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PHYSIOLOGICAL CRITERIA IN AID OF TAXONOMY OF ANGIOSPERMS

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ABSTRACT

In the past little attention was paid in the direction of utilising comparative biochemical studies towards a better understanding of Phylogenetic relationships of plants. In recent years with the advent of filter paper partition chromatography several investigators have, however, made comparison of chemical composition of higher plants within a particular taxon and between different taxa. Taxonomical correlations were attempted and the relation of conclusions arrived by this technique to the taxonomical concepts based on morphological studies were examined. In the present study a survey of the literature available in this field was made. The survey indicated that the data available do not as yet permit the drawing of significant conclusions but they are interesting enough to warrant further exploration to examine the potentialities of comparative biochemical technique in taxonomy. The available work is strongly suggestive that biochemical technique can be a useful addition to the conventional methods in the study of taxonomy.

INTRODUCTION

The impact of plant physiology and biochemistry on the solution of taxonomical problems has been little felt in comparison to that of the descriptive branches of botanical science. Nevertheless, the potentialities of comparative studies on the biochemical composition of individuals at different levels of taxa in establishing phylogenetic relationships are immense. Though the criteria obtained from comparative biochemistry and physiological approach may not in themselves form a basis for a convenient system of classification, they could make a significant contribution in areas where morphological evidence alone is too indistinct to decide on the affinities between taxa or within a particular taxon.

In dealing with living objects the study of function is as important as that of the morphological structure. Any system of classification based on simple comparative morphological descriptions although being practical is obviously subjected to certain drawbacks. The functional criteria should be sued to supplement the evidence available from other sources as well as to verify the conclusions arrived on the phylogeny by the descriptive approach.

Despite the underlying potentialities of the biochemical criteria, taxonomists have not made use of them largely owing to the lack of comprehensive data in this respect. The pioneering work of Molisch, Mez and others clearly emphasized that a better understanding of relationships could be gained through the judicious application of the knowledge of physiochemical and serological investigations (*cf.* Laurence, 1951). Unfortunately subsequent to these early attempts very little attention was paid in this direction until recent years.

With the advent of filter paper partition chromatography entirely new vistas have been opened up in the line of investigation on comparative biochemical composition of different genera and species of a family or families. Much of chemo-taxonomical work of the present day is due to this technique. Analysis of plants for chemical constituents such as soluble and insoluble carbohydrates, phenolic substances, free amino acids, fluorescent compounds, essential oils and organic acids, to mention only a few, were made in the past few years by several workers with attempts for taxonomical correlations in each case.

The aim of this study is to survey the various investigations available in this comparatively recent field of taxonomy. The work could conveniently be arranged under separate heads for different levels of taxa for which biochemical studies were made and taxonomical conclusions drawn there on.

SPECIES

A number of workers concentrated on the comparison of chemical composition of higher plants at the interspecific level.

Alston and Irwin (1961) studied the extent of variation in free amino acids and secondary substances (largely phenolic compounds) between five different species of *Cassia* using a 1—dimensional paper chromatographic technique.

The five species chosen by them belonged to four sections of the genus. Results indicated that the extent of variation in 'Secondary' substances (Phenolic compounds) was more sharp than in amino acids, and that the pattern of difference of the latter have a greater taxonomic potential for the species examined by these authors *C. occidentalis* and *C. lindheimeriana* belonging to sections Oncolobium and Chamaefistula respectively were reported to have exhibited a similar chromatographic pattern. These two sections are also similar in their morphological characters. On the other hand *C. corymbosa* also belonging to sections Chamaefistula showed a different pattern from that of *C. lindheimeriana*. The authors did not draw any definite conclusions but suggested an equitable evaluation of biochemical data relative to morphological and cytological data, as the central theme in such studies.

Roberts et al (1958) using paper chromatographic methods studied a number of species belonging to the section *Thea* of the genus *Camellia*. Here again phenolic substances such as depsides, flavonols and leuco-anthocyanins were examined in the different varieties of the classical tea plant *C. sinensis* and between species of *Thea* group as well as the other sections of the genus. They found that the triglycosides of quercetin and kaempferol and an uncharacterised substance which they named as IC were proved to have considerable taxonomic interest. Important differences between varieties were seen in these compounds. Large amounts of triglycosides are characteristic of china variety. The pattern of triglycosides in tea plants, which have mixed features of the varieties, closely agree with taxonomical conclusions arrived through morphological studies. The chemical investigation of tea plants provided a useful support for the taxonomist. Species within the section of *Thea* had close similarity in chemical characteristics.

Constituents of essential oils were compared between some species of *Elsholtzia* by Fujita (1960). He found that *E. ciliata* and *E. oldhami* contain elsholtzia ketone while the latter species also contains naginata ketone which proved to be precursor of Elsholtzia ketone. But *E. oldhami* var. *argyi* contains in addition to the two above mentioned ketones d-linalool also. Thus this variety can be distinguished from *E. oldhami* and *E. ciliata*. Another species *E. nipponica* differs from the others in having geraniol and p-cymene.

Proteins were examined for the organ as well as taxon specificity for several species belonging to the family Viciaceae by Kloz et al (1960) using serological methods. Results obtained by them showed that protein characters were specific for each organ while species specificity being preserved.

Chemical investigations were found useful in distinguishing species of *Baptisia* by Alston and Turner (1961). Chromatographic evidence in four of species tried showed at least one substance not present in any of the other species. Such species-specific chemical substances were used by them to analyse complex hybridisation pattern.

In the line of methodology Ellison and Turner (1961) have found that a diagrammatic technique would serve a highly useful purpose for presentation of biochemical data for systematic work. The affinities of chemical composition between two species are expressed percentages along radii of a circle, and when the points are connected the affinities are shown in polygons. The authors have attempted this with respect to genus *Bahia*.

GENUS

Studies of chemical composition at intergeneric and intertribe level were carried out by MacLeod and McCorquodale (1958). These authors have made determinations of water soluble carbohydrates including sugars, oligosaccharides, and water soluble polysaccharides in the seeds of twenty-two species of Gramineae. The species selected were of different genera representing eleven tribes of the family. Their study showed that two tribes, Bromeae and Hordeeae are uniquely characterised by the type of sugars found in their seeds, the former containing fructosans but lacking raffinose and the latter containing fructosans as well as raffinose. It is possible to distinguish the species belonging to these tribes from the rest of the grasses by their sugar content. They form natural tribes. A high content of mannan makes *Nardus* occupy an isolated position. *Anthoxanthym* and *Holcus* belonging to Aveneae completely lack β-glucosan and strongly differ in this respect wth *Avena* and *Arrhenatherum* of the same tribe. It is suspected whether the former genera should be included in Aveneae. On the other hand they show affinities to Agrostideae and *Phalaris*.

The investigations of MacLeod and McCorquodale clearly showed that biochemical criteria could form a powerful tool in establishing affinities.

The usefulness of accumulation of an organic acid, tartaric acid as a taxonomic characteristic was examined in the family Geraniaceae by Stafford (1961). *Erodium* and *Geranium* were found to be non-accumulators of tartaric acid while majority of species of *Pelargonium* are high accumulators. A few species of *Pelargonium* are, however, low in tartaric acid content. Stafford concludes that since there is a need for improving the existing classification of Geraniaceae the criterion of tartrate accumulation may be used in addition to the other characteristics.

Hydrocarbon constituents of wax coatings of the leaves of thirtyfive species belonging to four genera of the sub-family sempervivoideae of Crassulaceae were studied for a taxonomic survey by Egglinton et al. (1962). The analytical technique used by them was gas-liquid chromatography. These authors found that hydrocarbon pattern was a stable character, independent of age and season and that it was characteristic of each species examined. An uniformity of pattern of distribution of alkanes was present within each of the genera, *Monanthes, Greenovia* and *Aichryson* and also within sections Urbica and Holochrysa of the genus *Aeonium*: There was found a close correlation between the botanical classifications and hydrocarbon pattern.

Fowden and Steward (1957) studied fifty six genera belonging to Liliaceae for nitrogenous compounds. Free amino acids identified and unidentified were compared for different genera. Some attempts for taxonomical correlation were made and in their opinion the ability to synthesize or accumulate certain compounds occurs widely throughout plants and that the factors governing their distribution do not operate at generic or specific level.

Nine species belonging to four genera of Iridaceae were investigated by paper chromatography by Riley and Bryant (1961). Fluorescent compounds, altogether about twenty spots, were distinguished in the nine species studied by them based on the difference in Rf values and the colour under ultraviolet light. Chromatographic pattern of fluorescent compounds of *Watsonia ardernei* and *W. vanderspuyae* had very close similarity to one another while *W. fourcadie* is not closely related to those but more so to *W. wilmaniae*. Two other species, *W. pyramidata* and *W. wordsworthiana* are not related to other species. The divergence in pattern was more sharp when comparison was made of species from genera *Dietes* and *Babania* with species of *Watsonia*. Thus it was clearly seen that chemical pattern of species of different genera differed more than those from the same genus. The indication is that such factors operate at intergeneric level rather than at interspecific level.

FAMILY

A few workers have also studied comparative chemical composition of plants at the level of different families with a view to find out taxonomical relationships.

The relationship of Hamamelidaceae to the families in Rosales and also to amentiferous families was investigated my Shaw and Gibbs (1961). A number of chemical characters such as leucoanthocyanin test, hydrogen cyanide test and presence or absence of glucitol and sedoheptulose were used. The authors had assumed that those families which show close correspondence in chemical criteria are most closely related. Fourteen families belonging to Hamamelidaceae were tried and on application of the above criteria Shaw and Gibbs were of the opinion that an order 'Hamamelidales' may be proposed to include Hamamelidaceae, Platanaceae, Myrothamnaceae and probably Cunoniaceae. Rosaceae will form a homogeneous group characterised by occurrence of glucitol and cyanogenetic glycosides if the sub-family Chrysobalanoideae is excluded. According to the chemical evidence Hamamelidaceae and Rosaceae strongly differ which sufficiently warrants their inclusion in separate orders. Similarly the authors found that if Garryaceae, Leitneriaceae and Juglandaceae were to be excluded amentiferous families would form a natural group.

Again, the chemical composition of surface lipids was found to be characteristic of species by Purdy and Truter (1961). Although they could not identify the actual components of lipids the analysis of lipids indicated that out of ten monocotyledonous plants examined each showed a pattern characteristic of its own. Fourteen species belonging to different genera of various dicotyledonous families also had a specific pattern for each plant.

Recently Bate-Smith (1962) has made quite an extensive study of the phenolic constituents present in the leaves of plants of dicotyledonous families. A chromatographic survey of leucoanthocyanins, flavonols, hydroxycinnamic acids and their substitution derivatives was made. Leucodelphinidin, leucocyanidin, myricetin, quercetin, kaempferol, ellagic acid, caffeic acid and p-coumaric acid were found to be the eight commoner phenolic constituents of plants and all the rest of the compounds of phenolic nature being derived from them by various substitution reactions. It was assumed that the greater the divergence in the structure of phenolics from the common constituents, the more likely they are to be of value as indices of affinity between species in which they are found. Families that are positive to leuco-anthocyanin and trihydroxy substituted derivates are expected to be closely related than to those that are negative. Leguminosae alone among the families of dicotyledons contain all the classes of phenolic constituents. Leguminosae have affinities with a large number of families and this agrees with the notion that Rosales occupy a central position in dicotyledons. The author found that Rosales, Parietales, Myrtales, and Ericales to Ebenales have families in which leuco-anthocyanin and trihydroxy substituted derivatives are concentrated. Subsidiary constituents are found to be more useful in establishing relationships within the family and the genus than at higher levels of taxa.

DISCUSSION AND CONCLUSIONS

The above account suggests that biochemical technique is yet another useful tool in the hands of modern taxonomist and it may prove an important addition to the conventional methods of studying plant taxonomy. It cannot replace the existing methods but when in combination it would help solve matters of indecision regarding phylogenetic relationships of different taxa. Because of the meagre data at present available on the biochemical composition it is premature to draw any significant conclusions but the results certainly warrant further detailed exploration of the potentialities of this method. A considerable attention has also to be paid to find out how far a particular chemical constituent is a stable character in respect to age, development, and climatic conditions.

That the study of comparative biochemistry is of considerable value in understanding the problems of phylogeny needs little emphasis. Biochemical evolution is understood to have taken the line of progressive loss in the capacity to synthesize specific constituents. Loss in the synthetic ability is to be traced to the absence of the specific enzyme responsible for the synthesis (*c.f.* Hutner, 1953) Bate-Smith (1962) based on his studies of phenolics could draw certain conclusions on the phylogeny. The subsidiary constituents are supposed to have arisen as a result of evolutionary loss of enzymes carrying out specific steps of oxidation and that such steps are irreversible. In other words those families that possess the subsidiary phenolic constituents would have been derived from those that form the regular end-points. Much scope lies in this field for the future investigations. The frequent occurrence of enzymic machinery in plants for the multiple pathways leading to the same metabolic products may also be of evolutionary significance.

It would appear that those substances which are relatively more dynamic in the metabolism of plants will offer a less taxonomic potential. This is evident from the work on comparative studies based on free amino acids and organic acids. On the other hand phenolics and surface lipids, hydrocarbon constituents, fluorescent compounds etc. are expected to offer much greater potential in taxonomical investigations.

That any sharp qualitative differences in the comparative chemistry may not operate at an inter-specific level is also borne out specially by the work of Alston and Irwin (1961). This is not true, however for certain compounds like hydrocarbons and surface lipids, which appear to be specific for species. In general, at higher levels of taxa divergence in chemical pattern is apparent.

In micro-organisms, particularly in respect of bacteria, a number of physiological characteristics such as oxidase reaction have exclusively been used for taxonomical classification. Steel (1961) has made a thorough study of large number of strains of different families. The production of indophenol due to oxidation of dimethyl-p-phenylenediamine and a-naphthol was used as a critical test. It shows the presence of cytochrome oxidase. A comparative study of intermediary carbohydrate metabolism in acetic acid bacteria as a tool for interpretation of phylogenetic relationships was made by De Ley (1961). Also rates of growth, catalase activity, nicotinic acid production were taken into account in classifying mycobacteria by Csillag (1961).

Such studies as those on micro-organisms are lacking for higher plants. The distribution of terminal oxidases such as ascorbic oxidase among different plants if investigated on a large scale may prove very important for establishing affinities. Although cytochrome oxidase appears to be ubiquitous in its occurrence in higher plants certain plants may seem to specialise in other systems. For instance Ascorbic oxidase is commonly present in members of Cucurbitaceae; similarly variations in carbohydrate metabolism, capacity to accumulate one or the other metabolite which again depends on the relative rates of the enzymic composition can all be used for purposes of finding out the natural relations or difference among plants.

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