PLANT CHEMOSYSTEMATICS

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Preface

Since we have dedicated this text to our late good friend and colleague, Professor Ralph E. Alston, it seems appropriate to relate briefly here our professional relationship with the man and his work and how this led to our mutual interest in the field of chemosystematics generally.

Professor Alston came to the University of Texas in 1957 with training as a physiological geneticist and only cursory interest in the field of systematics. His doctorate work dealt with flavonoids and their general control, a fairly narrow research specialty at the time. But the man was bigger than his field of specialization. His purview of things biological was quite exceptional and few, if any, scientific papers escaped his broad-ranging, imaginative but critical mind. This was important, for the "field" of chemosystematics in the mid-1950s consisted of a smatter of isolated papers in a range of botanical, chemical, and zoological journals by workers essentially unknown to each other. To recognize the *emergence* of this new field required wide interests and a sound training across a broad front of biological science.

An equally predisposing factor in Ralph's interest in chemosystematics was his great delight in field work. He loved the outdoors and preferred to be involved with problems in which he personally undertook field observations, population sampling, etc. This joy in field work developed relatively late in his short career, mostly after he became involved with the junior author on populational studies of natural hybridity in the legume genus *Baptisia*. Nevertheless, he was also a tireless laboratory worker, and was, in every way, a firm believer in getting at the molecular basis of biological problems.

The work on *Baptisia* seemed sufficiently impressive to Professor Alston so that he put almost full effort into this area of research. Beginning in 1959 until his premature death at the age of 42 in 1967, he lived and breathed this field (literally, for he spent much of his time working in an inadequately vented laboratory running hundreds upon thousands of two-dimensional paper chromatograms in his efforts to make certain that species, as populations, were understood, both as to the uniformity and variability of flavonoids). Because of his convictions and the satellitic, mostly verbal, stimulation of one of us (B.L.T.), these two workers contrived to write what might rightfully be called the first textbook on chemical systematics in the broad sense (Alston and Turner, 1963a). This was soon followed by a veritable deluge of texts on the subject, usually edited versions in which chemical experts were called upon to expand, chapter by chapter, upon their own particular chemical interests (Swain, 1963; Leone, 1964; Swain, 1966; Hawkes, 1968; Mabry *et al.*, 1968; Harborne and Swain, 1969; Harborne, 1970; Bendz and Santesson, 1974). A notable exception has been the six volume compendium of Hegnauer (1962–1973), but unfortunately this ambitious and remarkable compilation by a single individual was published over a ten year period in German, and further, it did not set out to cover the subject in an academic or conceptual sense. Gibbs (1974) has completed a less ambitious compendium with greater emphasis upon the taxonomic implications of microchemical data for flowering plants, especially at the ordinal level, and more recently, Smith (1976) has completed a text which covers a broad spectrum of conceptual concerns.

At the present writing, one cannot help but be impressed with the large number of biochemical articles having to do with *systematic problems* which appear almost daily in botanical (and other) journals. And *that* has been the most conspicuous change in the decade or so following the appearance of the text *Biochemical Systematics*: more and more the plant systematist is carrying out the chemical work necessary to help resolve his particular taxonomic problem.

And that is the purpose of this particular book, to review briefly, and hopefully succinctly, the state and potential of this approach to plant systematics up to the end of 1982. We make no pretence of covering the literature relating to zoological and prokaryotic systematics, for that would be a laborious undertaking requiring more sophisticated knowledge of relationships among these groups than we possess. However, the underlying principles and methodological approaches to such studies are virtually the same and, in this sense, the present text can be considered a revised version of *Biochemical Systematics*, in spite of the fact that only two chapters (2 and 3) are recognizable in the present version.

January, 1984

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Introduction

1
The Biochemical Background

The great advances in biochemistry which have come in the last few decades have impressed both the informed layman and the scientist. The scientist who has made an effort to acquire more than a passing acquaintance with the subject is appreciative of not only the elegance of method and the intellectual challenge of the field but, in addition, the implications, sometimes of even a philosophical nature, of these discoveries to other subdivisions of biology. For instance, the biochemical unity disclosed incidentally along with the elucidation of basic pathways of metabolism is as effective a support for Darwinian evolution as is comparative anatomy. Without a fossil record, and assuming that evidence from comparative anatomy were in some way unavailable, comparative biochemistry would have already established unequivocally the same concepts of evolution which now exist.

Four levels of biochemical unity may be recognized which, collectively, provide a framework for evolutionary theory. Starting with the most fundamental they are: (1) biochemical unity as expressed in the basic similarity of the hereditary material of all organisms; (2) biochemical unity as expressed in the group of co-enzymes which are essential to many of the basic biochemical processes; (3) biochemical unity as expressed in the similarity of metabolic pathways, particularly those involved in energy exchange, of different organisms; and (4) biochemical unity as expressed within major taxonomic groups in the common presence of certain structural components such as chitin, cellulose, and so on. At all of these levels there is also some degree of diversity. For example, while deoxyribonucleic acid is present in the chromosomes of diverse species, the same sequence of nucleotide subunits is unlikely to be expressed even in two individuals of a single species. All of this knowledge has a direct bearing upon phylogeny in its broadest meaning. At least, all of the facts have potential phylogenetic significance; those that emphasize unity, to relate species, and those that emphasize diversity, to separate species.

In recent years many books have been written about various aspects of the broad subject of biochemistry in relation to evolution. Typical among books by individual authors is that of Jukes (1966) entitled *Molecules and Evolution*. Molecular evolution has also been reviewed in such multi-author works as Ayala (1976). Comprehensive coverage is provided in a volume of a general treatise (Florkin and Stotz, 1974) in which biochemical evolution in plants is reviewed by T. Swain. Speculation about the origin of life and the role of symbiosis in plant evolution is also almost entirely centred on questions relating to changes at the molecular level (Margulis, 1981).

Dating back many years before the beginnings of enzyme chemistry and studies of metabolic pathways are numerous investigations of the distributions of various substances, initially in higher plants and now including fungi and bacteria as well. Such investigations often had pharmacological and other economic objectives, but some of the earliest workers were interested in correlations between the distributions of substances and the taxonomic treatments of the species investigated (for review, see e.g., Gibbs, 1963). Subsequent workers have continued to note such correlations or even to make a tentative taxonomic judgement based on their chemical results. Periodically, belief in the utility of biochemical data for systematic purposes has been reiterated. Although a wide range of biochemical characters are available (Table 1.1), biochemistry has not yet been responsible for any major advances in our knowledge of phylogenetic relationships. Yet, inexorable progress in the accumulation of biochemical data, many of which are already seen to be of phylogenetic importance, points to an obligatory integration of these data in systematics. The systematist does not have the prerogative of evaluating the purely chemical aspects of data, but he has a responsibility to be alert to progress in biochemistry, particularly when discoveries bear potentially upon phylogenetic considerations. Biochemistry relates to phylogeny at several levels, only one of which involves the taxonomic distribution of specific compounds. Certain approaches discussed in chapter 4 may seem to be remote or even irrelevant, but it is worth reiterating that no approach should be discouraged provided it is theoretically sound though its practical value may eventually prove to be slight.

It is not the purpose of this book to develop a case for the use of biochemical data in systematics since this has already been done. Rather, it is hoped to establish a better perspective concerning the place of biochemistry in systematics. There is a need for an exploration of some theoretical and intellectual aspects of the subject, the development of a basic rationale, an integration of certain chemical and biological aspects, an analysis of the advantages and limitations of the biochemical approach, a broad and essentially critical analysis of existing work. We have attempted to accomplish this series of objectives.

Table 1.1 Biochemical characters of application in plant systematics

Secondary Metabolites
Volatiles (plant scents and odours)
mono- and sesquiterpenes, aliphatic and aromatic volatiles, amines, sulphides, isothiocyanates
Defence agents (bitter principles and toxins)
alkaloids, cyanogens, non-protein amino acids, iridoids, sesquiterpene lactones, diterpenoids, steroids and triterpenoids.
Colouration (pigments)
anthocyanins, betalains, yellow and colourless flavonoids, quinonoids, carotenoids
Storage metabolites
fatty acids, sugars, polyols, cyclitols, alkanes, polyacetylenes
De novo *defence agents*
phytoalexins
Variations in Metabolism
Primary pathways
photosynthesis (C_3, C_4, CAM), lysine biosynthesis, tyrosine biosynthesis
Secondary pathways
shikimate isozymes
Degradative pathways
conjugation, detoxification, aromatic cleavage
Macromolecules
Proteins
variation in amino acid sequences, electrophoretic mobility, isoelectric focusing, serology, etc.
Nucleic acids
variation in base sequences, hybridization and base ratios
Polysaccharides
variation in sugar components, linkages, branching, etc.

We do not believe that biochemistry represents a panacea for all systematic problems. It is quite clear from many experiments reported in later chapters that the available biochemical characters (Table 1.1) can often do no more than provide supplementary data for phylogenetic considerations. However, profound and far-reaching new insight into phylogenetic relationships is potentially available in biochemistry especially at the macromolecular level and intensive study of DNA and protein sequences (see chapters 17 and 18) is already providing much new information in this area.

Nowadays, much is spoken and written about what is popularly known as molecular biology and its relationship to descriptive or classical biology. It is possible that some individuals regard these two categories as mutually exclusive. It is true that in this age one person rarely acquires eminence in both areas. However, there are many who can excel in performance in one area and be intellectually in contact with the other. It is the purpose of this book to contribute to an integration of these disciplines by providing the groundwork for a more effective utilization of biochemical data in systematics than has previously existed. One might go as far as to say that recent developments in molecular biology have re-emphasized once more the central role that systematics has in biology today.

2
Taxonomic Principles

I. Introduction

Taxonomy is one of the oldest fields of biological science. Organisms, and their relationships to other organisms, have occupied man's thinking for hundreds, if not thousands, of years. In order to classify, even at the most elementary level, man had to recognize (or identify) organisms. To do this he was prone to observe, make comparisons and, to some extent, integrate data, and develop generalizations therefrom. It can be argued that taxonomy was almost synonymous with biology in its beginning as a science. The identity of organisms occupied the thinking of early biologists. To derive order out of the multitude of forms in existence, these biologists were primarily concerned with writing descriptions and giving names.

Many non-taxonomists, including biologists and other scientists, believe that the sole function of the taxonomist is to describe and name species. Although this is still an important function of taxonomy, it is not its beginning or end. Taxonomy, like other areas of biology, has kept pace with the mainstream of biological progress.

A well-trained worker in taxonomy today must have a broad background in the fundamental concepts and basic working techniques of a number of disciplines. He not only has to be familiar with the special disciplines of his own field, but also should have some familiarity with cytology, genetics, statistics, anatomy and, it is hoped, biochemistry. Without such breadth the worker is often confined to a rather narrow avenue with much diminished perspective. If he is to synthesize and integrate the data provided by classical methods and augment his knowledge with new kinds of evidence he must be, as he was in the beginning of the natural sciences, one of the better informed and widest-read of all biologists.

Taxonomic thought, as indicated in more detail below, changed radically with the advent of Darwinism. Taxonomists not only have incorporated various new morphological approaches (for example, embryology and palynology), but also have accepted enthusiastically the contributions from genetics and cytology. In the present text we are attempting to inform the interested taxonomic worker of some present trends and developments in biological thinking which are or may become relevant to taxonomy.

Certain biologists attempt to discredit taxonomy as a 'classical' or dead field. This is unfortunate since taxonomy offers a conceptual approach to biology at the organism level such as chemistry offers at the molecular level. Both taxonomy and chemistry are unifying fields. The former, based on evolutionary principles, provides a framework to account for morphological variation and its mechanisms at the organism and population level, while classical and theoretical chemistry provide a systematic framework to describe and in part comprehend variations in the organization of elementary particles.

Although the term taxonomy has long been used to cover systematic work in the inclusive sense, more recently a number of new approaches has occasioned the advent of new names, such as systematics, biosystematics, and so on. In the present text we have used the terms interchangeably and in the inclusive sense. Regardless of their appellation, all such workers are, in fact, taxonomists; perhaps a bit more modern by employing experimental procedures but otherwise attempting to solve the same problems, namely, to show relationships and to classify accordingly.

Placed in its proper perspective then, taxonomy becomes the framework or the ordered arrangement of innumerable observations and bits of information. This order is as useful for biochemical data as it is for morphological features. Indeed, it would seem almost indispensable for the former since the seemingly unlimited number of molecular configurations might lose much of their interpretative significance without such a foundation.

Taxonomists generally fall into one of two sorts: (1) those who are primarily interested in the biological units, particularly with respect to their identification, distribution and proper description; and (2) those who are less concerned with the names and descriptions of categories and more concerned with evolutionary histories, the relationships of categories one to the other, and the mechanisms of speciation. In taxonomy, as in most other fields, there are specialists, some who are involved with floristic work, some with identification, some with phylogeny, and some with evolutionary mechanisms. There is room for all, in spite of the fact that different approaches might seem to be more significant at different periods of time. Ultimately all of the information must be consolidated into any unified system of classification.

II. Taxonomic categories

A. Formal categories

There has been much misunderstanding about the nature of biological categories. Such terms as species, genus, tribe, family, order, and division have no specific meaning to most non-biologists and frequently disputed meaning among biologists. The categories may be regarded as highly arbitrary.

Fig. 2.1 The argument that species are arbitrary constructs of the human mind might lead to strange cage-fellows.

Any attempt by man to categorize natural variation must be arbitrary with respect to a terminological system. This does not mean that the natural entities which are being classified are, in themselves, arbitrary or subjective. If evolution is accepted as the general mechanism for the origin of extant taxa, it necessarily follows that the hierarchy of formal categories erected by man do stand in certain positions relative to each other.

It is often argued that the biological categories, in that they are classified by man, are completely subjective in nature. What is often overlooked here is that the subjectiveness is in applying the terminology; the objectiveness of the category under consideration, from a biological point of view, is real. If the biological entity were completely subjective, then, to use a far-fetched analogy, one might well expect individuals of the species *Homo sapiens* to be at least occasionally caged with *Gorilla gorilla* (cf. Fig. 2.1).

Nevertheless, at least a few professional plant systematists despair at attempts to define species. Thus, Levin (1979) states that 'Plant species lack reality, cohesion, independence and simple evolutionary or ecological roles', which is an overstatement, for most plant systematists have little hesitation in using the term 'species', describing their characters or pointing out to yet others their ecological place in this or that ecosystem. In fact, there is considerable concordance among plant systematists working in the field as to the *population* units that make up this or that genus. No doubt this has prompted Cronquist (1979) to codify taxonomic or phenetic species as '. . . the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means.' This is a reasonable working definition but one which tacitly assumes that the biological species concept (i.e. an interbreeding, self-sustaining, population unit that does not normally outcross or exchange genes with yet other population units at any one geographical site), is valid at least as to its theoretical foundation, or else no such unit might be found in nature. Grant (1981) has discussed the biological species concept in some detail and, suffice to say, we largely subscribe to his point of view.

Fortunately, however, most humans are not concerned with semantic problems and, though not trained in taxonomy, they find no difficulty in understanding the biological concept of a species, at least in practice.

The professional biological classifier has been said to arrive at his classification through a process popularly known as the taxonomic method. Several attempts have been made to define or otherwise explain the taxonomic method, but most definitions or descriptive attempts fall short of their mark. Although most taxonomists have a fairly good idea what is meant by this method, they find it difficult to express. Flake and Turner (1968) addressed the problem as follows: