37TH PROFESSOR SIR A.C. SEWARD MEMORIAL LECTURE

Links with the past in the plant world : cuticles as recorders of diversity, kerogen formation and palaeoatmospheric CO_2-level

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The study and interpretation of plant cuticles may lead to the establishment of three important (taxonomic, biochemical, physiological) links with the past in the plant world : (1) Cuticles reflect characteristic patterns of epidermal cell organization; on the basis of cuticle analysis natural plant genera and families can be established as a necessary taxonomic background for recognizing past episodes of biodiversity change. (2) Cuticles have a high fossilization potential, due to the presence of resistant biopolymers in the cuticular matrix; chemical analysis of fossil and present-day cuticles can help to clarify interrelationships between extant biomass, kerogen and fossil fuels. (3) Cuticles bear the plant's stomata, essential for photosynthesis; stomatal frequencies are dependent on ambient CO_2 concentration, so that calculation of stomatal indices has the potential of determining palaeoatmospheric CO_2-levels within the time-scale of the last 10 million years.

Key-words—Morphology, Cuticle, Kerogen, Palaeoenvironment.

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साराँश

पादप जगत् का अतीत से सम्बन्ध : विभिन्नता, केरोजन निर्माण एवं पुरावायुमंडलीय कार्बनडाइऑक्साइड-स्तर के सूचकों के रूप में उपचर्मों की भूमिका

ऍच० विशर

पौधों का उपचर्मों के अध्ययन तथा इनकी व्याख्या से पादप जगत का अतीत से तीन प्रकार (वर्गीकरण, जैवरासायनिक, कार्यिकीय) से सम्बन्ध स्थापित किया जा सकता है: (1) उपचर्म बाह्यत्वचीय कोशा संगठन का लाक्षणिक स्वरूप प्रदर्शित करती हैं, उपचर्म विश्लेषण के आधार पर जैवविभिन्नता की अतीत की घटनाओं के अभिनिर्धारण हेतु प्राकृतिक प्रजातियों एवं कुलों को सुनिश्चित किया जा सकता है। (2) उपचर्मीय द्रव्य में प्रतिरोधी जैवपोलिमरों की उपस्थिति के कारण उपचर्मों में अश्मित होने की अधिक क्षमता है। वर्तमान जैवद्रव्य, केरोजन एवं अश्मित ईंधनों के मध्य अन्तरबन्धुता सुनिश्चित करने के लिए अश्मित एवं वर्तमान उपचर्मों का रासायनिक विश्लेषण सहायक सिद्व हो सकता है। (3) उपचर्म पौधों में रन्ध्रों की जन्मदाता है जो प्रकाशसश्लेषण के लिए आवश्यक हैं, रन्ध्रों की संख्या कार्बनडाइऑक्साइड की सांद्रता पर निर्भर करती है और इसी के आधार पर ही पिछले 10 करोड़ वर्षों में पुरावायुमंडलीय कार्बनडाइऑक्साइड स्तरों के अभिनिर्धारण में सहायता भी मिली है।

IN 1911, the year of Birbal Sahni's arrival in Cambridge, Albert Charles Seward published his little book *'Links with the Past in the Plant World'*. His object in writing it, was primarily to call attention to contemporary questions related to the relative antiquity of existing plants, and to illustrate the nature of the evidence afforded by the records of the rocks. Eighty years later, the title of Seward's book, as well as its content, still reflect the very basic philosophy with respect to relevant research and training in palaeobotany. Seward's message to all generations of students of fossil plant remains is clear. Whether these students now classify themselves as palaeobotanists, palynologists, organic geochemists, coal petrographers or organic petrologists, they should always attempt to establish links between past and present plant life. Moreover, they should develop appreciation, understanding and a solid degree of familiarity with many aspects of progress and priorities in biological and geological research.

At the Laboratory of Palaeobotany and Palynology, ever since its foundation by Professor F.P. Jonker, we attempt to take Seward's message seriously. In our research and training projects, we try to have an open eye for links between past and present, as well as for the major issues that require integration of data and models based on the study of fossil plant remains from both the terrestrial and the marine biosphere. By way of illustration, in this Seward Memorial Lecture, I shall concentrate on a summary of one aspect of our research: the study of cuticles.

Cuticles reflect characteristic patterns of epidermal cell organization of land plants. They have a high fossilization potential. They bear the plant's stomata, essential for photosynthesis. Let us, therefore, predict and see whether these three factors may enable the establishment of taxonomic, biochemical and physiological links with the past in the plant world.

A TAXONOMIC LINK WITH THE PAST IN THE PLANT WORLD

Seward concluded his little book by quoting Darwin: 'We need not marvel at extinction'. Now, eighty years later, it would be appropriate to add '... but we should thoroughly explore its causes and effects', or, following one of the recommendations to the earth science community within the framework of the International Geosphere and Biosphere Program (Hsü & Henken-Mellies, 1990), '... but we should explore the consequences of reduction of biodiversity, with reference to records of past catastrophes'.

In the past decade, considerable attention has been given to the study of the Cretaceous-Tertiary extinction interval. Only recently, the prominent Permian-Triassic extinction events are attracting renewed attention. Late Permian extinctions reflect the most profound change in the marine biosphere of all time. Analysis of marine biodiversity levels suggests that about half of the Late Permian invertebrate families and more than 90 per cent of the included species may have become gradually or stepwise extinct over a period of a few million years. At the Permian-Triassic junction, however, sustained diversity decline was dramatically punctuated by a final extinction pulse. This terminal Palaeozoic biotic crisis affected benthos, nekton and zooplankton without obvious selectivity.

In remarkable contrast, a concomitant decline in terrestrial biodiversity is not generally emphasized. Although tetrapods, insects and plants are known to have suffered considerable extinction during Late Permian times, their diversity level is considered to remain relatively balanced as a result of rapid replacement by newly originated taxa. Notably the major categories of vascular plants, with the exception of the cordaitaleans and some groups of pteridosperms, such as the glossopterids, show a high persistence-capacity of families and genera at the time of sustained biodiversity decline in the marine biosphere (Traverse, 1990). Similar to the situation at the Cretaceous-Tertiary boundary, however, there is every indication that at the very end of the Palaeozoic also plant life was seriously affected by a biotic crisis. At the Permian-Triassic junction, especially the palynological record testifies a marked floral turnover. In widely separated areas, diversification among gymnospermous pollen types rapidly declined. Concomitantly, lycopodiophytic microspores frequently become characterizing elements of palynological assemblages. In the Italian Alps, for example, this reflection of a floral turnover occurs in the same stratigraphical interval where typical Permian faunas abruptly disappear (Visscher & Brugman, 1988).

The palynological information suggests that especially conifers and pteridosperms should be considered as categories of organisms that do have experienced extinction at the end of the Palaeozoic. There is strong evidence that the terminal Palaeozoic biotic crisis is causally related to a significant destruction of standing biomass resulting from mass mortality among arborescent plant life.

Why is it then that we do not recognize a floral change in the plant megafossil record? Apart from inaccurate dating of terrestrial sedimentary sequences, we believe that this is largely due to inadequate taxonomy. Most latest Permian and earliest Triassic plant genera are form-genera by definition or by implication. Natural families, comparable to the families of the present-day land plants have hardly been defined. Thus in routine palaeobotanical research, one may identify the same genera and the same families on both sides of the Permian-Triassic boundary.

Inspired by the well-known pioneer work of Florin on the application of cuticle analysis in gymnosperm taxonomy, we started to explore the potential of cuticles in recognizing more natural taxa among Late Palaeozoic and Early Mesozoic gymnosperm remains from Europe. In other words, we wanted to try to describe Late Permian plant taxa as if they were present-day plants, hoping that such research may ultimately provide an answer to the question : '*do cuticles help to identify past episodes of diversity change?*

The outcome of our studies seems to give a positive answer. Notably in conifers the epidermal structure may vary according to family, genus and species, and hence proves to be of great potential in natural classification. Like in extant conifers, reproductive organs provide the most successful characters for delimiting natural genera and families. In absence of organic connection, through cuticle analysis detailed epidermal studies provide a reliable alternative for the necessary correlation of vegetative foliage and reproductive organs (Clement-Westerhof, 1984, 1987, 1988; Visscher *et al.*, 1986; Kerp, 1988, 1990; Kerp *et al.*, 1990; Poort & Kerp, 1990; Clement-Westerhof & Van Konijnenburg-Van Cittert, 1991).

The reconstruction of the genus *Ortiseia*, and its subsequent definition in terms of a natural conifer genus provides an illustrative example of the potential of cuticle analysis (Clement-Westerhof, 1984). *Ortiseia* was originally described as a form-genus based on well-preserved vegetative remains with preserved epidermal structure from the uppermost Permian in the Italian Alps. Larger plant fragments are very rare in the type strata. However, bulk-macerated samples yielded cuticle residues from which large numbers of small (less than 1 cm) but recognizable plant fragments can be isolated. These cuticular fragments include both vegetative and reproductive remains of conifers, such as isolated leaves, minute twigs, ovuliferous dwarf shoots, seeds and microsporophylls.

Through detailed comparative cuticle analysis it was possible to recognize three distinctive species within *Ortiseia*. All three species could be described in terms of characteristics of both vegetative and reproductive structures. The organization of the ovuliferous dwarf-shoots of the three species could be reconstructed in detail on the basis of their cuticle remains. The dwarf-shoots are flattened and composed of 20-30 sterile scales and a single megasporophyll, bearing one inverted ovule on its abaxial surface. Since conifer taxonomy is largely based on the organization of ovuliferous organs, the form-genus *Ortiseia* became 'promoted' to the status of a natural genus. Also the study of the cuticles of the ovules, seeds and polliniferous organs of the three species, has contributed to the new, natural concept of *Ortiseia*.

The results of the work on *Ortiseia* prompted a re-study and re-evaluation of the Late Carboniferous-Early Permian genera *Walchia* and *Ernestiodendron*. Florin's classic concept of the organization of the ovuliferous dwarf-shoots for these genera had to be fundamentally revised. The genera appeared to be related to *Ortiseia*. Together, the three genera formed a sound basis for diagnosing the Walchiaceae. The accuracy of the diagnosis is comparable to those of families of extant conifers. The family is unique among conifers. It could be demonstrated that the ovules are characterized by the presence of a pollen/archegonial chamber and that the pollen is in effect prepollen.

Subsequently, other natural taxa of Late Palaeozoic and Early Mesozoic conifers have been similarly recognized. Also pteridosperm taxa may be distinguished through bulk maceration of what was earlier called 'unidentifiable plant debris'. The methodology is time-consuming, and more work has to be done before we have a complete inventory of the Late Permian flora from Italy. Yet the methodology is rewarding, especially now that we can see that none of the so far recognized natural conifer genera (e.g., *Ortiseia*, *Pseudovoltzia*, *Majonica*, *Dolomitia*) and families (Walchiaceae, Majonicaceae) survive the terminal Palaeozoic extinction event.

A BIOCHEMICAL LINK WITH THE PAST IN THE PLANT WORLD

At present, successful oil and gas exploration is largely dependent on reliable conceptual models of the formation of kerogen, the sedimentary organic matter, insoluble in common organic solvents, that generates under thermal stress a variety of hydrocarbons. In organic geochemistry and petroleum geochemistry it is generally claimed that kerogen formation is mainly the result of a series of consecutive and random repolymerization and polycondensation reactions of a small fraction of the degradation products of buried biomass. Such newly formed so-called 'geopolymers', are thought to continuously undergo chemical transformations, by which they become increasingly condensed and insoluble.

However, comparable to the study of coal, microscopic analysis of kerogen constituents frequently reveals their structured nature. When using electron microscopy, even in the presumed 'structureless' or 'amorphous' organic matter of palynofacies classification, various biological structures attributable to phytoplankton and bacteria can be recognized. It may be assumed, therefore, that kerogen is largely composed of resistant biopolymers, rather than newly formed 'geopolymers'. Consequently, the conventional chemical concept of kerogen formation may need serious reconsideration. Through their physical properties (e.g., fluorescence of the maceral cutinite) and distinctive morphological features, cuticle remains can be easily distinguished in macroscopic and microscopic sedimentary organic matter of various age. Thus, cuticle research became confronted with an another challenge, namely the development of a palaeobotanically acceptable model of kerogen formation, emphasizing a biochemical link with the past in the plant world. In other words : '*do cuticles help to clarify interrelationships between extant biomass, kerogen and fossil fuels?*

In collaboration with the Organic Geochemistry Unit of the Delft University of Technology, a research project was initiated, aimed at the comparative microscopical and chemical study of cuticles of fossil and extant plants. The outcome of the research was surprising. It was an example of 'the past as a key to the present'. After recognizing that fossil cuticles are largely composed of a previously unknown biopolymer, it became clear that this resistant polymer represents a significant proportion of the biomass of present-day cuticles (Nip *et al.*, 1986a,b, 1987, 1989; Tegelaar *et al.*, 1989b, 1991, 1993).

Analysis of cuticles was performed by Curie-point pyrolysis coupled with gas chromatography and mass spectrometry. The pyrolysis-gas chromatography trace of material of the Permian peltasperm *Autunia conferta* appeared to be typical of those obtained from Late Palaeozoic and Mesozoic cuticles. The principal constituents of the pyrolyzate were identified by mass spectrometry as a homologous series of *n*-alkanes, *n*-alk-1-enes and *n*-α,ω-alkadienes, chain lengths ranging from C_6 to C_{29}. Such a pyrogram is quite unlike those obtained from other natural biopolymers or degraded biopolymeric products.

In the extant plants, waxes and cutin are generally considered to be the two main components of cuticles. Although there was little published information on the subject, there seemed to be sufficient indirect evidence that the aliphatic pyrolysis products generated from fossil cuticles could not have derived from either waxes or cutin.

Thus, in order to resolve the problem of the origin of pyrolysis products identified in fossil cuticles, the cuticles of a variety of taxa of recent plants were analyzed. A step-by-step procedure was followed : analysis of the intact cuticle, analysis after extraction of waxes, and analysis after extraction of waxes and saponification of cutin. The results can be well demonstrated on the basis of pyrolysis-gas chromatography traces of cuticles from *Agave americana*. After removal of waxes and cutin, pyrolysis results obtained from the cuticular residue show a marked resemblance to pyrograms obtained for fossil cuticles.

Further research fully confirmed the presence of a previously unknown highly aliphatic biopolymer in the matrix of many present-day plant cuticles. This biopolymer was termed *cutan*. However, it was also found that in some plants this new biopolymer is absent. Apart from waxes and some cellulose, a cuticular matrix can consist either of the biopolyester cutin, our newly found polymethylenic biopolymer cutan, or (in most cases) a mixture of both polymers.

The biological significance of the presence or absence of cutan is still unclear and awaits more detailed investigation of further species. The geological implication, however, is evident. There is every indication, that among the main structural components of cuticles, only cutan is highly resistant to diagenetic processes. When studying the fate of the components of the cuticular matrix in relation to time, we see that cutin can still be identified in Late Miocene plant cuticles from Europe. Highly modified, cutin-derived components are sometimes present in Paleogene material. Mesozoic and Palaeozoic cuticles are in general exclusively composed of cutan. The high preservation potential of cutan results in its selective preservation and consequent enrichment during the processes of sedimentation and diagenesis.

The fate of the cuticle components can serve as a model for kerogen formation. By determining the preservation potential of a large number of other biomacromolecules present in land plants and phytoplankton, it became apparent that the dominant aliphatic moieties in kerogen can be explained by the selective preservation and selective enrichment of resistant aliphatic biopolymers occurring in extant biomass.

The resistant aliphatic biopolymers are now thought to be the major precursors of *n*-alkanes in crude oils. This view is supported by the results of simulation experiments. At relatively low temperatures, the homologous series of *n*-alkanes generated from artificially matured cutan of cuticles from *A. americana* exhibit an internal distribution pattern that is virtually indistinguishable from that of *n*-alkanes in some crude oils from Indonesia (Tegelaar *et al.*, 1989c). Cuticle research thus formed the basis for a new conceptual model, describing the interrelationships between extant biomass, kerogen and fossil fuels (Tegelaar *et al.*, 1989a).

A PHYSIOLOGICAL LINK WITH THE PAST IN THE PLANT WORLD

From the issue 'energy resources' back to 'global change'. Within current international research programmes aimed at our understanding of the causes, processes and limits of variability in global environmental change, there is an ever-increasing demand for information on past composition of the atmosphere. Because of the interest for greenhouse warming, documentation on past CO_2 concentrations is especially welcome.

Through the study of ice cores from Antarctica, palaeoatmospheric CO_2 levels are well documented for the past 150,000 years. Before this time, CO_2 levels cannot be directly measured but only inferred from forward modelling of the CO_2 global budget or from proxy records based on geochemical or paleontological observations. The geochemical approach concentrates on the interpretation of changing $^{13}C/^{12}C$ isotope ratios in the marine carbonate record and, more recently, on $^{13}C/^{12}C$ analysis of terrestrial soil carbonates.

Any relevant paleontological approach is highly dependent on the ecophysiological interpretation of morphological or anatomical characters of fossils. Considering the fundamental role of leaf stomata of land plants in the interaction between biosphere and atmosphere, cuticle research is now challenged to explore a physiological link with the past in the plant world, by answering the question : '*do cuticles reflect changes in atmospheric CO_2 concentration observably?*'

It may be predicted from physical models of CO_2 fixation in land plants with C_3 metabolism (Farquhar & Sharkey, 1982), that the areal density of leaf stomata is responsive to changes in atmospheric CO_2 concentration. An important corollary of such a response would be that analysis of stomatal frequencies on fossil representatives of extant plant species has the potential of determining palaeoatmospheric CO_2 levels.

Under non-limiting light and water conditions, leaves have an optimal CO_2 fixation rate at a stomatal conductance to diffusion of CO_2 that maintains the CO_2 concentration in the leaf interior at the break point between saturation and limitation of the CO_2 receptor molecule ribulose biphosphate. The stomatal conductance to diffusion, the reciprocal of resistance, is a complex parameter dominated by the areal density and the apertures of stomata. An increase of atmospheric CO_2 concentration may require a lower value of the stomatal conductance to maintain optimal CO_2 fixation. Hence, if we assume that differences in stomatal frequency are part of the phenotypic variability of a plant species, such a lowering could well be reached through a decline of stomatal density.

This prediction is corroborated by the results of the study of herbarium material collected over the last 200 years. The British physiologist Woodward presented conclusive evidence that man-induced CO_2 increase has demonstratably resulted in a mean reduction of 40 per cent of the stomatal density for European temperate forest trees. Such a decline was confirmed by experiments under controlled ambient CO_2 levels. We decided to extend Woodward's (1987) approach to other time-scales. Here, I shall summarize the results of a recent study, suggesting that quantitative analysis of fossilized cuticles may help to identify changing CO_2 levels within the time-scale of the last 10 million years (Van der Burgh *et al.*, 1993).

Because of the limited material available, in fossil studies a direct interpretation of stomatal densities in terms of palaeoatmospheric change may give unreliable results. Within individual species of living plants the number of stomata per unit area is known to vary significantly in response to environmental factors other than ambient CO_2 level. A decreased stomatal density may be the result of decreasing light intensity or increasing humidity. However, such changes go hand-in-hand with a reduction of the number of epidermal cells. In contrast to the stomatal density, therefore, an expression of stomatal frequency as a percentage of the total number of epidermal cells represents a satisfactory constant for any one plant. Experiments have shown this stomatal index to be generally reliable, except in extreme conditions under which the plants probably would not have survived in nature. In fossil studies, therefore, if sufficiently well-preserved cuticles are available, reliable stomatal indices can already be calculated on the basis of a series of measurements on preparations from one or two leaves.

We calculated stomatal indices for *Quercus petraea* from five successive Late Neogene time-intervals characterized by contrasting climatic conditions. *Q. petraea*, the Durmast oak, represents an essentially European species with a present distribution from southern Scandinavia to the Mediterranean region. This deciduous oak is one of the few present-day angiosperm species in Europe which is known to extend back as far as to the Late Miocene.

We analyzed cuticles of leaf compressions collected from clay intercalations in the terrestrial Upper Miocene and Pliocene of the Lower Rhine Embayment, that is to say, the south-eastern margin of the Neogene North Sea Basin in Germany and adjacent parts of The Netherlands. Ages of source strata correlate with five successive time-intervals between ~10 Ma and ~2.5 million year, that can be regionally recognized on the basis of palynological information.

Stomatal indices calculated for fossil *Q. petraea* from two Late Miocene intervals, as well as an Early and a Late Pliocene interval, show mean values between 9.5 and 11.5. In marked contrast, the mean value of 16.2 for leaves from a latest Miocene interval is significantly higher. To test the validity of this difference, cuticles of an extinct species, *Fagus attenuata*, were analyzed from different intervals. Again, the stomatal index for latest Miocene leaves exhibits an increase of ~40 per cent relative to values for material studied from other intervals.

The changes in stomatal index for *Quercus petraea* show a clear covariation with temperature-related climate shifts inferred from the Late Miocene-Pliocene palynological record of the Lower Rhine Embayment by Zagwijn and Hager (1987). Comparison with the extensive record of plant megafossils, notably fruits and seeds, confirms that changes in quantitative composition of pollen assemblages reflect broadscale vegetation change characterized by an alternation of mixed broad-leaved evergreen/deciduous and mixed broad-leaved deciduous forest types.

Climatic interpretation indicates warm-temperate to subtropical conditions during the Late Miocene and Pliocene intervals characterized by low stomatal indices for *Q. petraea*, and a prevailing temperate climate during the latest Miocene interval with high indices. From the differences in the thermal regimes that constrain the areal distribution of modern Northern Hemisphere analogues for the two contrasting forest types, it can be estimated that the Late Miocene-Pliocene vegetation changes imply fluctuations of 2-3°C in the regional annual temperature.

In addition to the analysis of fossil material, we calculated stomatal indices for herbarium specimens of *Q. petraea* covering the last 120 years. The mean stomatal index for 1990 shows a decrease of ~55 per cent relative to the mean value calculated for leaves collected in 1870. The different indices for nine data-points indicate an approximately linear decrease of stomatal frequencies. Recent stomatal indices for *Q. petraea* are similar to those for fossil material from the warm-temperate to subtropical Neogene intervals. On the other hand, 19th century indices prior to accelerated industrialization appear to be comparable to the latest Miocene stomatal record from a temperate climatic interval.

The recorded changes in the stomatal index for an individual plant species confirm the predicted potential of stomatal frequencies as proxy indicators of fluctuations in paleoatmospheric CO_2 concentration. Calibration of the stomatal indices against the historical relation between stomatal frequency and CO_2 levels enables quantification of the Late Miocene-Pliocene palaeoatmospheric signals. The relative changes so far observed suggest that the corresponding global CO_2 concentration has fluctuated between boundary values of ~280 ppmv and ~370 ppmv. The fluctuations are of a similar magnitude as the change influenced by man during the past century. Covariation with climatic changes is in support of a causal relationship between CO_2 regime and temperature in Late Miocene-Pliocene times. If we accept the reality of such a relationship, present-day low stomatal indices can be considered to foreshadow global warming.

The fossil leaf record is characterized by its generally discontinuous nature. The relatively low number of data-points available in any one sedimentary sequence will limit the extent to which detailed patterns of palaeoatmospheric change can be reconstructed exclusively with the aid of quantitative cuticle analysis. Yet, there is every indication that this novel application of cuticle analysis may promote many collections of fossil leaves to become invaluable palaeoatmospheric databases that can provide the quantitative information required for testing and refining palaeoclimatic modelling exercises.

With respect to the Lower Rhine Embayment, a research-project has been initiated to trace additional data-points that can be used to test whether the Miocene-Holocene curve of regional relative temperature inferred from the more continuous palynological record consistently reflects palaeoatmospheric change.

CONCLUDING REMARKS

I hope to have demonstrated the potential of cuticles as recorders of diversity, kerogen formation and palaeoatmospheric CO_2-level. More importantly, I hope to have demonstrated, even by restricting myself to a single aspect of palaeobotanical research—the study and interpretation of cuticles—that the further establishment of 'links with the past in the plant world', has a tremendous potential in current research related to relevant scientific and political issues, such as 'global change' and 'energy resources'. We should anticipate that a new generation of students of macroscopic, microscopic and molecular plant remains will become increasingly involved in multidisciplinary research projects. At present, therefore, perhaps the greatest challenge in palaeobotany remains adequate multidisciplinary training.

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