

THE ROLE OF CHEMISTRY IN PLANT SYSTEMATICS

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ABSTRACT

Although the hybrid discipline of biochemical systematics has been a distinct field of study for only about ten years, it has had a telling impact on taxonomic practice. Nevertheless, the chemical approach, with its emphasis on the exact, tends to exclude much of the wealth of natural phenomena that are the backbone of taxonomy. It is necessary for the chemist and biochemist to acquire a better understanding of the nature of classification before undertaking surveys. More thought on infraspecific variation is also required, and the tentative nature of most phylogenetic proposals needs to be more widely appreciated. These problems are discussed in relation to recent studies involving different types of chemical compounds, including proteins, and suggestions are made for future priorities in chemotaxonomic research.

INTRODUCTION

Although the use of chemical characters in taxonomy and systematics has a very long history, the emergence of the hybrid discipline biochemical systematics or chemotaxonomy† as a distinctive and developed field of study dates back only about ten years. It was marked by the publication of such works as Alston and Turner's pioneer text *Biochemical Systematics* (1963), the symposium edited by Swain, *Chemical Plant Taxonomy* (1963), another by Leone, *Taxonomic Biochemistry and Serology* (1964), Mentzer and Fatianoff's *Actualités de Phytochimie Fondamentale* (1964) and, of course, by the first volumes of Hegnauer's *Chemotaxonomie der Pflanzen*, 1962–1965.

I well recall something of the exciting atmosphere during that formative period in the early 1960s as I was then engaged with my colleague Peter Davis in writing a textbook of Angiosperm taxonomy¹ and our decision to include a chapter summarizing the rapidly expanding literature on the use of chemical

† The terms 'chemical taxonomy' or 'chemotaxonomy' are most unfortunate and should not be used since 'taxonomy' means the study of classification, including its bases, principles, procedures and rules, a definition going back to A. P. de Candolle (1813) and followed by most recent authors, e.g. Davis and Heywood¹, Mason², Simpson³. The prefix 'chemo-' is either therefore superfluous or meaningless, cf. Merxmüller⁴. On the other hand, the term 'systematics' refers to the scientific study of the kinds and diversity of organisms and of all or any of the relationships between them. 'Biochemical systematics' is acceptable, although by no means perfect as a descriptive term for the field under consideration and can be defined quite simply as the use of biochemical data in biological systematics. Such a definition may not meet with universal approval but will be justified in a later section of this paper.

data in taxonomy was regarded by some as distinctly *avant garde*. It was, in fact, the only general text on taxonomy at that time to recognize that a new field was being born.

Since those days there has been one of those explosive developments which characterize present-day science: countless books, symposia, review papers, journals and research articles on various aspects of chemistry and taxonomy have been published. Today chemical approaches to taxonomy are routine and many of the analytical paraphernalia required are to be found in botanical laboratories throughout the world. Often the sheets of chromatography paper outnumber the herbarium sheets! Not only has the apparatus of chemistry been imported but so has the terminology, and taxonomists now have the additional hazard of having to familiarize themselves with TLC, R_f values, electrophoretograms, etc., not to mention the somewhat chaotic nomenclature of chemical compounds.

It is not my intention to present yet another review giving, in greater or less detail, examples of the ways in which chemical characters may be used in systematic studies of plant groups. Our field is in danger of being over-reviewed and too many reviews tend towards self-congratulation and complacency. A more significant factor is that we tend to adopt a one-sided approach in such reviews in that we tend to overlook the problems of the chemist or biochemist in the relationship and concentrate on those of the taxonomist. This is unfortunate in that although the field we are concerned with is basically systematic and taxonomic, many of those contributing the chemical data are chemists or biochemists with no taxonomic background. There is a dual problem then—on the one hand, the taxonomist has to assess the value, limitations and problems of taking another set of data into account in making his assessments; and, on the other, the chemist has to try to understand the aims, methods and special problems of the taxonomist if he is to avoid becoming no more than a data-provider. In some ways the problems in this situation are more demanding for the chemist than for the taxonomist since the latter has a long tradition and experience of absorbing new kinds of information in making his taxonomic decisions and interpretations. The aim of the next section is to discuss the relationship between the two sciences of taxonomy and chemistry in detail.

TAXONOMY AND CHEMISTRY—THE UNLIKELY MARRIAGE

In a series of posthumously published essays entitled *The Relations between the Sciences*, the Cambridge zoologist Pantin⁵ analyzes the differences and similarities between the different departments of scientific knowledge. His analysis, which is very relevant to our theme, is summarized by the quotation he gives from A. V. Hill—'Physics and chemistry will dominate biology only by becoming biology'. As Pantin notes, if one considers what practising scientists actually do, one is puzzled at the diversity of their activities and it is difficult to see what it is they have in common. Consider, for example, the taxonomist identifying and classifying plants in a herbarium and the chemist in his laboratory: they seem to be doing very different things indeed. One could, of course, suggest that the taxonomist is not a

scientist or is, at most, a descriptive scientist, unlike the chemist who is an experimentalist, but such oversimple classifications are misleading if not invalid. Various attempts have been made to classify the sciences—the descriptive versus the exact, the observational versus the experimental, the physical (including chemistry and mathematics) versus the biological, but such divisions are arbitrary and artificial and used only for practical convenience. In practice, we tend to adopt a linear classification of the sciences, arranged according to their degree of exactness, with taxonomy at the bottom of the ladder and mathematics at the top. We know, of course, that the relationships between the sciences are in fact multidimensional with many cross-links, but there is a linear series we can recognize which is of great significance, namely that as one moves from say, biology to physics or mathematics, the sciences become generally less complex.

It is, as Pantin⁵ says, 'the richness and complexity of their phenomena which distinguish sciences such as biology and geology from the physical sciences. *Physics and chemistry have been able to become exact and mature just because so much of the wealth of natural phenomena is excluded from their study.* There is no need for the physicist as such to go to biology for data until in the last resort he has to take into account the fact that the observer is a living creature. I would call such sciences 'restricted'.'

Biology, on the other hand, is an unrestricted science in that scientists in this field must be willing, if they are to advance their science, to venture into every other scientific discipline. This has always been true to some extent, even in the earlier essentially descriptive and observational phases of biology, in that the richness of the natural phenomena available for study allows and indeed forces the biologist to select different sorts of observations to test his hypotheses. He can in fact observe natural experiments. It is this ability to seek correlations in his observations of the natural world that gives the biologist and the taxonomist such a powerful weapon. It is, one could say, the basis of the taxonomic approach. The great corpus of taxonomic information and the classifications on which we still rely today have been built up very largely on such a basis of field observation and repeated confirmation of what has been observed. The role of evidence selected from descriptive biology, geographical distribution and geology in the formulation of Darwin's evolutionary theories is well known.

In recent decades biology has become increasingly complex and unrestricted by precisely this process of embracing the physical, chemical and mathematical fields, to such an extent that it is now an almost intolerably difficult subject in which to advance. As a consequence we now have the curious phenomenon of the division of biology into the more exact and restricted branches such as biochemistry, biophysics, biomathematics, cell biology and molecular biology, where more or less precise experiments or mathematical models can be established, and the inexact areas of traditional biology which are now somewhat disparagingly referred to as Natural History. We have, in other words, the division of restricted and unrestricted science *within* biology and even nowadays within taxonomy itself. Just as successive sciences have become progressively more experimental and quantitative, so taxonomy can be regarded as a series of such successive sciences since it is dependent for its advance on the application of techniques

developed in other fields⁶. Chemistry and biochemistry have played a major role in this evolution of taxonomic approaches.

Nonetheless, the marriage between chemistry and taxonomy is somewhat surprising and in the next section I shall consider the nature of the taxonomic approach.

THE NATURE OF TAXONOMY

Taxonomy is basically a means of handling and processing data obtained from whatever source, for a variety of purposes—practical, academic, evolutionary, etc. It has no data of its own. It is dependent on human judgement in its procedures, no matter how precise the data (including chemical) may be, to a degree which is alien to more rigorous scientists such as chemists and biochemists. Not without justification has it sometimes been termed a scientific art. One can seldom, if ever, talk about *correct* classifications, only about those which best fit the available facts and aims of the particular classification sought. It is often remarked that classifications should make sense, by which it is meant that they should not run counter to our overall knowledge and experience of the groups being studied, or put positively, they should demonstrate and indeed be based on a maximum correlation of the attributes of the entities contained in the classification.

Formal taxonomy

Formal taxonomy is the delimitation and description of taxa at all levels and the presentation of the results in highly structured and processed forms such as Floras, monographs, revisions and keys, so as to facilitate recognition and discrimination. This kind of taxonomic activity is basic and essential. It is the role of the taxonomist to define the parameters of the units within which the consumer, be he ecologist, chemist or physiologist, works. For practical reasons this formal framework is expressed morphologically although all kinds of evidence may be used in delimiting the units (taxa) in this framework. Man cannot produce or utilize generally useful classifications in a visual vacuum. Even were we able to wipe the slate clean and remake all our classifications *de novo*, it is virtually certain that our new classifications would be morphologically expressed. One fundamental reason for this that is so obvious that it is often overlooked, is that we have to go out and find our taxa in nature (or in a restricted sense in the herbarium or botanic garden) and being visually gifted we have to look for them. It is perfectly possible to define taxa on the basis of nonmorphological features, good examples being morphologically indistinguishable cytotypes, but in practice we have to include such taxa within a single morphological group for the purposes of initial recognition. The same applies of course to chemically distinct but morphologically inseparable races. Fortunately non-morphological data, from whatever source, tend to correlate with morphological features. This in itself raises problems—we could ask whether it is worthwhile expending a great deal of time and effort simply to confirm what we already know. Should we not restrict the use of chemical data *in this kind of taxonomy* to those cases where there are intractable problems which conventional data do not permit us to clarify satisfactorily?

It is important to distinguish between the different levels of formal taxonomy because the problems they present are quite different, the amount of effort put into them differs, and the role of chemistry at each level differs.

The level of the family and above

The families and higher taxa are normally taken for granted by the taxonomist in his routine daily work. Very few taxonomists work at the family level. None of the major families has been revised as a whole in recent years and there is little likelihood of this happening in the future because of the sheer size of the task of considering all the tribes and genera concerned (cf. Jacobs⁷, Watson⁸). The total number of flowering plant families is 250 to 350 so that the scope for the world's taxonomic labour force of several thousands is not great if delimitation of families were to be a common activity. This is not to say that the limits of all angiosperm families are clearly defined: on the contrary there is great difference of opinion about the delimitation of families in parts of the Magnoliidae and in the areas of the Rosaceae and Leguminosae (cf. Heywood⁹). Often one is concerned here with status and relationships (not to mention phylogeny—see below) rather than delimitation. For example, in the Leguminosae (Fabaceae), there is general agreement that the Papilionoid, Mimosoid and Caesalpinoid groups should be recognized, but not about the status they should have—subfamily or family.

There is a confusion in some chemists' minds about this aspect of the taxonomist's activities, a confusion engendered partly by taxonomists themselves. This confusion stems from the failure to distinguish between: (a) work directed towards the relationships (natural, phylogenetic) between families and other higher taxa and arrangements which express these relationships; and (b) actual circumscription and description of these taxa.

The role of chemistry in deciding upon the circumscription of higher taxa is somewhat limited. Likewise, chemical data are of restricted value in the conventional type of practical description which is used for identification purposes, as in Floras. In more extensive systematic treatments there is no reason why chemical information should not be included. In recent years sufficient data have been accumulated to allow us to talk about the chemical profile of a family as in the Umbelliferae¹⁰ and Leguminosae¹¹.

The level of the genus

Taxonomists undertaking monographic or revisional work tend to concentrate at this level of the hierarchy. In many ways decisions on generic status are amongst the most difficult that the taxonomist has to face since there is no way of defining a genus in a way that is not equally applicable to the subgenus or section.

There are many thousands of genera and only a small proportion of them has been revised this century. In the majority of cases we still have to use generic revisions or concepts established by de Candolle, Bentham and other classic 19th century authors whose work was based primarily on morphology. Just how unsatisfactory this is is seldom appreciated. Bentham and Hooker¹² in their monumental work *Genera Plantarum* adopted in general a broad

generic concept and frequently dismissed in comments many of the smaller, recently described genera. A similar approach was followed by Engler and Prantl¹³ in their *Die natürlichen Pflanzenfamilien*. On the other hand de Candolle recognized many of the small genera and added further ones of his own. The phenomenon of 'splitting' and 'lumping' was therefore well established in the 19th century. In the Compositae (Asteraceae) and other large families this is particularly evident: the monographers of that family, Cassini and Necker, used what by any standards is regarded as a narrow generic concept which was largely followed by de Candolle.

A group of the Compositae in which I have been interested for the last 25 years is the Anthemideae DC. This tribe is conventionally divided into two subtribes, the Anthemidineae and Chrysanthemineae, on the basis of presence or absence of receptacular paleae (scales). This has the effect in keys and in linear sequences in Floras of separating genera with or without scales quite widely, irrespective of their other characteristics, so that otherwise similar genera are not considered together.

In the Chrysanthemineae the major genera are *Chrysanthemum* and allied groups, *Matricaria*, *Abrotanella* and *Artemisia*, while in the Anthemidineae the major genera are *Anthemis* and allied groups, *Eriocephalus*, *Santolina*, *Athanasia*, *Anacyclus* and *Achillea*. The development of generic concepts in the *Chrysanthemum* complex in the last 100 years is almost bewilderingly complex although fairly representative for the family¹⁴. The details cannot be given here but whereas Bentham¹² recognized 5 genera and Hoffmann¹³ 4, a recent generic revision¹⁵ recognizes 14. It is interesting to note that this rearrangement of genera has been based largely on the evidence derived from carpology and embryology¹⁶⁻¹⁹ and detailed morphological study. The effect has been to break down a large heterogeneous assemblage into a series of medium sized (e.g. *Tanacetum*, *Leucanthemum*) and small genera which are more or less homogeneous in terms of floral features, cypselar structure and anatomy, embryology, etc.

Phytochemical data have recently been published which tend to support this new generic disposition. If we consider this in some detail it will illustrate some general problems of the use of chemical data in taxonomy. Although various chemical compounds had been reported previously in members of the group, the first major survey was that of Bohlmann and collaborators²⁰ who surveyed the polyacetylenes in 40 species of Anthemideae. His plants were raised from seed obtained from Botanic Gardens and he employed the nomenclature and taxonomy given on the seed packets. To the taxonomist using his data this posed a number of difficulties: even assuming that the original identifications were correct (an unwise assumption), the names had to be interpreted in the light of current classifications and this is always dangerous since, for example, the same specific epithet can occur in different genera and refer to different species and without seeing specimens we cannot be certain which. When, however, the names in Bohlmann's lists had been translated into the various genera currently recognized, his data provided support for the recognition of some of them although a few of his chemical groups were heterogeneous taxonomically.

Another, partly overlapping selection of species of Anthemideae has been surveyed for flavonoid aglycones in the leaves by Greger²¹ with similar

taxonomically valuable results. At Reading, we have surveyed the flower and leaf flavonoid patterns in 21 species of the *Chrysanthemum* complex²² and found that they too are useful for delineating the segregate genera. Similar considerations apply to the *Anthemis* group of genera where the chemical data support the recent separation of smaller groups such as *Chaemaemelum* (*Ormenis*), etc. from *Anthemis*.

The total sampling of the Anthemideae from a chemical point of view is still extremely limited and it will take a very considerable time before we can talk with any certainty about the chemical characterization of the various genera; many have not yet been studied at all although further work is in progress. A certain amount can be done using herbarium material, but surveys of living plants are necessary and a major difficulty is simply that of obtaining viable seed. This is where so many biosystematic investigations fall down. The percentage of wild species available in Botanic Garden seed lists, although improving, is still extremely small and in nearly every large genus only a very few species can be obtained through this method. Only common species tend to be collected; the less common are listed occasionally; the rare species seldom occur. Moreover the number of gardens issuing such lists is small²³ and tend to be concentrated in a few European countries: few lists are issued by gardens in the United States or in tropical countries. When one is considering a large genus containing species of wide distribution, extensive and highly expensive field work is needed to obtain an adequate representation of seed material and even then species restricted to inaccessible areas are rarely obtainable.

In our work on the Umbelliferae (Apiaceae) at Reading on which we have been engaged for the last 7 or 8 years this still remains an intractable problem. We are dealing mainly with the tribe Caucalideae which contains 18 to 20 genera and 80 to 100 species, and is thus a relatively small group, yet we have still been unable to obtain seed of any representatives of 5 of the genera and of the remainder only about half the species, despite strenuous efforts and assistance from colleagues in many countries. In the context of the Mediterranean, for example, it is difficult to obtain seed from North Africa, some of the Middle East countries, Anatolia, and even from many parts of southern Europe. In south-west Asia it is even more difficult and there is little hope of obtaining samples from the vast areas of central Asia.

It is as well that workers in chemosystematics be aware of these problems before undertaking surveys which require material from a wide geographical distribution range. Unless one is prepared to make great efforts over a number of years to obtain material, the study is likely to remain tantalizingly incomplete and be of very limited use in comparative terms. On the other hand it must be recognized that an incomplete but representative sampling of genera is often of value since the role that chemical characters can play in helping to resolve the intricate problems of generic delimitation in critical groups is often to suggest, on the basis of a limited sample, where the taxonomist might look for discontinuities and useful variation patterns. In other words, chemistry may not give the answer but rather suggest to the taxonomist where, with further work of a non-chemical nature, it may be found. Another point worth stressing is that the chemist should obtain the best taxonomic advice possible so that within the limits of the material

available, as representative a sample as possible should be made and if possible critical or 'linking' groups included.

Returning to the Anthemideae, this tribe illustrates the well known phenomenon of the cluster pattern whereby one finds a small number of large genera surrounded by a series of satellite genera containing often only one or two species. Other examples that have been studied chemotaxonomically are to be found in the Fabaceae (Leguminosae). There are, for example, the nodal genera *Genista* and *Cytisus* in the tribe Genisteae each with their cluster of small genera, some of which (as in the case of the Anthemideae) interconnect the two, forming a pattern of reticulate variation. Although the Genisteae have been extensively surveyed chemically, the results are somewhat inconclusive. Faugeras and Paris²⁴ present the results of an alkaloid survey of 65 of the 158 European species showing that four basic groups could be distinguished (1) 'Cytisus', including *Cytisus*, *Chamaecytisus* and *Lupinus*; (2) 'Genista', containing *Genista*, *Laburnum*, *Teline*, *Chamaespartium*, *Echinospartum*, *Gonocytisus*, *Retama*, *Spartium*, *Petteria* and *Ulex*; (3) *Calicotome* and *Lembotropis*, (4) *Adenocarpus*. In a survey of the polyphenols of a large sample of the same tribe Jay, *et al.*²⁵ found that the 'Cytisus'-'Genista' complex as a whole is chemically very homogeneous and provides no justification for the recognition of two central groups or for their dismemberment into smaller genera. In this case, the results from different classes of secondary chemical constituents are appreciably dissimilar and not in agreement at some of the critical taxonomic levels. Depending on the constituents studied, the taxonomist, looking for support for a particular generic treatment, could come to quite different conclusions. This is in no way surprising, but does underline the need for extensive sampling in terms of classes of compounds as well as of species.

The level of the species

The greater part of the work in formal taxonomy consists of deciding upon species limits, identifying material as to species and preparing Floras and keys which permit rapid determination of species. Such work ranges from the sophisticated and highly skilled to the superficial and provisional. It should be noted that species delimitation or recognition is not dependent on detailed generic studies—in other words, to refer again to the Chrysanthemineae, it is not necessary to decide upon the generic status of say, *Coleostephus*, *Glossopappus*, *Lepidophorum* or *Leucanthemum* before being able to identify a species in this group, since they can all be included in the genus *Chrysanthemum*. The circumscription of species remains the same no matter what genus they are placed in.

The evidence used for the circumscription and recognition of species is basically morphological, although cytological and occasionally anatomical or palynological information may be used. In addition, field observations of variation coupled with studies on breeding systems and crossing experiments are often employed in arriving at decisions on specific status. The results are almost invariably expressed morphologically, no matter what the evidence used has been, and only in critical groups are micromorphic characters, such as seed surface, pollen or stomatal size or leaf histology used for diagnosis. Chemical data can be employed at the specific level and

there are numerous examples of this in the literature but the purpose of chemical surveys of a range of related species is usually to work out generic or infrageneric groupings, not to decide upon the validity of the species themselves.

Most problems of species delimitation can be solved without recourse to chemical data, as Turner²⁶ rightly points out. Moreover, for purely practical reasons, chemical characters are no more feasible for routine, general purpose identification than chromosome numbers. Exceptions are odour and taste which have a limited use in some groups.

Chemical studies may be of particular value in helping to solve population problems at and below the species level especially in situations where hybridization or introgression is occurring or is suspected to occur. Classic examples are the investigations of Alston and Turner²⁷ in *Baptisia*, Emboden and Lewis²⁸ in *Salvia*, and more recently of Adams, Flake, von Rudloff and Turner^{29, 30} on *Juniperus*. The results may serve to establish which members are hybrids by the general principle of 'chemical complementation'³¹, especially in complex situations where morphological features are difficult to interpret. This is dependent, of course, on the parents possessing distinct and recognizable chemical profiles which is, unfortunately, not always so. Chemical data may also help to demonstrate that suspected hybrids are not in fact so, as in the case of *Hedeoma*²⁶. Here it was suspected that *H. drummondii* and *H. reverchonii* were hybridizing so as to produce a group of intermediates often referred to as *H. serpyllifolium*. A gas chromatographic study by Irving of the volatile constituents revealed, however, that 93% of the identified compounds in the three taxa were composed of a different structural class—monocyclic terpenes in *H. drummondii*, acyclic in *H. reverchonii* and bicyclic in *H. serpyllifolium*. The latter therefore appeared to have a 'biological identity' of its own and not represent a hybrid. Hybrids were in fact rare and from experimental crosses were shown to be morphologically more or less similar to *H. serpyllifolium*. Turner comments that 'if one were using purely cytogenetic findings of the type so common in the biosystematic literature (i.e. in many experimental studies production of the morphological intermediate is often all that has been required to 'prove the case' for hybridization), it might be concluded that what appears to be the case, visually speaking isn't.' This of course invites the response, 'why should one?' There are other similar cases, as in *Spergularia*³², where careful morphological study combined with crossing experiments and cytology, disproved the hybrid nature of suspected hybrids.

In fact, chemical data can sometimes tip the balance in a complex situation but no generalizations can be made. Sometimes they are of little value and very extensive sampling is required in all cases. As in most taxonomic situations the researcher has to decide, in the light of the resources and time available, on which technique to expend the greater part of his effort. Certainly a greater use of chemical data is to be encouraged but they may not always prove of value and references such as Turner makes to 'the cynic steeped in the morphological approach' are gratuitous.

Similar considerations apply at the infraspecific level and I find it difficult to accept fully Turner's statement that at this level morphological ('megamorphic') data are increasingly difficult to assemble without bias on

a population level because of phenotype variability, and that on the other hand, chemical data can be readily compared on a character-to-character basis, making statistical comparison easy, and can be accumulated without bias.

While it is true that chemical characters are not initially visible to the naked eye and are sampled on a blind basis without bias, the plants which bear the organs to be sampled and the organs themselves have to be sampled visually and this introduces as much bias as in other classes of character. Likewise, while it may be true that environmentally-induced phenotypic variation makes morphological sampling difficult (although this depends on the kind of character used), it must not be assumed that chemical compounds are not subject to such environmental factors. On the contrary there is much evidence to indicate that they are susceptible to considerable variation in response to the metabolism of the plant and to numerous external factors as well as showing organ to organ, nutritional and seasonal differences (cf. Erdtman³³ and Scora and Malek³⁴). Moreover, there is very little information available in individual cases about such variation whereas, the parameters of morphological information are often much better understood as the result of repeated observation and general experience. Another point that has to be borne in mind is that we can often assess the significance in biological terms of morphological variation—this is part of the accumulated experience of a skilled taxonomist—whereas we have very little knowledge so far, except in general terms, as to the biological meaning of chemical data. These points are discussed more fully in a later section.

It is certainly not my purpose to minimize the role that chemical data can play in formal taxonomy but rather to indicate their limitations and their lack of privilege. In individual instances they may be more valuable than other classes of data in helping the taxonomist to arrive at a decision but optimistic generalizations are misleading and do no service to those of us who would like to see the more general application of chemical techniques.

It is in considering relationships and analyzing variation patterns that chemical data have often proved of exceptional value as will be illustrated in the next section.

STUDY OF VARIATION PATTERNS AND RELATIONSHIPS

The museum or herbarium taxonomist is often required to spend the greater part of his time on the various kinds of formal taxonomy outlined above, as well as in curating collections and in general administration. His primary tasks are, therefore, to deal with identification of incoming material, the preparation of Floras, and, if he is fortunate enough to have the time, the preparation of revisions. If he belongs to a well-staffed institution he may be able to devote some of his time to going beyond the purely formal side of his work to study variation patterns and relationships of various kinds, including phylogeny.

It is not, perhaps, widely known just how critical the manpower situation is in many herbaria especially the major ones which are responsible for much of the formal taxonomy produced. A thought-provoking survey of these problems, supported by extensive statistics, is given by Shetler³⁵ who notes

that as a group the world's largest herbaria appear to be seriously understaffed.

The academic taxonomist, by which term I mean a full time teaching member of a University staff with a research activity in taxonomy or a related field, is, on the other hand, usually more privileged than his herbarium colleague in that he can choose his own field of research and is not tied to an official programme or policy. His curatorial responsibilities are usually small (or he has none) and if he is able to label himself a cytotaxonomist or biochemical systematist or biosystematist, etc., he is often able to avoid the chores of the herbarium taxonomist and look upon himself as an experimental scientist. There are, of course, numerous exceptions to the above generalizations but they are on the whole valid. The situation is well summarized by Shetler³⁵.

Not just a few curators are virtually enslaved by the sheer burden of the routine daily transactions and public service, when in fact they should be practising science. At the same time [as the growth in the herbarium over 400 years] science, too, has changed, so that altogether the forces of change and growth have conspired to make it difficult for today's herbarium botanist to be both curator and scientist. Descriptive taxonomy is a fairly natural and easy by-product of curatorial activities, and it thrives on a constant inflow of new material. To the biosystematic, ecosystematic, or experimental taxonomist, however, curation is largely an encumbrance, a service to perform as the price of being a professional taxonomist.

One consequence of this situation is that the majority of major advances in systematics and taxonomy this century have come from the academic taxonomists who have the freedom (*not* the leisure!) to apply techniques from disciplines which have been developed in the main outside taxonomy or even outside biology. The history of taxonomy and systematics this century has followed this pattern—as witness the application of cytology, genetics, chemistry and biochemistry, electron microscopy, statistics and computer technology. We have to consider the role of chemistry in this context, noting in passing that it, along with so-called numerical taxonomy, is only the latest of a series of new approaches to systematics which have yet to be digested.

The whole of biological research today is undertaken in an evolutionary context, whether this be explicit or implicit. I have noted on previous occasions, e.g. Ref. 36 how little evolutionary theory has affected what formal taxonomists actually do, although it has provided them with a new intellectual framework and new actual or potential goals. As has been frequently noted (e.g. Turner³⁷) phylogenetics or evolutionary information seldom affects the recognition or circumscription of taxa at any level but does have an important role to play in their arrangement and relationships.

When taxonomists are freed from the restrictions imposed by making practical working classifications ('general purpose' classifications) and identification aids, they are able to consider the intellectually more exciting questions: how did the species/genus/family originate? Where? What biological or evolutionary mechanisms does it illustrate? We want to study, in other words, variation patterns, pathways (evolutionary, biochemical, etc.) and all kinds of regularities or irregularities shown by our material. Living organisms being such complex systems, we are trying to find some fixed point

in the bewildering array of organic variation, some pattern, some regularity in the complexity.

It must be emphasized that not all this can be usefully grouped under the heading of phylogeny, at least not in the strict sense of the term. Different authors stress different aspects of phylogeny—some emphasize patristic relationships (common ancestry), others cladistic relationship (branching pathways) and there is a complex literature on the subject which cannot be reviewed here (see Heywood³⁸, Hennig³⁹, Crowson⁴⁰ and the somewhat polemical series of papers in *Systematic Zoology* during recent years). Phylogenetic relationships in fact comprise various components—patristic, cladistic, chronistic, together with other phenomena such as anagenesis, convergence, parallelism, etc. Seldom is relevant information available on all these factors and the taxonomist has to content himself with some degree of evolutionary content in his classifications and arrangements. In this rather vague sense, phylogeny can be regarded, to quote Turner's³⁷ definition, as the arranging of taxa such that their taxonomic position one to another best reflects the genetic ancestry of those taxa. Clearly such a definition offers a wide scope and there is much to be said for adopting the recommendation that the terms 'evolutionary relationship' or 'evolutionary arrangement', which are deliberately vague, be used instead.

The role of chemical data in 'evolutionary' and 'phylogenetic' studies varies (as in the case of formal taxonomy) according to the level of the hierarchy involved. As a general rule it can be said that the higher the taxa the less valuable a contribution chemistry can make although a further distinction has to be drawn between micromolecular and macromolecular compounds (cf. Turner³⁷).

It is at the species and population level that biosystematic and, by definition, micro-evolutionary studies are most meaningful since it is within these limits that experimentation can take place (crossing experiments, pairing relationships, artificial synthesis of polyploids, etc.). Not surprisingly it is also at this level where chemical data have been found to be of outstanding value in assessing relationships. Examples are the studies of Johnson^{41, 42} on protein electrophoresis in solving problems of species relationships in wheats (*Triticum* spp): he found strong evidence that 'electrophoretic methods may provide a simple approach to the question of single versus multiple origin, which takes on a practical significance in the tetraploid wheats and cottons, for example'. Similarly Smith⁴³ has studied serology and species relationships in *Bromus* and concluded that although serological evidence had no greater value than any other kind for taxonomic purposes, 'in evolutionary studies it may contribute more reliable information, than, for example, comparative morphology, because it is less likely to be affected by environmental variation. Serological data relating to protein similarities are a readily tapped, independent source of facts about plant relationships'.

Similar studies have been carried out in other groups and reference may be made to the symposium volume *Chemotaxonomy and Serotaxonomy* edited by Hawkes⁴⁴. Essential oils are also readily utilized in relationship studies at the species and population level. They have the advantage that they occur widely, show considerable chemical diversity and can be relatively quickly extracted and analyzed on a large scale. The papers by Zavarin and

von Rudloff in this symposium are excellent examples as are those of the Texas group on *Juniperus virginiana* already mentioned above. Another outstanding investigation using essential oils in the analysis of taxonomic and evolutionary relationships is that of Scora and Malik on *Citrus* spp. They studied 13 essential oil components in the rough and smooth lemon groups and other major *Citrus* taxa and analyzed the results by computer discriminant analysis to give a statistical divergence diagram from which conclusions as to the possible origins of the various groups were drawn.

Other classes of micromolecular or secondary compounds, notably the flavonoids and alkaloids, have been employed in similar studies to those just described although the analytical techniques are different. Chromatographic patterns of species and hybrids in *Baptisia* have already been referred to and other examples are summarized by Turner⁴⁵.

ANGIOSPERM PHYLOGENY AND EVOLUTIONARY TRENDS

When one considers evolutionary or phylogenetic trends and relationships above the species level in the angiosperms, one is entering into a field where speculation, intuition and inference play a major role. In the absence of detailed fossil sequences, phylogenetic reconstruction on the basis of information derived from extant plants is not impossible, but it has to be admitted that it is often, if not usually, tentative and hypothetical. Although precise methodologies and principles for 'phylogenetic classification' and derivation have been proposed by zoological writers such as Hennig, it should be observed that (a) they are controversial and highly debated by zoologists themselves, (b) they are difficult to put into practice due to their lack of explicitness or their non-operational nature (cf. Hull⁴⁶), (c) there are real differences between angiosperm and animal groups which cast doubt on the applicability of these techniques and principles to the former.

There are, in fact, major differences between plant and animal groups in respect of their mode of life, patterns of population structure and reproduction, and apparent adaptive significance of taxonomic characters as has been pointed out by several authors⁴⁷⁻⁴⁹. At the higher taxonomic levels major adaptive shifts may be recognized as having played a significant role in the origin of taxa but as Stebbins points out⁴⁹, an important difference from animals is that relatively few of these adaptive shifts were unique events, most of them having occurred repeatedly in different evolutionary lines. He notes that

This tendency for extensive parallelism and convergence, as a result of which similar structures and modifications arise many times independently, is particularly conspicuous in the angiosperms, and is now being recognized as one of the chief obstacles in the way of achieving a satisfactory classification, and of tracing out the course of their evolution. In the past, characters such as syncarpy, sympetaly, and epigyny were believed to have occurred only once or a few times during the evolution of angiosperms, and so were regarded as reliable criteria by themselves for recognizing orders or even subclasses. As knowledge increases, however, more and more separate lines can be recognized in which these trends have occurred independently of each other. Moreover, in some instances they appear to be associated with the origin of major subdivisions of angiosperms, in others with the origin of individual families, in still

other examples with the differentiation of genera, and occasionally, even with the differentiation of species within a genus.

The problems of working out the probable course of evolution in the absence of extensive fossil series by inferences based on comparison of characters shown by contemporary organisms, are still largely unresolved. They involve arranging characters of present day organisms in series (character gradients or semophyloeses), deducing the direction in which such trends took place in historical time on the basis of what palaeontological, phytogeographical and ecoclimatological information can be obtained, and then inferring on the basis of correlation between several independent trends what the probable course of evolution of the organisms and groups bearing these characters might have been. It is essential to emphasize the distinction that must be made between the evolution of individual characters and the evolution of taxa, and analogously between the degree of evolutionary advancement or primitiveness of a character and that of a taxon which has, of course, to be an average condition of those individual characters studied.

Many evolutionary trends have been described in the literature (for a summary see Davis and Heywood⁵⁰) but the question of the relationship between 'advanced' and 'primitive' characters and ancient or recent groups is highly controversial and not yet fully resolved (see Eyde⁴⁸ for a useful discussion).

Evolutionary trends in chemical characters have been postulated in several recent papers. Harborne⁵¹, for example, makes a case for the special role of flavonoid characters as phyletic markers. On the basis of the distribution of yellow -flavonoids and -flavones and comparison of the patterns with Cronquist's classificatory scheme, he found that substitution of —OH in the 8-position appears to precede substitution in the 6-position in the dicotyledons although the functional significance of this was not clear. One must point out, however, that the direction of this trend was suggested by a previously published evolutionary scheme and cannot therefore be taken as independent evidence to support it. It would be a different matter if one could establish the evolutionary sequence of these compounds on chemical and biosynthetic grounds and then find that the sequence agreed with an existing systematic-evolutionary scheme. Otherwise there is a strong element of circularity in the argument. A much quoted example of the value of chemical information deserving phylogenetic emphasis is the betalain story⁵²⁻⁵⁴ where it has been found that the betalains and anthocyanins are mutually exclusive in distribution and is proposed that the betalain-containing families in the Centrospermae be treated as a separate phyletic group from the anthocyanin-containing families. Even here, it is the general systematic context that suggests to us how to weight the chemical evidence, and as further non-chemical evidence is examined in detail the picture may be gradually modified. Indeed, Benke and Turner⁵⁵ have recently published ultrastructural work in sieve-tube plastids which led them to comment 'it seems reasonable to admit that the Caryophyllaceae, in spite of their anthocyanin pigments, are indeed closely related to the chemically constituted Centrospermae' although this need not negate the treatment proposed by Mabry *et al.*⁵². The point that has to be made here is that no matter how

convincing chemical or any other class of data may be, only a balanced correlative study of all the available evidence is likely to lead to a satisfactory evolutionary and systematic interpretation. Chemical approaches to evolutionary studies are strongly to be encouraged as they are likely to suggest many exciting new leads in our research for relationships. But in our enthusiasm we must not overlook other evidence or lose sight of the fact that much angiosperm classification rests on a very shaky foundation. So often one is dealing with precise chemical characters in an imprecise taxonomic-evolutionary framework. It is scarcely possible to have a purely chemical phylogeny since it is organisms that evolve, not just the chemical compounds they produce. In a word, you cannot work out the phylogeny of groups that *have* no evolutionary coherence.

It is not fully appreciated outside the taxonomic fraternity just how tentative and inferential is the phylogenetic content of recent systems of angiosperm classification such as those of Cronquist⁵⁶, Takhtajan⁵⁷, Thorne⁵⁸, Soó⁵⁹, and others. As already mentioned, what phylogenetic ideas are expressed in such systems do not normally affect the circumscription of the families and orders: the criterion of reasonable monophyly of these taxa is inferred from phenetic evidence. It is the arrangement of the groups relative to one another and the sequences which purport to reflect their evolutionary relationships and derivations that constitute the main phylogenetic content of such systems.

We have to remember that we are considering evolutionary relationships of contemporaneous families, some of which are relatively young, some of which are relatively old in origin, but both containing genera and species which may be young *or* old. Unfortunately we have little direct information as to the relative age of the angiosperm families: probably the best data available are those derived from fossil pollen grains supported by some macrofossil evidence which are summarized in an excellent review by Muller⁶⁰; even these data are very incomplete and not always in agreement with evidence from other sources.

Although there is a certain measure of agreement between most recent systems of angiosperm relationship as regards the placing of key groups, it is entirely possible that they are fundamentally incorrect on various major points such as the monophyletic versus polyphyletic origin of the angiosperms as a whole, and the basal position of the Ranalean complex (recently contested on chemical grounds by Kubitzki⁶¹), as well as on numerous less traumatic matters. Future research may hold many surprises for us. There is, in fact, a high degree of selectivity in the preparation of angiosperm systems, and much relevant information is ignored or even deliberately passed over if it does not fit in with the prejudices of the author concerned. A major defect is that the evidence and bases for the construction of the systems are not clearly and explicitly laid out fully and fairly. This is not surprising when one considers the vast corpus of evidence that would have to be taken into account and correlated if the task were to be approached scientifically—a task far in excess of the capabilities of a single individual. I wonder if it is not significant that no serious cooperative attempt to tackle this problem has been made or suggested. My own view is that it is not a primary concern of evolutionary-minded taxonomists today to work out the phylogenetic

tree of the angiosperms and other groups. It is not a major preoccupation whether the Magnoliaceae is moved up, down or across the system! Such a goal is far too limited and I believe that too much time has already been wasted in such attempts. Indeed the construction of phylogenetic schemes of the angiosperm is a much overrated pastime which has been pursued far too unscientifically for far too long. What is more, it is highly debatable whether such an activity should be given a high priority when we consider the urgency of the tasks facing taxonomists and biologists as a whole today.

Certainly one welcomes clear and scientific approaches to the study of evolutionary pathways and mechanisms which are vastly more important to biology than the correct evolutionary placement of taxonomic groups in a system. It is the processes rather than the highly schematized product that we should be concerned with and chemistry will almost certainly have a major role to play here.

THE NATURE AND HANDLING OF CHEMICAL INFORMATION

Until recently, chemical data available for use in classification have been on too small a scale in most cases to pose problems of handling. Now that we increasingly find ourselves with chemical data not only in quantity but of different sorts, we have to consider carefully the ways in which they can be best utilized, correlated and presented, alone or in conjunction with other classes of data as, for example, in taximetric studies. This is a subject which deserves a review in itself and I can only draw attention to a few of the problems. If we take a simple situation first, spots on a chromatogram can be handled as presence or absence characters and simply tabulated as is common practice, or if one is comparing the results from a limited number of taxa, the chromatographic 'profiles' or 'patterns' can be compared visually, albeit imprecisely, for purposes of identification. Grant *et al.*⁶², for example, found it possible to recognize each taxon in a TLC study of fluorescent compounds in *Lotus* by their individual pattern of spots and colours. For precise comparison, however, a simple matching coefficient of association⁶³ was used and a phenogram based on cluster analysis of the coefficients produced. In this case, each distinct colour at any particular R_f value was regarded as a separate character.

Again, in the case of gel electrophoresis, band patterns are often characteristic for each taxon in the sense that they can be recognized visually, on the basis of certain conspicuous features, as we have found, for example, in the Umbelliferae where genera and even tribes can be readily recognized by their typical electrophoretogram pattern⁶⁴. It is when one tries to break down such patterns into elements that we run into difficulties. Various methods have been used:

- | | |
|---|---|
| (a) the number of fractions (i.e. bands in the gel) | } as an approximate estimate of the relative amount of protein in each fraction |
| (b) the density of the bands | |
| (c) the width of the bands | |
| (d) the position of the bands. | |

The number of interpretative problems here is vast and it is not surprising that some authors simply refer to general banding patterns and conspicuous features rather than attempt a detailed analysis. In their work *Gossypium*,

Cherry and collaborators⁶⁵ noted that it was difficult to distinguish the number of individual protein bands contained within specific large major bands. These large dense-staining bands consisted of a number of individual protein bands which could not be resolved because of high concentrations of proteins with similar rates of movement. Although further dilution of the major bands did show some increased complexity, many of the minor bands became unresolved. In a number of cases, however, bands of different mobility tended to overlap one another indicating that similar proteins within the bands are present. Thus, regions which look *unrelated* may contain similar proteins but in much higher concentrations, or may contain similar proteins along with a number of dissimilar proteins.

Conklin and Smith⁶⁶ used the matching of band positions between the zymograms of each species of *Datura* as a method of estimating closeness of genetic relationship amongst the species in the genus. They comment that 'the application of this method, which is based on molecular characteristics can be compared with phylogenetic relationships that have been arrived at by the use of more classical criteria'. The method they used to provide this estimate of relationships was the hypergeometric distribution which involved a computer programme. Various other methods have been employed such as simple percentage similarity values e.g. Whitney *et al.*⁶⁷, and Ziegenfus and Clarkson⁶⁸.

There are two aspects of this general problem that need to be studied: (1) the selection of what are to be regarded as characters, which has to be based on chemical criteria and taxonomic theory (nature of unit characters, information content, homology and analogy, etc.); (2) the selection of a statistical or numerical procedure to work out the most satisfactory method of comparison. This brings one into a field of almost bewildering complexity where expert advice and assistance is essential. For a brief but highly technical review of the field I would recommend the recent paper by Cormack⁶⁹ and the ensuing discussion which is reproduced. To give but one example, he lists eleven indices of similarity that can be used in taximetrics ranging from Euclidean distance and City-block metrics to the Canberra metric and simple matching.

We are still at a very early stage in both these areas and I would like to see much more research as far as chemical data are concerned. What is not widely enough realized is that in such 'numerical' approaches, one has a great deal of choice as to the selection and handling of characters and the kind of answer (i.e. form of the classification) one wants to produce. Reference should be made to a paper by Runemark⁷⁰ in which he critically reviews statistical methods employed in the comparison of different chromatograms. Some examples of sophisticated data handling in chemosystematic studies have been published recently and I would refer to the outstanding work of Adams and Turner on *Juniperus* where in the study of volatile terpenoids of leaves by gas chromatography their numerical approach included analysis of variance, contour mapping of characters and numerical classification²⁹.

There is one danger to which I would draw attention: a table of statistics or a complex diagram is no substitute for thought! It is often, I suspect, only the compilers of such intricate schemes who can understand what it is they are trying to convey and there are many cases where the significance and

interpretation of the data can be more helpfully summarized in a few lines of text. This is certainly true of some of the numerical taxonomic models published in recent years—they may be superb representations of mathematical concepts but their biological meaning is far from clear. The same applies to relationship diagrams in taxonomy in general where in so many cases no clear indication (if any) is given as to the meaning of the axes or parameters.

VARIABILITY OF CHEMICAL CHARACTERS

One of the main tasks of the taxonomist is to assess the extent to which characters are susceptible to environmentally-induced variation. In the case of morphological characters this can be exceedingly difficult and time-consuming but repeated observation has allowed taxonomists to build up a vast body of experience. On the other hand, although some classes of chemical compound have been shown to demonstrate less inherent variability than others, there is little accumulated experience as yet and insufficient attention is paid to this problem in many chemosystematic studies. A notable exception is the work of Scora and Malek on *Citrus*³⁴ already referred to and the following quotation serves to outline the kinds of problem involved:

Essential oils are influenced by the metabolism of the plant and by many outside factors... we first investigated the influence of climate, of tissue maturation, of senescence-delaying sprays, of rootstocks, of polyploidy, and of nutrition upon the essential oils. A biosynthetic study with radioactive isotopes was also carried out in order to learn about the sequence of oil formation in plants. After ample study on population diversity and investigation of all plant organs, from the germinating seed tube to leaf abscission, we now have some understanding of the behaviour of the individual oil's components and some of their physiological interrelationships. All these investigations have begun to provide data that will enable the selection of the taxonomically most reliable essential oil components.

The need for detailed knowledge of seasonal variation as well as within-tree and within-populations variability is also stressed by von Rudloff⁷¹ in his work on volatile oils in *Picea glauca*. His paper gives many references to similar studies in this and other groups. Spontaneous and induced variation in leaf constituents in the grass *Hierochloe* analyzed by chromatography are discussed by Weimarck⁷². Fortunately not all compounds show extensive variability but much further attention to these problems is needed to establish the facts in particular situations.

PRIORITIES FOR FUTURE RESEARCH

The biochemical systematist, like many other biologists, is apt to proceed in his research as though the source of raw materials were unlimited. This is not surprising when we reflect that there are 250 000 species of angiosperms and only a small fraction of these have been studied chemically to any degree. The progress of civilization is, however, rapidly changing our pattern of plant resources and at a rate which is difficult to assess.

Conservation of natural resources is a major preoccupation of all of us today and is now a matter for governmental concern. The situation in many parts of Europe is already critical. To give just three examples: (a) on the basis of a recent survey of the rare endemic species of Europe by Dr S. M. Walters it has been calculated that up to 15%, i.e. 500 are actually or potentially in some danger of extinction; (b) the impoverishment of the flora of Belgium, during the last hundred years was recently surveyed by Delvosalle *et al.*⁷³ and the magnitude of the disaster came as a surprise—not only have many species been lost but three-quarters of the stations of rare plants have disappeared; and (c) the flora and vegetation of hundreds of miles of the Mediterranean coastline has been eliminated by property development, largely for tourists.

What is happening in other regions of the world is perhaps even more dramatic. Although I do not align myself with the prophets of doom in such matters, I cannot fail to be impressed by the statement of such sober and respected scientists as P. W. Richards who writes as follows in a paper given at the Centenary Symposium of the Jardin botanique nationale de Belgique on nature conservation⁷⁴

... all over South America, as in tropical Africa and Asia, the forest is retreating and a man-made landscape taking the place of the climax plant communities. The rapid acceleration of this process in recent decades is partly due to the introduction of new tools such as power saws and bulldozers which make the clearing of the forest easier and quicker, but even more it is due to the inexorable demand for land by expanding human populations. Accurate and meaningful figures for the rate at which tropical forests are disappearing are hard to obtain, but it is *probably no exaggeration to say that if recent trends continue there will be hardly any primary forest left anywhere in the tropics by the end of this century*. . . It is now evident that if the process continues unchecked, *man will have destroyed the tropical forest, which in many of its features seems to have changed very little since the early Tertiary period, in barely 200 years.*

Similar quotations for other areas could be given from other papers in the same symposium. And Turner⁷⁵ has estimated that we have only 30 years left if we are to assemble reasonably representative collections of the world's species in populational form, with detailed field data, before decimation or destruction prevents this. There is little point however in discussing time-tables since the general picture is clear enough.

In the above context and in the light of what is discussed in the main body of this paper I should like to suggest the following priorities for chemosystematic research.

(a) Extensive sampling of as many species, genera and families as possible, at a population level when possible, so as to find out quite simply what chemical compounds they contain, while we still have the chance. There is a need for stocks to be built up via seed banks and botanic gardens. Just as we have somewhat belatedly realized the need for conserving gene pools for future breeding programmes, so there is a need for chemical compound banks (in the form of plants or seeds) to be built up. There are, I believe only five seed banks in the world; chemists could well add their support and influence in this area.

(b) Closer cooperation between chemists and taxonomists including

discussions on methods of analyzing and comparing chemical data in systematics and evolution, including numerical techniques. Just as chemists do not tolerate slipshod chemical work by taxonomists, so taxonomists have a right to demand the highest standards in taxonomic work put forward by chemists. Research teams seem to be only a working solution to this problem.

(c) Detailed discussions between chemists and taxonomists as to the most useful methods of storage and presentation of data given that the traditional format of Floras, revisions and other conventional taxonomic publications, are not designed to incorporate such information on any substantial scale.

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