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EPIDERMAL AND CUTICULAR STUDIES OF LEAVES

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Abstract

The gross morphological characters are regarded as one of the most important tools in plant taxonomy. They have been used for many years in making taxonomic and phylogenetic interpretations. However, for a better understanding of taxonomic relationships, cuticular and epidermal characteristics of leaves also have played an important role. Their use has been on the increase in the rceent years.

This review gives a comprehensive account of epidermal and cuticular features of leaves in taxonomic studies. It also includes a detailed account of their historical background, the methods used in the study of these features, and their application in many taxonomic, paleobotanical phylogenetic, and recently, ecological studies.

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One of the responsibilities normally assumed to pertain to the field of plant taxonomy is the assemblage of a sufficient body of data about plants to permit construction of a more nearly correct phylogenetic arrangement of the members of the plant kingdom. This 'body of data' has in the near past been based primarily upon characteristics of a gross morphological nature. This has served amazingly well in a great many of the plant groups largley because morphological structures have been demonstrated to have a genetic basis. Within some families the plants are of such a nature that knowledge of morphology alone leaves much to be desired and has been viewed as inadequate.

The gross morphological characteristics of the leaf appear to have been used for identification purposes as long as man has been identifying plants. With increased sophistication of classification systems it has become increasingly important to have more elaborate means of identification. The leaf has not lost its importance as a taxonomic tool, but rather has proven to be more useful when a fuller understanding of all its characteristics are known and appreciated.

The epidermal characters, if properly interpreted, are being regarded as important taxonomic tools. They should not be dissociated from supplementing the morphological features which have so long been used in the taxonomic and phylogenetic interpretations. Cuticular features such as stomatal frequency, stomatal index, type of stomatal complex, trichome types, and others are helpful in understanding relationships which could not be explained otherwise. Fritsch (1903) believed that the characteristic structure of the stomatal apparatus in many plants was undoubtedly due to an inherent property in the plant and he considered them to be of great importance in the characterization of some orders . . . "Since their mere occurrence and constancy are genetically controlled, it should be helpful to utilize these features of the cuticle as taxonomic tools, which may be used in phylogenetic comparisons."

Stebbins and Khush (1961) rightly remarked that another point "which makes the study of the stomatal complexes a desirable tool in taxonomic research is that genera and even families show great constancy for their possession of a particular complex, yet there is considerable variability from one higher taxon to another. Thus, at the level where classical methods of cytology and genetics cannot be applied, this study of stomatal complexes should help us to understand true evolutionary relationships of monocotyledons." Finally, Stace (1965a) has indicated that, "a soundly based theory of culticular patterns can be put to a good number of uses besides the obvious applications in identification, taxonomic research, and phylogenetic investigations. These include peat stratigraphy, pharmacological analyses, and animal foodstuff research."

A review of the literature of cuticular studies and of the epidermal features reveals a wealth of data and great interest in a number of facets concerning these features of leaves. This review does not attempt to cover the ontogenetic or physiologic aspect of the stomatal apparatus, but pertains basically to the taxonomic applications of surface features.

According to Stace (1965a), one of the earliest reference to leaf cuticles was the note by Brodie, who in 1842 remarked, "when the sand stone is freshly broken the epidermis of the fossil frequently peels off." The first significant work on the systematic treatment of cuticles was that of Bornemann (1856), in which he described the fossil cuticles of cycads. He was possibly the first person to realize the nature of the cuticles and explained the relationship between them and the leaves which bore them: "the outline of the cell walls of the epidermis is almost always shown on the cuticular membrane by a network of dark brown lines, which apparently represent the cell walls, but which are much thinner than these would have been. These brown lines are to be regarded as parts of a homogeneous cuticle . . ." (translation). One of the earliest systematic studies on the surface features of angiosperms was the investigation by Prillieux (1956) on the types of peltate trichomes found in various members of the Oleaceae. He examined some 23 taxa and was able to separate all 23 on the structure of these trichomes alone.

The first taxonomist to make consistent use of anatomical characters for his diagnoses was apparently Bureau (1864) in his revision of the Bignoniaceae, although these characters formed but a very minor part of the desc iptions. Weiss (1965) produced a monumental account of the sizes, numbers, and distribution

of stomata in a large number of dicotyledons. Strasburger (1866) was also interested in the development of stomatal complexes. Schenk (1869-71) recognized the importance of surface features and included some drawings of cuticles in his studies, but none of these was of the angiosperm.

Campbell (1881) gave a rather good description of the typical epidermal cells and the cell make up of the stomatal complex of *Tradescantia vulgaris.* For this work he used very young leaves and examined the whole leaf without removing the epidermis. Although he quite adequately described the epidermal cells, guard cells, and accessory cells, no mention was made of possible morphological or phylogenetic relationships. Bokorny (1882) surveyed the distribution of and occurrence of pellucid spots in dicotyedonous leaves. Zeiller (1882) added to the total data by presenting several interesting facts concerning the cuticles of fossil ferns and conifers. De Bary (1884) made a rather extensive study of the anatomy of ferns and gave some excellent descriptions concerning stomatal ontogeny. Atkinson (1893) extended this work on the biology of ferns when he made use of a collodion method to investigate several structures on the fern. Bachmann (1886) also recognized the importance of leaf features by giving a beautifully illustrated account of all types of peltate hairs known, in systematic arrangement.

Grob (1896) again pointed out the value of epidermal characteristics as a taxonomic tool. His work was primarily concerned with the grass family and the epidermal characteristics of some members of that family. Anderson (1897) was also concerned, in a comparable manner, with the stomatal arrangement on the bud scales of *Abies petinata.* Minden (1899) thoroughly covered the different types of water-scereting organs (e.g. water stomata, hydathodes) found in dicotyledons. It was perhaps this productive period which led Fritsch (1903) to summarize the most important systematic anatomical characters to which taxonomists might pay more attention, and in many instances his comments still apply today.

Buscaliono and Pollacci (1902) first employed a collodion film method for making epidermal impressions. The prime use they made of these impressions was the determination of the stomatal numbers of the leaves of various plants. Nathorst (1907-12) was also one of the early workers on cuticular studies. His "Paläobotanische Mitteilungen" represents the first work which reported a long series of cuticular studies on a large number of species. Lloyd (1908) conducted a series of physiological investigations involving the stomata but made his observations simply by peeling the epidermis from the leaf surfaces. Solereder (1908) had investigated the development of stomatal complexes and the several patterns of ontogeny were demonstrated to be constant, reliable characteristics even for relatively large groups of plants. His encyclopaedic volumes on dicotyledonous systematic anatomy represented the epitome of anatomical works at that time. Harshberber (1908) published a very interesting work describing comparative leaf structures in sand-dune plants found in Bermuda. The works of Rehfous (1914, 1917) also presented some interesting data concerning stomatal structure and special emphasis was given to members of the Celastraceae.

The application of epidermal characteristics and taxonomic study seems to have been much more natural in dealing with fossil plants than in dealing with living specimens. Holden (1915) made rather elaborate use of cuticular characteristics to help identify a group of fossil conifers. She found that certain features in the edipermis were fairly constant, whereas others were inconsistent not only in a genus or species, but even on the same individuals. Holden concluded that the number and general distribution of stomata (upper to lower surface of leaf) were quite variable within a species, although wall features of the epidermal cells, arrangement of stomata, and the degree of lignified lamellae of guard cells were constant. She referred to the phylogenetic value of these features but indicated that they had little value. It seems clear from her work that she considered cuticular investigations quite important to the taxonomist for accurate specific diagnoses, but that their value as tools in evolutionary studies were limited, at least, in the coniferales.

During the 1920's the cuticle became a widely investigated topic. Rea (1921) published the results of investigations with *Campanula rotundi-folia* L. which described the stomatal structure and patterns. Also included was discussion of the effects of environment upon the development of stomates. The work of Piper (1922) brought to light a rather interesting series of investigations making use of minute leaf characteristics. Piper was concerned with the correct identity of *Berberis aquifolium* vs. *Berberis nervosa* vs. *Berberis repens* Lindl. He referred to a Dr. Stopf who, in 1919, made the observation that "the characters of the presence or absence of papillae is, indeed, a very great help in discriminating otherwise doubtful species." He referred here to the lower epidermis of the *Berbris* species. Piper also referred to an investigation carried ont in 1916 by Dr. Albert Mann on three variants of *B. aquifolium.* Mann concluded that the epidermal feature should not justify any specific distinction among the three variants. As a result of a series of rather lengthy examinations Piper finally concluded that Stopf was correct and stated "it seems that in all critical cases it furnishes a definite basis o fdetermination. The lower epidermal cells in both species project on their free sufraces as low papillae." This is, seemingly, the first case where epidermal characteristics were used as the delimiting factor between species.

Bandulska (1923, 1924, 1926, & 1931) was probably one of the first investigators who worked on the modern dicotyledonous cuticles. She described cuticles of many modern dicotyledonous leaves, as well as of coniferous leaves found in Eocene deposits in Bournemouth, England. Lee and Priestley (1924) performed some rather elaborate investigations concerning the structure and function of the cuticle.

Parkin (1924) took a slightly different approach when he investigated the possible relationship of stomata to phylogeny. He quotes Hutchinson's paper concerning phylogenetic classification of flowering plants in which he calls attention to the fact that many arborescent families are characterized by having stomata with the subsidiary cells parallel to the pore. He discussed Hutchinson's findings and suggested that the significance of the different types of stomata was not understood.

Buscalioni and Catalano (1925) also made some investigations concerning the phylogenetic significance of the cuticle particularly with respect to a specific type of stomata in the genus *Acacia.* Lang (1926) contributed a significant piece of work that has found many applications. He provided a cellulose-film transfer method for the investigation of several aspects of plant fossils. This method has been much used in investigations dealing with the cuticle. Westermeier (1926) also gave note of epidermal features but did little else with the idea. His work reported the results of a study in which the characteristics of the epidermal cells and the structure of the stomata had the most decisive influence on the properties of the plant, and that the relationship was linked with the total productivity and yield of the grain. Weber (1926) wrote a rather elaborate paper in which he described the various changes which occur in the shape of the guard cell nucleus of *Tradescantia virginiana* L. while the stomata were open or closed. Ohga (1926) was involved in investigations of leaf surfaces and described the stomatal distribution of several Machurian plants by use of a variety of impression techniques utilizing collodion, proteein, starch, new skin, and gum. One of the good early works which attempted to use stomata density at a taxanomic tool was that of Timmerman (1927). These data indicate a potential usefulness of stomatal density, but in light of present information tend to be over optimistic.

Ohki (1927) introduced a completely new variation on the use of the leaf for taxonomic study. In a series of investigations he made use of what was called a spodogram of leaves. When the leaves were charred the ash pattern of the epidermis clearly showed the form and arrangement of epidermal cells and the stomata. In several Japanese plants of the genus *Sasa* he found these spodograms to be of analytical help in the separation of species.

One of the most important works dealing with leaf surfaces was that of Salisbury (1927) in which he discussed stomatal frequency. He described these frequencies in terms of "Stomatal Index", although Loftfield (1921) and Gupta (1961a, 1961b) also worked along similar lines. Salisbury's stomatal index was expressed as: $I_s = \frac{100S}{E+S}$ where S = stomatal number per unit area, and E = number of epidermal cells in the same area. By means of this index it was demonstrated that the "proportion of stomata formed in the epidermis is not greater for sun leaves than for shade leaves, but that there is a high, positive, correlation coefficient between the number of stomata and the number of epidermal cells per unit area. Similarly, the increased stomatal frequencies in plants grown on dry soil as compared with those grown on wet soil, of small leaves as compared with large leaves, and the slight differences between the yellow and green regions of variegated and harlequin leaves, are all shown to be due chiefly to differences in spacing of the stomata and not to differences in the proportion of stomata developed. This appears to be true also for the variations in frequency in different parts of the same leaf." He also found that humidity, nutritional conditions, size of leaf, age of leaf, position on the leaf, whether woody or herbaceous, aquatic or terrestrial, and the upper vs. lower surface could be responsible for altering the stomatal index.

Walton (1927, 1928, 1930) was one of the early investigators to develop a quick method for removing a plant fossil from sections of fossil petrifactions. He employed a cellulose ester in his method which consisted of applying the ester to the fossil material, allowing it to dry, and then peeling it from the petrifaction. Papadopoulos (1928) also made use of cuticular data when she compared the characteristics of the leaflets of the parent plants of cycads with those of their hybrid offspring. She found that the stomatal pattern and arrangement was particularly useful in separating the parents and the various types of hydrids in an examination of over fifty hybrids she found that the structures of the leaflets resembled those of one parent or the other, while other structures showed characteristics of both parents. In many instances it was found that the F_1 plants inherited leaf characteristics rather equally from both parents.

While conducting research on *Rumex acetose* L., Peterson (1929) concluded that the number of stomata was a definitive characteristic of a plant comparable with any other quantitative characteristic, and as such, the number of stomata was the product of the interaction of the organism and the environment.

Barnes and Duerden (1930) devised a technique by which cellulose transfers from rocks containing fossil plants could be prepared. Their method consisted of etching the fossil rock surface. The celluloid solution used by them was made up of celluloid in absolute alcohol with ether subsequently added. They further improved this technique by using other replacements and additions to the solution. Hoskins (1930) suggested a cellulose film transfer method for use on thin rock sections. With this film extremely thin sections of petrifactions could be fixed on a glass slide which provided a clear observation of the section.

While working with the leaves of *Citrus* and related taxa, Hirano (1931) added a rather interesting piece of information. He found that the number of stomata in forty species of *Citrus* and twelve related taxa appeared to be related to the place of origin. He demonstrated that the tropical species generally had more than 500 stomata per square millimeter of leaf while the extratropical ones had a decidedly fewer number. He also noted that the plants in this group with the greatest resistance to cold had the smallest number of stomata on the leaves. He then made the rather interesting comment that the density of stomata seemed to be more related to spring rainfall than to many other factors.

Florin's (1931) name is important in the field of cuticular studies because his works included descriptions of the cuticles of many species of modern conifers with particular reference to stomata. In addition, his investigation dealt with fossil gymnosperm cuticles.

The French researcher Martens (1931a, 1931b, 1933a, 1933b, 1934a, 1934b, 1934c, & 1938) conducted an extensive series of investigations on the cuticles of the petals and staminate hairs of *Tradescantia.* He described the markings which he observed on the walls of these cells. He was concerned about the possible relationship of the markings and protoplasmic streaming. Martens determined that epidermal cells were covered with a "cutine" which had the peculiarity of being present as layers of constant maximal thickness. During certain stages of development more "cutine" was produced than was needed to cover the surface with a smooth layer, thus folds were produced in the "cuticula". The form of these folds was determined by the rate of formation, as well as by the direction of cell stretch during the formative period. Therefore, the straight and undulated parallel striations seen in many of the cells of *Tradescantia* with which he worked should be the result of a one-sided stretch in the direction of the striations. He further described a three phase growth pattern of the cells which resulted in a definite pattern of markings on the "cuticula". He referred to this pattern in the cells of *T. virginica* as a "reticulat" pattern.

Ashby (1932) performed a series of experiments on *Larrea tridentata* (DC). Cov. and *Ligustrum* sp. designed to furnish information concerning water loss by the leaves. As part of his work, he was concerned with the density as well as the anatomy of the stomata. Since stripping the epidermis proved to be an unsatisfactory procedure, he resorted to dipping the leaves in a cellodion solution in ether and subsequently removing the film when it had dried. This was found to be quite satisfactory for his work.

Prat (1932) published some significant work on the epidermis of Gramineae, in which he was able to separate all of the tribes on the basis of the hair types produced. The work of Avery (1933) added information concerning the morphology and ontogeny of leaf tissues. He used conventional staining and sectioning techniques in his studies. He stated that the unequal rates of division and cell enlargement between the mesophyll cells and the epidermal cells resulted in the irregularities in the lateral walls of the epidermal cells.

The separation of diploid vs. triploid apple varieties was studied by Nebel (1934). The lengths of the stomata were measured from free hand sections immersed in 3 per cent sugar solution. He found that the triploid forms without exception had the larger stomata, but advised caution in using stomata length as more than a preliminary guide.

Long and Clements (1934), in making stomata counts, also resorted to the use of films of cellulose nitrate and cellulose acetate. They suggested that such films possessed an exceptional range of application to plant organs and parts, to various types of fossils, and to the hard parts of animals. They also demonstrated that the number of stomata varied with the position on the leaf and with environmental conditions.

Wicks (1935) carried out an investigation involving stomatal counts, distribution patterns, and relationships in *Haemanthus* and *Brunsvigia* in which he pointed out that stomatal frequency generally increases from the base to the apex and from the median line to the margin of the leaf. He also found the stomatal index values to be more constant than the stomatal frequencies.

The arrangement and orientation of the stomata of several monocots and dicots were quite extensively discussed by Smith (1935). The suggestions were made that (1) stomatal orientation may be in some way an expression of environment, or (2) that "stomatal orientation may be an hereditarily determined character and in no way affected by the environment". He also pointed to an evident correlation between the orientation of the stomata and the direction of the veins. In *Sambucus nigra* L. he indicated a correlation between the direction of the long axes of the stomata and the direction of the underlying vascular tissue, which might suggest that during differentiation the veins exerted a directional effect on the orientation of the stomata. He indicated that this relationship was a much more important factor than the possibility of orientation being an intrinsic character of the epidermis itself. A clear statement of observational methods was missing from his paper, but it would appear that cleared whole leaves and epidermal strips were used.

According to Edwards (1935), structural differences in the cuticle always provided a means of distinguishing isolated leaves in the cases of convergance of leaf-form ,and the distribution of cuticular elements provided a means of discriminating between closely related taxonomic groups which were hard to differentiate by purely structural characters. He also stressed the importance of statistical characters like the size and frequency of elements, although the latter were not too reliable. On the other hand, the ratio of stomata to the epidermal cells (the stomatal index) was less variable than stomatal frequency alone. In taxonomic work, the use of the sum total of various available characters was regarded as a satisfactory basis, but not if individual cuticular and other epidermal features were considered in isolation. Edwards referred to the criticism made by Gordon (1939) who did not seem to be in favor of cuticular characters being used in a classification system. His objection was that since vegetative characters were not used in the identification and classification of flowering plants, they should not be regarded as very reliable. Gordon felt more confident in the determination of gymnosperms than flowering plants, by using vegetative characters. Edwards suggested that since gymnosperms were so few in number, cuticular characters could be studied intensively and could be put to taxonomic use. At the same time there was no reason why intensive cuticular studies of angiosperms coupled with fossil angiosperm cuticles could not be used for the same purpose. He cited Baranou's (1924) findings in which he admitted that the number of stomata was inconsistent, not only in different stages of the plant, but also on the same leaf from the base to the tip of the leaf. On the other hand, he found that the stomatal number in the median region of a leaf agreed closely with the average stomatal number calculated from various parts of leaves of all levels.

Gross leaf, sepal, and ovary indument characteristics were carefully described and used for identification purposes in the genus *Tradescantia* by Anderson and Woodson (1935). Glandular vs. eglandular pubescence was considered to be of prime importance to them; but the location of hairs, whether on the upper or lower surfaces, present or absent, near the tip or base, and whether on veins or not has also been used as important considerations in the identification of many plant species.

Darrah (1936, 1939) introduced the parlodion-castor oil peel technique which is commonly used in paleobotanical investigations. Although he did not suggest its use on living materials, it was eventually so used.

A very fine description of the formation of the guard cells, accessory cells, and epidermal cells of Bamboo was prepared by Porterfield (1937). The preparation of material was the standard use of sections which had been fixed, embedded, sectioned, and stained; so that this was basically a cytological-anatomical work.

Sax and Sax (1937) employed *Tradescantia canaliculata* Raf., *Secale*, and several other grass species to make a comparison of stomatal size and distribution in diploid and polyploid plants. They found the parlodion method of Darrah (1936) to be a more useful peel technique than the collodion process used by Long and Clements (1934). By use of these peels they made counts from five areas of the leaf at X300-400 magnification and converted these counts to number of stomata per square millimeter of leaf surface. From their work the following conclusions were reached: (a) a comparison of diploid and tetraploid races of *T. canaliculata* showed a high degree of correlation between chromosome number and size of pollen mother cells, microscopes, stomata, chloroplasts and stomata frequency, (b) the tetraploids had about half as many cells per unit of area as the diploids, and (c) in diploids and tetraploids of *Tradescantia* a positive correlation was found in stomatal frequency.

Van Iterson (1937) became very much interested in the work of Martens and virtually repeated the whole series of experiments. He sustained a majority of Martens' results and then added some interesting ideas of his own. He noted that the time and place of development of the cell helped to determine the exact pattern of the cuticular striations. He found the markings to be so exact in distances apart and pattern that he concluded their formation to be due not only to the mechanical factors of time and rate of growth, but also "to structural peculiarities in the cuticular layers." No indication was given either by Martens or Van Iterson concerning the controlling stimuli behind either the mechanical factors of time and rate or the structural peculiarities in the cuticular layers. If the assumption can be made that the genetic make-up of plant cells acts as the control mechanism here, as it does in most facets of cell development, then the size and shape of epidermal cells and the cuticle produced by them should demonstrate the constancy characteristic of genetic control.

A more orthodox epidermal technique was suggested by Blaydes (1939) who used phenolic Bismard Brown Y to stain the cellular material. This method was suggested as a means of preparing permanent slides which had good photographic characteristics. Drawert (1941) also used an epidermal strip method in the study of *Tradescantia virginica*. He used acetic toluidinblue solution which tended to stain the outer cell membranes.

The work of Watson (1942) contained an excellent review of the literature concerning the shapes and associated causes of shapes of epidermal cells. His work was primarily with juvenile shoots of *Hedera helix* L. He used 5 mm. disks of leaf material which had been killed, fixed, treated with hot Javel water, stained with saturated aqueous Nile blue sulphate, and then mounted in paraffin oil. This work showed that the cause of the waviness of the lateral walls of upper epidermal cells lies in the outer free wall. The origin of this waviness was considered to be due to the unequal plasticity in the various parts of the outer wall. It was pointed out that this waviness was not found in the majority of monocots.

A very excellent paper depicting the origin, structure, and function of the cuticle of angiosperms was published by Priestley (1943). He made extensive use of the polarizing microscope in his research work which added a new perspective to culticular studies. There was also a very excellent literature review as part of his paper.

The works of Rowson (1943a, 1943b) with *Cassia, Atropa,* and *Erythroxylum* introduced what appeared to be some very worthwhile information to the area of leaf study methods. He used both entire leaves and portions of leaves which had been cleared in chloral hydrate and mounted in dilute glycerine to make counts of the number of stomata and of epidermal cells. He used a microscope with a 4 mm. objective and a 10X eyepiece and counted twenty randomly selected fields from five leaves. In senna he found no significant variation in stomatal indices from different positions of the same leaf. In the leaves of belladonna it was found that stomata counts were unsatisfactory as a differential character. He did ascertain that a stomatal index determined from the ration $I_s = e/s$ where e is the number of epidermal cells and s is the number of stomata was a very useful number. He found this index would separate the leaves of *A. lutescens* from *A. belladonna* and *E. cocca* from *E. truxillense* in either the entire or in the ground condition.

A modification of this same type or work was done by Kritikos and Steinegger (1948, 1949a, 1949b), and Steinegger and Kritikos (1949). These workers made an extensive series of investigations on the genus *Lobelia* in which they determined such factors as epidermal cell number, stomatal number, and stomatal index. They were able to distinguish more than eleven species as well as separate diploids from tetraploids of one of the species of both stomata count and stomatal index number. They determined the stomatal index by use of the equation $I_s = \frac{s}{e+s}$ x 100 where s is the number of stomata and e is the number of epidermal cells. The major difference in this index as compared to Rowson's seems to be that this one is in terms of percentage.

Flint and Moreland (1946), Prant (1948), and Suryanarayana and Krishnoswamy (1948) have produced works utilizing epidermal and stomata characters to describe sugar cane, corn, and other grass species. Suryanarayana and Krishnoswamy introduced a stripping method utilizing 1:4 or 1:5 sulphuric acid plus potassium dichromate crystals and a final staining with phenolic safranin. Oppenheimer (1949) used strips of tomato fruit epidermis fixed in dioxane to study the opening reactions of stomata on tomatoes. Poplavaskia (1949) has described the distribution of stomata in a wide variety of plants.

Foster (1949), and Metcalfe (1946, 1950, 1955, 1956, 1960, & 1963) employed the patterns of stomatal development, their spatial relation to neighboring cells, and the presence or absence, number and arrangement of subsidiary cells in taxonomic and phylogenetic studies. Metcalfe actually included features of the epidermis as part of the description of a number of the plants with which he worked.

During the period between 1950 and 1960 a number of investigators published the results of their works on leaf characteristics, epidermal and stomatal characteristics, and the methods used for study. A check of this material shows that little really new was added, but rather there seemed to be a testing period for the techniques already developed. Works concerned primarily with technique include those of Crisp and Thorpe (1950) who used gelatin imprints of the cuticular hairs of insects; Hetzer and Volle (1950) on the compositae; Carolin (1954) who evaluated stomatal size and density; Hernandez's (1954) on chromosome number vs. stomata size; Mueller, Carr, add Loomis' (1954) cuticular structure investigations utilizing polyvinyl alcohol; Wagner (1954) with *Asplenium;* Skoss (1955) with cuticular structure vs. environment; Bennett and Furmidge's (1956) investigations of deposits on plant surfaces using cellulose acetate impressions; Joy, Willis, and Lacey's (1956) rapid cellulose peel method for paleobotany; North's (1956) acetate film method of stomata measurement; Vazuero's (1957) application of the Jeffrey method to leaves; Juniper's (1958, 1959a, 1959b, 1960) carbon replica method for cuticular ultra-structure studies; Sivadjian's (1958) collodian ricine peel method; and Schieferstein and Loomis' (1956, 1959) study of culticular layers.

The application and interpretation of epidermal-cuticular information with respect to taxonomy and phylogeny was demonstrated by Khush and Stebbins (1959) in their work concerning the guard cell complex of monocots. On the basis of this study, they found five distinctly different guard cell complexes as follows: (1) two guard cells and four well-differentiated subsidiary cells, with two to five additional subsidiary cells intergrading with epidermal cells: (2) two guard cells and four well-developed and sharply distinct subsidiary cells; (3) two guard cells and two well-developed subsidiary cells placed lateraly to the guard cels; (4) two guard cells and two small subsidiary cells placed at the ends of guard cells; (5) two guard cells without any subsidiary cells. In the material examined, they found that the kind of complex was constant within a genus and "usually constant within the tribe or family."

Since 1960, leaf epidermis-cuticular studies appear to have concentrated in three areas. The first of these has involved techniques for the study of the leaf and its epidermal features. The second has involved the application of these techniques in a large variety of plant families, and the third has been concerned mainly with the utilization of epidermal-cuticular information with respect to taxonomy and phylogeny. Among the published studies involving techniques are the following: Clarke (1960) demonstrated a lactic acid treatment of leaves to give strips of epidermal tissue; Lonert (1960a, 1960b) demonstrated the use of plastic cover slips to make surface replicas; Sarvella, Meyer, and Owings (1961) made use of Scotch brand plastic tape to show leaf surface features; Sinclair and Dunn (1961) introduced the use of Archer's (1950) herbarium plastic for leaf peels; Zelitch (1961) introduced the use of silicon rubber impressions to indicate stomatal condition: Aalders and Holl (1962) again used cellulose acetate peels on leaves of *Vaccinium;* Will (1962 'demonstrated how adhesive tape could be used for stripping the leaf epidermis; Waggoner and Zelitch (1965) demonstrated a tdouble transfer method using a silicon-rubber material to make the original impression from which a cellulose acetate transfer was then made of the impression. The final touch was added by Stoddard (1965, 1966) who published two articles which announced - without benefit of references - the brand new method of "fingerprinting" leaves by the use of fingernail polish (cellulose acetate).

Among the works dealing with epidermal-cuticular investigation in plant families was the interesting work on the Solanaceae by Romanovick (1960). Webber (1960) found that in the Araceae there were at least two patterns of "ribbing" in the cuticle. He also pointed out that epidermal cells demonstrated contour differences which varied from slightly raised or dome shaped in the center to papilla or projections that were larger and "button-like". Sinclair and Dunn (1961) presented a comparative study of surface features involving the families Nyctaginaceae, Araliaceae, Anacardiaceae, Orchidaceae, and Commelinaceae. Other works include: Gulyas (1961) on *Selaginella*; Stone (1961) on ploidy vs. stomata size in hickories; Gupta (1961b) with Solanaceae to determine what he called "Absolute Stomatal Number"; Argus' (1962) work with stomata and taxonomy in *Salix*; Bobrov's (1962) investigations with cycads; Campbell's (1962) research project on the genus *Datura*; *Watson* (1962, 1965) and his research on Epacridaceae; Dilcher's (1963) investigation with Eocene leaves; Rao (1963) and his work on the cuticles of *Hevea*; Ahmad (1964a, 1964b, 1964c, & 1964d) and his cuticle investigations with the Solanaceae; the work of Stace (1961, 1965) on the Combretaceae; the work of Dunn, Sharma, and Campbell (1965) which included 152 genera from 96 families including both monocots and dicots; Pant and Kidwai (1966) and their investigation in the Celastraceae; the several works by Mueller (1966a, 1966b, 1966c) on the cuticular patterns of *Vaccinium*; Sinclair's (1967) work on the genus *Tradescantia* where epidermal-cuticular descriptions for species along with a key were presented; Sharma (1967) and Sharma and Dunn's (1968) work which demonstrated the effects of environment on leaf features of *Kalanchoe*; the work on environmental modification of cuticular characteristics in *Pisum*; by Walker and Dunn (1967); and the biosystematic study by Keener (1967) on the genus *Clematis*.

The third, and most interesting, aspect of this field of leaf study deals basically with the application and interpretation of epidermal-cuticular information with respect to taxonomy and phylogeny. Only when this type of information is applied to the possible solution of large basic problems does the information justify the amount of time and effort expended. The work of Khush and Stebbins (1959) seems to have given impetus to this area of investigation. This was followed by the work of Jain and Stebbins (1959) concerning guard cell development patterns, Stebbins and Shah (1960) and Stebbins and Jain (1960) and their interpretations of the epidermis of monocots, and culminated with the work of Stebbins and Khush (1961) on the stomatal complex in monocots in which they tried to correlate their findings concerning stomatal complexes and subsidiary cell arrangement with the phylogenetic conditions of primitive vs. derived. Sinclair and Dunn (1961), while developing a technique for cuticular imprints, suggested that it might be possible to make a key to families, or genera, based on cuticular structures. They felt that a combination of pollen data and cuticular data could be used in phylogenetic and paleobotanical studies. Tumanyan (1963) used leaf surface information in dealing with the taxonomy of dicot leaves. The works of Campbell (1962) and Ahmad (1963) in the family Solanaceae have added support to the concept of phylogenetic importance. Campbell found that it was possible to separate 12 species of the genus *Datura* on the basis of epidermal characteristics. Ahmad worked with 17 genera and 26 species of Solanaceae from India and South America and suggested further subdivisions of the family and regrouping of different general under various tribes from leaf-cuticular data. Dunn, Sharma, and Campbell (1965) worked with peels from 152 genera of plants including both monocots and dicots. They state in their conclusion, "Since no two of the 152 genera studied had identical surface characteristics, we believe it may be possible to construct a key to genera based only on surface characters." Stoddard (1965) reiterated this same idea when he stated that "the features of the upper surface of the true leaves and the cotyledons, or both, have consistently been characteristic of the species or variety."

The several works of Stace (1961, 1963, 1965a, 1965b, & 1966) have added a tremendous amount to the total systematic consideration. He has stated that "leaf epidermal and cuticular characters are often of real value in taxonomic and phylogenetic investigations but that, as in the case of almost any other feature, the taxonomical level at which they supply useful information will vary greatly from taxon to taxon. Furthermore, the degrees of usefulness in identification and in phylogenetic considerations are by no means always directly related. Nevertheless their valid use in many phylogenetic problems is not to be doubted, despite comments to the contrary in the literature." Sinclair (1967) demonstrated that leaf surface characteristics were of much taxonomic value in the erect *Tradescantia* indigenous to the United States. He presented a leaf surface description for each of the species and found that it was possible to separate the members of the genus on the basis of the surface characteristics. Sharma (1968) demonstrated for *Kalanchoe* that not only was the length-width ratio of the leaf a reliable morphological measurement but also that the fine structural characteristics including pattern of stomatal development, stomatal index, stomatal size, trichome type and distribution, and the anticlinal wall patterns for both the upper and lower surfaces were useful in the determination of taxon.

It seems clear that leaf epidermal-cuticular characteristics, their descriptions, interpretations, and evaluations will become a real part of the descriptive characterization for many plant taxa. Such information will undoubtedly be of no greater or lesser value than other morphological traits, but may well prove to be of value in specific instances where other traits are of a more limited nature.

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