

RESEARCH NEWS AND COMMENTS

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The Clarkia "Fossil" Leaves: Windows to the Past

About an hour's drive out of Moscow, Idaho, towards the town of Clarkia, is a motorbike race track, called appropriately enough, the Fossil Bowl. The excavation of the track in 1972 by Mr. Francis Kienbaum, the owner of the property, unearthed a rare and remarkable fossil site later to be called the Clarkia Flora. A call to the nearby University of Idaho brought out a geologist, Dr. Charles "Jack" Smiley, who marvelled at the preservation of the fossil plant specimens and realized the scientific value of the site. His subsequent efforts to bring other scientists to study the site would lead to more than fifteen years of work and publications on this fossil flora.

In the summer of 1978, I visited the site with my colleague, Dr. Karl Niklas, a paleobotanist. Karl and I had just published on the chemical and ultrastructural preservation of green leaf compression fossils of the Miocene Succor Creek Flora of Oregon. These compressed leaves represented original green leaves preserved in the desiccant-like volcanic ash. Jack Smiley, hearing of our work, invited us to view the Clarkia site, with its profusion of equally well preserved and more accessible fossil leaf compressions.

As I stood at the Clarkia site, I turned and looked out over a quiet valley of green pastures growing over what had been a secluded bay of a Miocene lake bed, its age recently confirmed by potassium/argon isotope dating. Twenty million years ago the lake had been surrounded by deciduous trees including sycamores, oaks, chestnuts, tulip poplars, zelkovas and elms as well as evergreen trees including *Metasequoia glyptostroboides* (Dawn Redwood) and species of *Magnolia*. The closest living relatives of many of the Clarkia fossils now occurred only in the southeastern United States or more distantly in southern China.



Figure 1. The author (far left) standing before the Clarkia site during the first visit in 1978, discussing a fossil specimen with (from left to right), W. Rember (graduate student), Dr. Jack Smiley (site manager) and M. Huggins (graduate student).

Clarkia Lake had been formed by the blocking of what is now the St. Mary's River drainage by catastrophic volcanic activity. The lake apparently had been deep, cold and experienced little disturbance by strong currents such as in spring fed lakes. The lake probably lasted only a thousand years before filling in to form the valley before me. The Clarkia fossils are not torn or sheared but repose in the lacustrine shale matrix as if neatly laid and carefully covered with many layers of fine silt. The ease in prying the soft layers apart and exposing the fossils contrasts with the rock-like preservation of the Succor Creek specimens. The stark preservation of the Clarkia leaves in the cold, oxygenless sediments of the ancient lake was mirrored in the preserved original colors of the leaves, dark black greens, autumnal reds and browns and even a



Figure 2. Fossil oak species in freshly opened sediment layer. Note original sediment water layer on the fossil compression. Courtesy of K. J. Niklas.

film of moisture from the original lake water. With little apparent bacterial decomposition the internal cell wall, vascular tissue and chloroplast structure had also been preserved, unlike true fossils where the organic parts of the plant are replaced by inorganic minerals, much like petrified wood. Karl, Jack and I were to work on the paleochemotaxonomy of these fossils until the mid 1980s.

Late that fall of 1978, a Georgia colleague of mine, Dr. Michael Clegg, fascinated by the preservation of the many pigments, steroids, fatty acids and phenolics in the fossils, asked if DNA itself might also be present in these fossils leaves. I thought it highly likely. However, appropriate technology was unavailable at that time to exploit the possibility. By 1985, Clegg had left for the University of California, Riverside, and I was preparing to retrain in the molecular biotechnology which was becoming available to systematic and evolutionary biology. An invitation from Mike Clegg and his colleague, Gerard Zurawski, to learn the new technology in exchange for my taxonomic expertise in applying such data to taxonomic and evolutionary studies, soon had me

on my way to their California laboratories in fall, 1987.

At the end of my sabbatical in March 1988, Mike Clegg and I again discussed the possibility of sampling *Clarkia* fossils for DNA with the new biotechnology. I called Jack Smiley and arranged for a new trip to the fossil site in July 1988. After our arrival, Mr. Keinbaum's son bulldozed away the loose soil, exposing fresh layers of lucustrine shale containing the fossil leaves. Mike Clegg and Jack Smiley pulled out the fossils which I then photographed. After identification by Jack, Mike's two technicians, Mary Durbin and David Henderson, immediately scraped the leaf off the shale into a buffered extraction solution using the portable field lab set up at the site within a few feet of ongoing bike races. The extracts were stored on ice for transport back to Riverside. A farewell dinner with Jack Smiley and his wife, Peg, capped a most pleasurable field trip.

By September 1988, Dr. Ed Golenberg, a post-doctoral research associate working with Mike Clegg at Riverside, called—DNA in small quantities was preserved in many of the 54 fossils



Figure 3. Electron micrograph of cell cytoplasm of birch leaf (*Betula*). Note chloroplast membranes in lower right corner. Courtesy of K. J. Niklas.

we had collected—elation and cautious optimism.

At that time, a new technique called the polymerase chain reaction (PCR) became available which allowed for the duplication and amplification of small amounts of DNA to thousandfold amounts. By June 1989, Ed Golenberg had amplified, recovered and sequenced a large portion of a gene from a fossil *Magnolia*. The gene, called *rbcL*, is the large subunit of the chloroplast gene, RuBisCo, which codes for a major photosynthetic enzyme in the chloroplast. The gene was chosen because it is produced in large quantities in all green photosynthetic plants, making it more likely to be found in the fossil. Also, it is a slowly evolving gene which would give us a conservative look at evolution between the fossil and its living relatives. Numerical analysis of the sequence of nucleic acid bases of the fossil gene and that of its relatives confirmed the fossil to be a magnolia and that the fossil gene differed by only 17 nucleic acid bases from its nearest living relative. Our paper submitted to *Nature* in December, 1989, was accepted in February, 1990—jubilation.

“What is the significance of this work?” the reporter asked. Caught up in the race to be the

first to report on this startling work, it took a moment or two to put it all in perspective in terms of the real world. First, it was the oldest DNA *ever* recovered and sequenced. Second, we could now look back down the tree of evolution in vertical geological time to see where plant species diverged. Previous plant phylogenies



Figure 4. Dr. Jack Smiley, geologist (left center) explains the history of the *Clarkia* site to Dr. Mike Clegg (right center) and his technicians, David Henderson (left) and Mary Durbin (far right).



Figure 5. Dr. Edward Golenberg, who carried out the PCR amplification and sequencing of the DNA from the fossil *Magnolia*.

were based primarily on horizontal comparisons of living plants representing only the tips of the tree of evolution. Third, the analysis could be repeated with many other fossil species to compare rates of gene evolution. Fourth, the age of the fossil in millions of years is meaningful enough to allow scientists to use these rates to "set" the biological clock to calculate gene evolution in geological time rather than estimating from differences between contemporary species. In practical terms scientists involved in genetic engineering might now be able to predict how long transplanted genes and desired characteristics would remain stable in plants. Finally,

Additional Readings

Golenberg, E. M., D. E. Giannasi, M. T. Clegg, C. J. Smiley, M. Durbin, D. Henderson and G. Zurawski. 1990. Chloroplast DNA sequence from a Miocene *Magnolia* species. *Nature* 344:656-658.

Smiley, C. J., Ed. 1985. Late Cenozoic History of the Pacific Northwest. American Association for the Advancement of Science, San Francisco.



Figure 6. A fossil insect compression also found in the Clarkia sediments.

DNA, the ultimate evolutionary code, can unequivocally establish taxonomic and evolutionary relationships between plants, a significant taxonomic goal and equally important in choosing likely compatible genetic stocks for breeding programs.

"What are your future plans?" the reporter then asked. First, we intend to expand the surveys at Clarkia, especially with other scientists, to see if the rate of DNA base changes or substitutions is the same in all fossil species or does it differ from species to species. Perhaps other scientists will want to examine the fish and insects preserved in the Clarkia sediments. Also, we hope to locate older sites allowing us to push the geological reference points further back in time. The work establishes a new field, which we term paleomolecular evolution, using the "chemical bones" of the fossil DNA to establish precise evolutionary relationships between living and ancient plants. Most intriguing is that a modern technology gives us access to information uniquely preserved twenty million years ago when man himself had barely begun his formative ascent towards emergence as a rational organism.