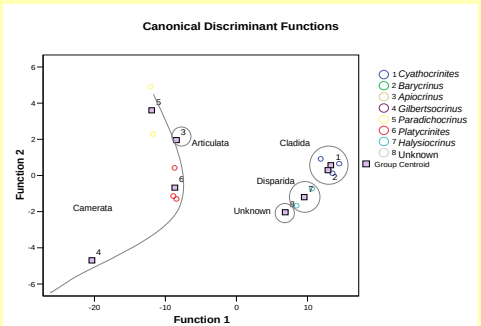
*Scale is in mm

Sample Selection

A Jurassic Crinoid, *Apiocrinus*, was selected to test the repeatability of extracting fossil organic molecules from crinoids.

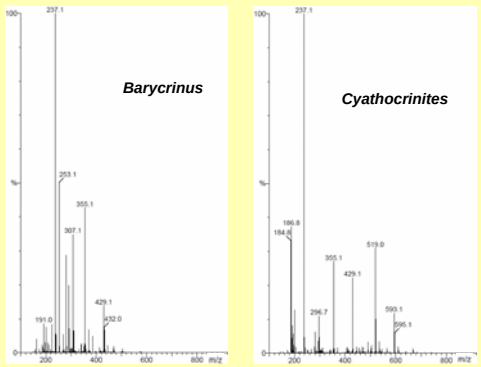
Mississippian samples are selected from two sites in Indiana.

- Isolated radial plates are used for analysis because they can be identified to the species level. The stems of *Gilbertsocrinus* are distinct enough to be identified and analyze stem concentrations of biomarkers. An identifiable basal circlet of *Platycrinites* has also been tested. These samples from different parts of the crinoid's skeleton may indicate differences in occurrence and concentration with anatomical location in the crinoid.
- Two genera (*Halysiocrinus* and *Cyathocrinites*), each occurring at both sites, are tested for consistency of the occurrence of the biomarkers within the genus. If there is a regional or environmental influence to the occurrence or concentrations of biomarkers in crinoids, that difference is expected to appear in the sample duplicates.
- Two genera (*Cyathocrinites* and *Barycrinus*) representing different families (Cyathocrinitidae and Barycrinidae) are tested to determine if there is a difference in biomarkers at the family level between crinoids.
- Two genera (*Paradichocrinus* and *Platycrinites*) representing different superfamilies (Hexacrinitacea and Platycrinitacea) of the subclass Camerata are tested to describe the difference in biomarkers in two superfamilies.
- Three genera (*Gilbertsocrinus*, *Paradichocrinus*, and *Platycrinites)* are tested to describe the differences of biomarkers in two orders of camerates (Diplobathrida and Monobathrida).
- Specimens from the subclasses Cladida, Camerata, and Disparida will be analyzed to describe subclass-level differences. (Articulata is represented by a species that is significantly older and so will not be included in this analysis).

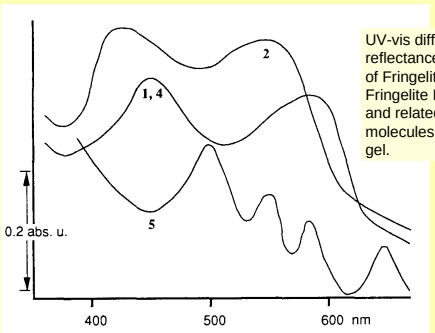


Color data (hue, chroma, saturation) when analyzed by Canonical Discriminant Function Analysis, shows that genera of crinoids sort into groups by subclass.

Sample Mass Spectral Data

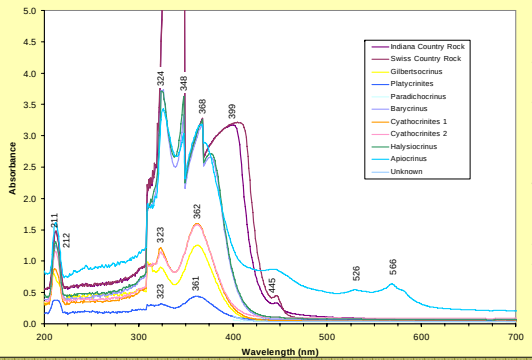


Difference crinoids show different mass spectra for solutions containing biomarker molecules. A similar peak between the two may be evidence of similar biomarkers present in the fossil, but note that many peaks are unique to the genera.



UV-vis diffuse reflectance spectra of Fringelite D (1), Fringelite H (2), and related molecules on silica gel.

Absorbance of Extracts in 4:1 Acetone: Methanol



Peaks in absorbance data at characteristic wavelengths indicate the presence of fringelite molecules in the *Apiocrinus* sample, but not in the Swiss Country Rock.

Fossil genera from Indiana localities show absorption peaks for different wavelengths, indicating that organic molecules in these fossils are not shared, and differences are intrinsic to the fossils themselves and not to the locality.

Results

- Coloration (chroma, hue and saturation) of the fossil specimens was determined by comparison to a Munsel color chart. When analyzed, these data showed that genera of crinoids do sort into subclasses based on color information.
- Organic molecules have been extracted from crinoids. Preliminary results show that these molecules exist in varying quantities in different genera of crinoids. These data also show that different molecules can be found in different genera of crinoids.
- Analysis by UV-Vis spectroscopy show different absorptions to light in each genus. UV-Vis spectroscopy indicates the presence of fringelite in the Jurassic *Apiocrinus sp.*, and further rules out inorganic metal oxides as the source of differential coloration in all tested preserved crinoids.

Future Goals

- Gain better identification of compounds in crinoid extract by use of HPLC / MS analysis.
- Analyze extracts of fossil by HCl to identify possible co-occurring inorganic metal oxides, and investigate the possibility of metals complexing with chelating quinone moieties.
- Enable identification of the fringelite compounds and quantitative measurement of their relative concentrations in different groups of crinoids.
- Produce biomarker index unique to genera or species.
- Use information about the abundance and identity of biomarker molecules in crinoids as a proxy for phylogenetic reconstruction.