GAS CHROMATOGRAPHY AS A TOOL IN THE BIOSYSTEMATIC STUDY OF CERTAIN MEMBERS OF THE ANDROPOGONEAE (GRAMINEAE)

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PREFACE

Biosystematists are always searching for a better tool, or a combination of tools, to aid them in their task of discerning the relationships among their specimens. This thesis is an evaluation of the usefulness of a new tool in systematic research. Even though we are dealing here with chemical characteristics, we are still only measuring the phenotypic expression of genes. Until the time comes when we can unravel the DNA helix and catalog all the genes in the cells of an organism, we will have to be content to study all the phenotypic characters we can find and attempt to establish the relationships accordingly. It is hoped, therefore, that this work will be of some value to those concerned with this field of study.

In the course of this work, I have become indebted to the members of my advisory committee: Dr. J. M. J. de Wet, Associate Professor of Botany; Dr. W. W. Hansen, Professor and Chairman, Botany and Plant Pathology Department; Dr. G. W. Todd, Associate Professor of Botany; Dr. L. H. Bruneau, Associate Professor of Zoology; Dr. J. S. Brooks, Professor of Agronomy; and Dr. J. R. Harlan, Professor of Agronomy; for their guidance and encouragement.

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CHAPTER I

FUNDAMENTAL PREMISES IN CHEMICAL TAXONOMIC STUDIES

During the last decade the generic group, Bothriochloininae, was studied in detail at Oklahoma State University. This has resulted in a fairly complete understanding of relationships between the various taxa included, based on data from cytological studies, genetic studies, geographical distribution and gross morphological studies. Therefore, this group offers an excellent opportunity to evaluate the usefulness of the relatively unknown and largely untried technique of systematic study, namely the use of gas-liquid chromatography.

Early in the research work, Celarier and Harlan (1955) noted that many of the grasses being studied contained aromatic essential oils. The resulting pungency of the foliage was later used (Harlan et al., 1961) as a key morphological characteristic in designating the assumed genomic constitutions of some of these plants. A cooperative project with a group of organic chemists at Oklahoma State University laid the foundation for the study reported herein. This also resulted in the isolation and identification of two of the essential oil fractions (Zalkow, Zalkow and Brannon, 1963; Zalkow, Shaligram and Zalkow, 1964) as intermediol and neo-intermediol, both sesquiterpene alcohols.

Essential oils of grasses, notably species of Cymbopogon (Andropogoneae) have long been of commercial importance (Guenther, 1950) as scenting agents in perfumes, cosmetics, soaps, detergents, sprays, disin-

fectants and polishes. They are also used as flavoring agents in cooking and in tobacco. One of the more interesting uses is as a source of material in the production of synthetic vitamin A. Guenther (1950) and Gildemeister and Hoffman (1956) discussed the oils of several grasses not used commercially, but of chemical interest. Among these are Andropogon intermedius (= Bothriochloa intermedia), A. intermedius var. punctatus (= B. intermedia), A. kuntzeanus var. foveolata (= B. pertusa) and A. odoratus (= B. intermedia). Earlier, Parry (1921) studied the essential oil of B. ischaemum. He reported only the physical properties of the oil and concluded that it had no value as a commercial perfume oil. However, Tucakov (1957) listed this species as one of the principle aromatic plants of Yugoslavia. Besides the two sesquiterpenes studied by Zalkow, Zalkow and Brannon (1963) and Zalkow, Shaligram and Zalkow (1964), the following volatile chemicals have been identified from these grasses: the acids (either free or as esters) acetic, butyric, caproic, caprylic, enanthic and p-methoxycinnamic; the alcohols borneol, camphene, an unnamed camphene-isomer, a decyl-isomer, geraniol, limonene, octyl and pinene; a decyl aldehyde; the ketone 4-undecanone; and an unknown cadinene-type sesquiterpene. No detailed taxonomic treatment of any grass genus employing characteristics of essential oils appears extant. However some chemical data that may be useful in the taxonomy of the more commonly studied pungent grasses are summarized in Table I.

The usefulness of gas chromatography, especially in studies of essential oils, was indicated by Alston and Turner (1963). They cited the fact that gas chromatography can detect chemical components in extremely low concentrations; emphasized that the gas chromatographic technique is limited only by its practical difficulties; and that essential oil

TABLE I

COMPONENTS OF THE ESSENTIAL OILS OF ANDROPOGONEAE GRASSES[1]

Name of grass[2]	Alcohols & Acids[3]	Aldehydes & Ketones	Terpenes[4]
Cymbopogon martini var. motia	Geraniol Linaloöl Terpineol Acetic acid Capric acid	Formaldehyde Isovaleraldehyde Citronellal Citral Methyl heptenone	Dipentene Ocimene Farnesol
Cymbopogon martini var. sofia	Geraniol Perillyl	d-carvone l-carvone	Dipentene Limonene Phellandrene
Cymbopogon flexuosus	Geraniol Linaloöl Nerol Methyl heptenol	Citronellal Citrals a & b Decylaldehyde Methyl heptenone Diterpene ketone	Dipentene Limonene Myrcene Farnesol
Cymbopogon citratus	Geraniol Linaloöl Terpineol Nerol Methyl heptenol Citronellol Isopulegol Capric acid Caprylic acid Isovaleric acid Citronellic acid Geranic acid Neric acid	Isovaleraldehyde Citronellal Citrals a & b Decylaldehyde Furfural Farnesal Methyl heptenone Diacetyl ketone Acetone Dihydropseudoionone	Dipentene Myrcene Farnesol Camphorene A bicyclo-camphorene

TABLE I (Continued)

Name of grass[2]	Alcohols & Acids[3]	Aldehydes & Ketones	Terpenes[4]
Cymbopogon nardus	Geraniol Linaloöl-like cmpd. Nerol Citronellol Borneol Thujyl Methyl eugenol Acetic acid Butyric acid	Citronellal Methyl heptenone	Dipentene Limonene Camphene Farnesol Sesquicitronellene
Cymbopogon winterianus	Geraniol Citronellol Methyl eugenol Eugenol Isobutyl Isoamyl Hexenol Hexanol Methyl pentanol Chavicol Cadinol Butyric acid Citronellic acid	Isovaleraldehyde Citronellal Citral Furfural Benzaldehyde Hexenal Methyl pentanal Vanillin Methyl heptenone Methyl cyclohexanone Diacetyl ketone	Dipentene Limonene Sesquicitronellene Elemol Cadinol Cymbopol γ-cadinene δ-cadinene Dicitronelloxide
Cymbopogon flexuosus f. albescens	Terpineol Borneol Acetic acid Butyric acid		Limonene Camphene
Cymbopogon caesius	Geraniol Perillyl		Dipentene Limonene

TABLE I (Continued)

Name of grass[2]	Alcohols & Acids[3]	Aldehydes & Ketones	Terpenes[4]
Cymbopogon coloratus	Geraniol Borneol Acetic acid Propionic acid Laurinic acid	Citronellal Citral	Limonene Camphene Caryophyllene
Cymbopogon goeringii	Terpineol Borneol Elemicin		Camphene Cadinene
Cymbopogon iwarancusa	Palmitic acid Decylic acid Octylic acid	d-piperitone l-piperitone	Carene
Cymbopogon polyneuros		Perillaldehyde	Limonene
Cymbopogon procerus	Geraniol Butyric acid Propionic acid Formic acid	Trimethoxy- gallic aldehyde	Pinene
Cymbopogon rectus	Geraniol Methyl isoeugenol Caprylic acid Butyric acid Formic acid Valeric acid	Citral Methyl vanillin	Pinene

TABLE I (Continued)

Name of grass[2]	Alcohols & Acids[3]	Aldehydes & Ketones	Terpenes[4]
Cymbopogon senaarensis	Acetic acid Caprylic acid Palmitic acid Decylic acid Octylic acid	Piperitone	Limonene Pinene
Andropogon connatus	Geraniol Dihydrocuminyl		Phellandrene
Andropogon fragrans	Geraniol Linaloöl Nerol Citronellol Acetic acid Abietic acid	Citronellal Citral Cuminic aldehyde Methyl heptenone Methone	Pinene
Andropogon intermedius	Acetic acid Butyric acid		Limonene
Andropogon intermedius var. punctatus	Decyl isomer Octyl Acetic acid Caprylic acid Butyric acid Caproic acid Enanthic acid	4-undecanone	

TABLE I (Continued)

Name of grass[2]	Alcohols & Acids	Aldehydes & Ketones	Terpenes[4]
Andropogon kuntzeanus var. foveolatus	Borneol		Camphene Camphene isomer Cadinene type cmpd. Pinene
Andropogon odoratus	Geraniol Borneol Butyric acid Methoxycinnamic acid		Camphene Pinene
Andropogon schoenanthus ssp. nervatus	Perillyl		Limonene
Andropogon sp.	Geraniol	Citral	
Andropogon sp.	Cineol	Citronellal	Dipentene Limonene
Vetiveria zizanioides	Palmitic acid Benzoic acid Vetivenic acid	Vetivone Isovetivone	Cadinene isomer Various vetivenols Vetivene

[1]Compiled from: Guenther (1950); Gildemeister and Hoffman (1956).

[2]Names are listed as reported, no attempt has been made to resolve synonymy.

[3]May be free or as esters.

[4]Including monoterpenes, sesquiterpenes and diterpenes other than those listed in other columns.

analysis involves a highly refined procedure. One of the first questions that appeared in our own work involved the repeatability of the chromatograms. Would consecutive analyses of the same sample show the same peaks? The answer to this question is seen in Plate I, Fig. 1, 2. There is some slight but insignificant variation in the peaks. The next question, can the chromatogram be repeated weeks later from the same sample? To find out, the sample was stored in a refrigerator and rerun (Plate I, Fig. 3) many weeks later.

The chemical taxonomist should know whether the chemical composition of a plant changes during the growing season, if transferred from one region to another or when the environment is changed in the same area (Mirov, 1963). Carvalho (1962) found that niacin content of coffee depended greatly on whether the plants were grown in the sun or in the shade. Riley and Isbell (1963) tested Haworthia plants grown under uniform conditions to eliminate these factors. They tested different leaves of the same plant; different plants of the same species; and plants at different times of the year. Using paper chromatography, they could not detect significant differences. In the essential oils of spruce trees, von Rudloff (1962 a) found some seasonal variation in the amounts of some components, but very little significant difference in the kinds of components. This was found also to be the case when old and young leaves of naturally grown and greenhouse specimens of Thuja were examined (von Rudloff, 1962 b). The chromatogram in Plate I, Fig. 4, was taken later in the season from the same plant as the sample shown in Fig. 1, 2, 3. Again slight differences in peak heights can be noted as well as a slight reduction in retention times. This latter situation is probably due to a slight fluctuation in gas flow at the

LEGEND TO PLATE I

Reproductions of selected chromatograms. Horizontal scale: One inch = 4 min. retention time.

Figure 1. Chromatogram of Capillipedium spicigerum. Note the distinct peaks.

Figure 2. Same as Fig. 1, sequential run showing reproducibility of peaks.

Figure 3. Same as Fig. 1, 2, made several weeks later showing lack of auto-decomposition upon storage.

Figure 4. Second sample from same plants as the sample shown in Fig. 1, 2, 3, collected late in the growing season.

Figure 5. Chromatogram of Bothriochloa glabra. From sample collected in the field during the summer.

Figure 6. Second sample from same plants as the sample shown in Fig. 5, collected from the plastic greenhouse in mid-winter.

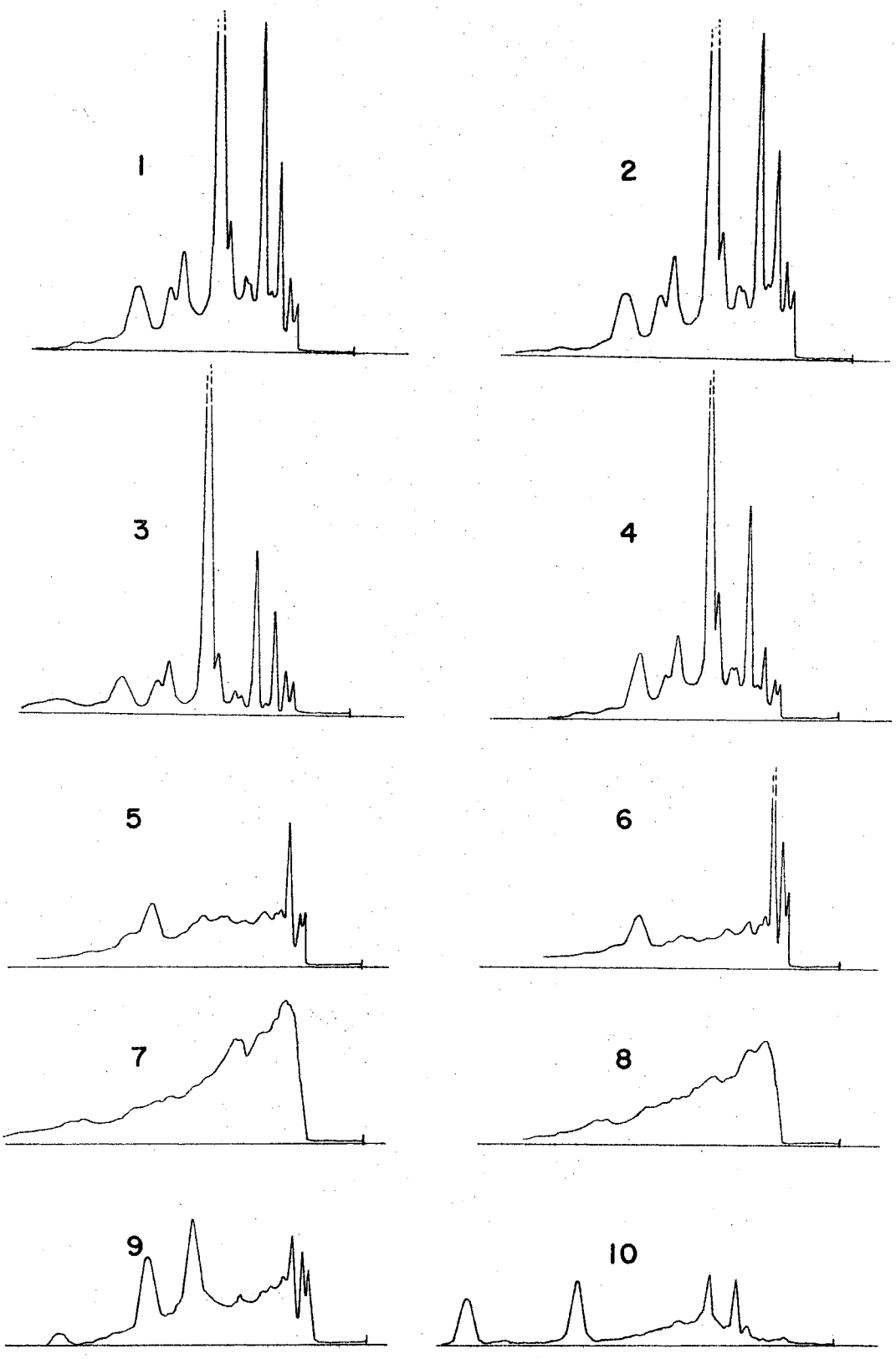
Figure 7. Chromatogram of Bothriochloa intermedia. Note tailing nature of the peaks.

Figure 8. Same as Fig. 7, after treatment with silica gel.

Figure 9. Chromatogram of Bothriochloa glabra. Sample run with butanediol succinate as the liquid phase of the column.

Figure 10. Same sample as that shown in Fig. 9, with phenyl type silicone oil as the liquid phase of the column.

PLATE I



time this chromatogram was made. However, attention should be called to the fact that the same chemicals are obviously present.

A further testimonial to the reliability of gas chromatographic determination of chemical composition may be seen by comparing the chromatograms of Plate I, Fig. 5, 6. Figure 5 represents a sample taken from the field during the summer growing season, whereas Fig. 6 represents a separate sample from the same plants during the winter; after they had been growing for some time in a plastic covered greenhouse. The component peaks are essentially the same.

Not all chromatograms obtained in this study are as nicely separated as those in the preceding figures. These chromatograms approach "brochure runs". A brochure run, according to Flynn (1964), may be one out of thousands selected by the chromatograph manufacturer to show what his instrument will do. Flynn pointed out that more often chromatograms may have, among other things, "...peaks that tail; drifting baselines" or that the "...baselines may be so flat and quiet that the operator has jiggled something to reassure himself that the instrument is operating".

The tendency to form these "tails" was particularly annoying. Issenberg and Wick (1963) analyzed an aqueous extract of banana oil and obtained a tailed chromatogram which resembled several obtained from ether extracts in our study (Plate I, Fig. 7). This evidence led to the hypothesis that perhaps water was somehow contaminating our samples. An attempt to dry the sample over silica gel resulted in the chromatogram shown in Plate I, Fig. 8. As can be seen, the tailing was not appreciably altered by this treatment. It now appears that tailing peaks are characteristic of certain kinds of plants.

Alston and Turner (1963), Alston, Mabry and Turner (1963) and von Rudloff (1963 a), among others, have pointed out that it would be extremely useful to know the exact chemical composition of spots on paper chromatograms, or of peaks on gas chromatograms. Bernhard and Wrolstad (1963) accomplished the identification of the terpene hydrocarbons of Schinus molle oil by cross reference of known and unknown fractions on three different columns. Accordingly, in our studies a second column recommended for essential oils was obtained. Correlated comparisons of chromatograms made with this column and the previous one were found to be difficult (Plate I, Fig. 9, 10) and it was discovered that separation of the chemicals was not as good on this second column as on the first. The use of the second column was soon discontinued.

In the work of von Rudloff (1963 a, b) it was pointed out that in essential oils the monoterpenes and some hydrocarbons elute from the column in the early part of the run whereas the sesquiterpenes are laggards. The same is assumed to be true for the oils of these grasses. The sesquiterpenes described by Zalkow, Zalkow and Brannon (1963) and Zalkow, Shaligram and Zalkow (1964) are known to come off after 20 to 30 minutes in our chromatograms and are therefore not included in the materials analyzed taxonomically in this research. Furthermore, Shulgin (1963) has emphasized that the cis-isomer of a stereo-isomeric pair of chemicals always precedes the trans-isomer in gas chromatography. Therefore, it is assumed that some of the detected peaks that show close overlap are such stereo-isomer combinations. While it would be advantageous to know the exact nature of the chemicals being detected, it may be pointed out that significant taxonomic inferences may be obtained without this knowledge. Recent examples are shown by the works of Alston,

Mabry and Turner (1963) and Stebbins et al. (1963).

Another question concerning the components of essential oils involves what happens to them when they are chromatographed. Von Rudloff (1961) believed, as did Jahnsen (1962), that the components were easily isomerized, autoxidized, polymerized and dehydrated. However, later, von Rudloff (1963 b) was able to show that in gas chromatography, less destruction of chemicals occurred than by the fractional distillation method of analysis. Since in so far as possible, all samples in this study were treated alike (reflux-distilled) it is believed that if any chemical change occurred it would have done so universally and therefore should not greatly affect the usefulness of these data.

The Bothriochloininae are characterized by a high degree of polyploidy, and Löve and Löve (1957) noted that drug content in Acorus was higher in the polyploids than in the basic diploids. Dent and Aldrich (1963) found that tetraploid ryegrass consistently had a higher soluble sugar content than diploids. How, then, might polyploidy be expected to affect the chromatograms in this study? In the first place it may be pointed out that the above authors were working with cases of autopolyploidy; whereas the ploidy mechanism in the Bothriochloininae is primarily allopolyploidy. Therefore we would expect examples of complementation, as defined by Alston and Turner (1963), or anomalous situations, in which completely new chemicals arise in hybrid offspring, like that described by Harney and Grant (1963; 1964) to be more frequent in natural polyploids.

No standardized procedure for the presentation of chromatographic data appears extant. Two dimensional paper chromatograms, sometimes called "fingerprints" are often pictured. Papers dealing with gas

chromatography often feature reproductions of chromatograms, however it is sometimes difficult for an inexperienced reader to interpret the chromatograms. In the following chapters on chemical taxonomy, the data are presented in tabular form, and by the reproduction of some of the chromatograms. In addition, the data have been calculated as percent-of-total-chemicals and plotted on a polygonal graph, such as the one presented in Plate II, to yield "profiles" which are more easily compared than the chromatograms.

Conclusions

1. Gas chromatography can be useful in taxonomic studies, since it is a technique that requires less material for detection than other methods of chemical analysis.

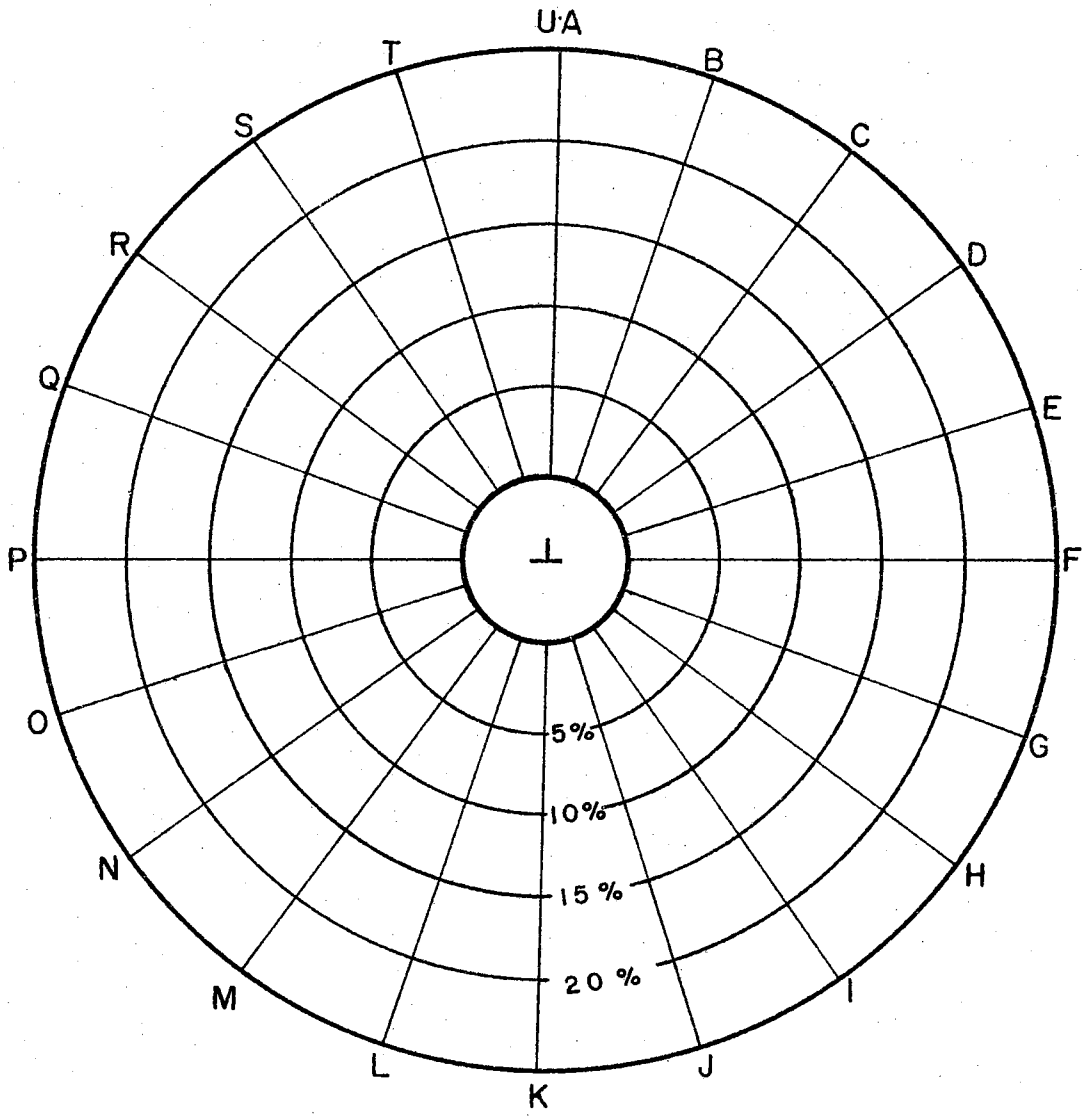
2. Chromatograms are reproducible from the same sample and from the same specimens at different times; therefore, it appears to be a reliable technique for use in a taxonomic study of the Bothriochloininae.

3. Changes in environment do not appear to seriously affect the kinds of chemicals in the essential oils of grasses belonging to the Bothriochloininae.

4. Inherent difficulties in interpreting some chromatograms may be due to the presence of chemicals characteristic of certain species of these grasses.

5. Useful taxonomic inferences are expected even though the exact nature of the chemicals is unknown and may even have been altered in the process of chromatography.

PLATE II



LEGEND TO PLATE II

Polygonal graph of the type used in making chemical profiles. Percentages shown are percent-of-total chemicals. The radii are lettered in the sequence the 21 chemicals elute from the chromatograph.

CHAPTER II

CHEMICAL PLANT TAXONOMY

More than twenty years ago, in a centennial tribute to Rafinesque, Haag (1941) stated: "We have fairly well classified botanically the plants which inhabit our world; relatively their chemistry and particularly their pharmacology have been ignored." More recently, Bonner (1963) suggested that botany is in danger of becoming a senescent science and that taxonomy is one of the most depleted disciplines within the field. We must agree, in part, with these authors that biochemistry is an important adjunct to classical botany, but it should never be allowed to supercede the latter entirely. This was the sentiment expressed in the majority of the answers to Bonner's paper. Laetsch (1963) said in effect, if the "classical" is allowed to die, who will tell the "moderns" what to work on, what they are working with and whether their results are biologically valid? Shetler (1963) and Smith (1964) presented the viewpoint that only a small percentage of the world's flora has been adequately explored, and that when attempts to form evolutionary systems are considered, the field has hardly been touched. These viewpoints were reiterated by Zuck (1964), and the statement of Smith (1964) that "...recent advances in the experimental fields have given taxonomy a new lease on life", is a valid one.

The first chapter of Stebbins (1950) emphasized that taxonomists interested in the evolutionary aspects of their subjects must use data

from many sources. There are three major objectives of plant taxonomy. First, the analytical or recognition objective, which embodies the accurate description of the floristic elements. Second, the synthetic or classification objective, wherein these elements are placed in some meaningful system. And third, the experimental or evolutionary objective, which is the determination of how well the system reflects the natural relationship of the floristic elements. It is the inaccuracies and the inability to prevent inaccuracies in the determination of evolutionary sequence which dictates the use of data from as many sources as possible.

Early History of Chemical Plant Taxonomy

Plants are usually classified on the basis of phenotypic continuities and discontinuities of morphological traits. A new tool is now available which is giving new insight into taxonomic problems. That tool is biochemistry. Actually the use of chemical data in taxonomy is nct new. According to Plouvier (1963), A. N. Monteverde in 1893 examined 797 species in 199 genera of the Scrophulariaceae with the aid of a simple test for the presence of mannitol and dulcitol, and suggested certain taxonomic revisions on the basis of his findings. Because of the scope of his work and the conclusions he made, Plouvier has claimed for Monteverde the title of "father of chemical taxonomy".

On the other hand, Bate-Smith (1962) gave credit to one James Petiver, who in 1699 wrote on "Some attempts made to prove that herbs of the same Make or Class for the generality, have the like Vertue and Tendency to work the same Effects." In his excellent review of the history of chemical taxonomy, Gibbs (1963) recognized Petiver, but also

mentioned that similar concepts were held by several early herbalists, notably Nehemiah Grew in 1673 and Rudolph Jacob Camerarius in 1699. The idea was next borrowed by A. P. de Candolle in 1804 and 1816 in some of his publications on medicinal plants. Gibbs stated that these ideas next appeared in England in the writings of John Lindley in 1850-1853 primarily by his translations of de Candolle's works. Thus, it seems there was an earlier recognition of the importance of chemical characters even though not a great amount of work of taxonomic import seems to have been done. The statement attributed by Gibbs to Helen C. de S. Abbott in 1886-1887 seems prophetic today: "There has been comparatively little study of the chemical principles of plants from a purely botanical view. It promises to become a new field of research."

Around the end of the 19th century there seems to have been a slight revival of interest in chemical taxonomy from the tropical garden at Buitzenzorg, Java in the writings of J. F. Eykman, P. van Romburgh, Melchior Treub, A. W. K. de Jong and R. A. Gorter (Gibbs, 1963). Mention should also be made of the work by Baker and Smith (1902) on the taxonomy of Eucalyptus, based largely on their essential oils. M. Greshoff in 1909 wrote a paper entitled "Phytochemical investigations of Kew" in which he pleaded that every description of a new genus or species should include a short chemical description of the plant (Gibbs, 1954). Guenther (1950) pointed out how Stapf was unable to detect any morphological difference in specimens of Cymbopogon martini, varieties motia and sofia, and how I. H. Burkill in 1909 was able to detect differences in the essential oils from these two grasses.

Wheldale (1911) expressed the hope that biochemical characters would some day shed light on the relationships in the plant kingdom.

Hegnauer (1961)was not able to substantiate the contention of Hallier in 1913 that possession of cyanogenetic substances would be an indicator of phylogenetic lines. However, Hegnauer did express the opinion that at the level of arrangement of certain families in orders, certain tribes in families, certain genera in tribes and certain species in genera, these data could be useful. One of the most extensive works of this time period dealing with chemical aspects of taxonomy is that of Reichert (1919) in which the configuration of starch grains were used for taxonomic confirmation of numerous plants. Earlier, Harz (1880), in a paper apparently overlooked by recent reviewers, on the basis of starch grain ontogeny presented a classification system for the family Gramineae that differs very little from present day systems based on cytogenetics and anatomy.

If the title of "father of chemical taxonomy" should go to Monteverde for the scope of his work, then the title of "God-father of chemical taxonomy" should go to McNair for his patient persistence. McNair (1916) started his interest by refuting the idea that certain chemicals (glucosides of fisetin, rhamnose and gallic acid) were the poisonous principles of poison ivy since poison oak did not contain these compounds, whereas two non-poisonous species of Rhus did. This argument gradually evolved (McNair, 1917) into the idea that saturated fats in plants are more primitive than unsaturated fats. By 1929, he had extended the idea that marine (therefore, primitive) animals had oils with lower melting points than terrestrial (advanced) animals, to a concept that tropical plants should contain oils with higher melting points than the plant oils of temperate climates. In McNair (1930 a, b) this concept was amplified to include gums, tannins, resins and starches.

Plant waxes were added (1931) and the addition of volatile oils, saponins, cyanogenetic glucosides and carbohydrates came later (1932). McNair (1934) summarized the available data and concluded with the hypothesis that temperate plants were primitive in nature and tropical ones more advanced. It is interesting to note that McNair was assimilating the papers of organic chemists newly aware of plant compounds and attempting to organize the data of the various authors' along taxonomical and ecological viewpoints.

The conclusions reached by McNair (1935 a, b) are the ones most often criticized by present day reviewers (Alston and Turner, 1963; Gibbs, 1963). McNair tried to show chemical support for the system of Engler and Prantl over Bessey's system. He proposed that woody plants were the most primitive (i.e., they had the simplest chemical compounds) and that they gave rise to shrubs and these, in turn, gave rise to herbaceous plants. The monocotyledonous plants were supposed to be more primitive than the dicotyledonous ones; but polypetaly was supposedly more primitive than gamopetaly; polycarpy more primitive than oligocarpy; apocarpy more primitive than syncarpy. In this way he supported some of the dicta of Bessey while refuting others.

McNair (1941, 1942) expressed the almost-Lamarckian idea that plants "...which do the hardest (most difficult) work have evolved to the highest positions". Therefore, plants which have the most complicated chemical compounds (and presumably work harder to make them) are the most highly evolved ones. To support this hypothesis, he cited the work of Baker and Smith (1902) and concluded that "Morphological and chemical phylogeny have their counterparts in ontogeny". His paper of 1945 is essentially a summary of all the previous ones in which it is often

implied, but never directly stated, that all chemical compounds result from the same process or processes and represent a progression from simple to complex products of the same reaction or reaction types. Thus, carbohydrates can be converted to saturated fatty oils and these, in turn, to highly unsaturated oils. It is further implied that environmental factors coupled in a limited way with hereditary factors affect the equilibrium of these reactions to cause a particular chemical compound to accumulate in any plant.

McNair is primarily criticized for allowing his enthusiasm for chemical taxonomy to lead him to some rather sweeping conclusions. Mirov (1963) defended McNair on the basis that it was not the latter's fault that the information available to him from the research data of botanists and chemists was often fragmentary and contradictory. Mirov stated that although some criticism seemed justified, basically McNair's "...conclusions still seem to be correct". An example of how sketchy this information sometimes was, is found in Wheldale (1911) in which she stated that the very important nucleic acid component, adenine, is found only in the genus Thea. Recently, Willaman and Schubert (1961) published a rather large catalog of plants known to possess alkaloids. Willaman and Li (1963) then analysed a 10% sample of these 3600 plants and their 2000 alkaloids to determine familial distribution and size of alkaloids. These data were correlated with habit, habitat and geographic location of the plants. They found that woody species from the temperate zone have larger alkaloids than herbaceous, temperate species; in fact, all woody species had larger alkaloids than all herbaceous ones. This, in part, vindicates McNair's claim. However, they did find that of the herbaceous species the temperate zone monocotyledonous ones have much

larger alkaloids than temperate dicotyledonous ones, while the tropical dicots have larger alkaloid molecules than the tropical monocots.

Blackman (1921) reviewed work that had been done in the exploration of the different carbohydrates, and stated that such information might be of importance taxonomically. Weevers (1943) pointed out that the same chemical compounds are often known to occur in widely separated plant groups. He felt, therefore, that caution must be used in drawing conclusions, since such cases of parallel evolution would negate broad generalizations. This cautious concern is still very much present in current research reports.

One of the primary reasons chemical taxonomy did not advance rapidly in the first half of the 20th century, was the difficulty encountered in detecting the various chemical compounds. Tests that had to be made were often long and tedious and required a trained chemist to perform them. Mirov (1963) suggested that botanists and chemists do not always understand each other or the objectives each is trying to attain. The chemist is often not sure of the identification of the plant materials he is working with and is usually unconcerned with this lack of knowledge. The botanist on the other hand, is not always concerned about the identification of the chemical compounds he is working with. Various techniques have been devised for use in chemical taxonomy.

Phytoserological Technique

This aspect of chemical taxonomy was adequately reviewed by Chester (1937). Essentially the technique consisted of the injection of test substances into an animal. These substances, being mostly foreign proteins, acted as an antigen in the stimulation of antibody formation.

The antibody-containing serum was then agglutinated with test substances from plants which were presumed to be closely related to the original antigens. The more agglutination that occurred the closer the presumed relationship of the tested substances and therefore of the plants which produced them. Chester pointed out that the technique was not a simple one, as it had to be skillfully used and the reactions had to be rigidly controlled.

Perhaps this is the reason phytoserology did not become important in America. Davidson and Thompson (1956) used the serological technique to study the geneology of certain corns of known ancestry. They found a very good correlation with the known ancestry except that they were unable to differentiate between popcorn and one of the dent corn hybrids. Fairbrothers and Johnson (1961) found that grass proteins do not readily cause antibody formation in rabbits and chickens. This may partially explain the results of Davidson and Thompson. Likewise a serological study by Masłowski (1962) on corn showed that there were no detectable differences in the endosperm of several varieties of corn. Davidson and Thompson, suspecting this to be the case, used only the young plant. Masłowski, too, was able to find varietal differences when the latter technique was used.

Although there appears to be little use of phytoserology in America at present, there is an active group in Czechoslovakia pursuing this area with a new addition to the technique. This is the use of electrophoresis to separate the proteinaceous fractions of the sera before making the agglutinin test, as fully described by Gysels (1963). Kloz (1962) and Kloz and Turková (1963) have thus found that in the Leguminosae, the tribe Vicieae is characterized by the presence of leguminoid and

vicilinoid proteins; tribe Trifolieae by vicilin-like proteins only; whereas tribes Genisteae and Phaseoleae lack both of these classes of proteins. The latter tribe on the other hand contains phaseolinoid proteins. In a similar study, Hall and Johnson (1962) were able to detect the parental proteins in an amphiploid hybrid of Stipa viridula and Oryzopsis hymenoides.

Chromatographical Techniques

Classical taxonomy of the lower plants, particularly bacteria and fungi has largely been based on intangible concepts, such as edibility of the fungi and color reactions to certain tests for the bacteria. Higher plants have been classified primarily on the basis of tangible morphological characters (Walters, 1963). The ideal system of classification would be one based on a complete understanding of the geneology (Swain, 1963). Mirov (1963) and Zuck (1964) believed that the very essence of nature will prevent us from attaining this perfect stage. Gibbs (1954) optimistically predicted that the goal of a "real phylogeny" might be reached chemically. In spite of some other optimistic expressions, biochemistry has been slow to take root in the field of taxonomy. Only in the last ten years has there been a concerted effort toward this end. According to Swain (1963), this has been caused by an awakened interest on the part of botanists in the chemical products of plants, coupled with gross advances made in the development of new analytical tools and techniques in the field of organic chemistry. Chromatography is one of these techniques.

The first known use of chromatography for the detection of a chemical compound, according to Block, Durrum and Zweig (1958), was when

Pliny the Elder, first century A. D., described the use of papyrus impregnated with an extract of gall nuts to detect ferrous sulfate. However, paper chromatography was not to find its place in the chemical world until 1850 when a German dye chemist, F. F. Runge, used it in a separation process (Dal Nogare and Juvet, 1962). In 1906, the botanist, Tswett, used a column to separate plant pigments, notably the chlorophylls and associated pigments. This was the first time the term "chromatography" was used.

Paper Chromatography--Paper Chromatography, as it is practised today, consists of several closely related techniques. Essentially, all involve placing the material to be separated as a spot on filter paper and allowing an organic solvent or mixture of solvents (e.g., the ones described by Blundstone, 1963) to pass over the spot. The mixed compounds are separated by their varying affinities for the solvent used. The filter paper may be cut in strips, for strip chromatography with either an ascending or descending solvent, or it may be used in sheets where the material is resolved in one direction, dried, then resolved in a second direction with a different solvent. This two-dimension chromatogram usually gives better resolution of the separated compounds. The chromatographed substances, which are not always as "colored" as the name implies, are detected by various techniques depending on the type of chemicals being examined. Ultraviolet light, ninhydrin or other indicators, such as the one described by Vincent (1962) may be used. It is well to note that these techniques do not determine the exact chemical identity of the substances; they merely detect spots. Various appropriate organic chemical analytical techniques must be used for identification.

A vast amount of work of a taxonomic nature has been done during the last decade utilizing chromatography. Hagen (1960) was confident that paper chromatographic studies would become an important part of taxonomy. When considered along with morphological data, he believed that a truer picture of evolutionary relationship would be the result, since not only the degree of relationship, but the direction evolution had taken, could be determined. The latter concept comes from the idea that each step in the biosynthesis of the detectable compounds, except for a few spontaneous reactions, is under enzymic, and thus genetic, control (Birch, 1963). As expressed by Nowacki (1963), the use of the "Neurospora" method would show which chemicals are progenitors of others and therefore which plants and species of higher categories were relatively primitive or relatively advanced. In this connection, Pecket (1960 a, b) believed that the anthocyanin content of leaves in Lathyrus would show a better phylogenetic picture than those of the flower. His reasoning appears to be that there would be less selection by insects for leaf compounds than flower compounds, or in other words, the study of flower pigments would tend to make the plants of this genus appear more closely related than they were in fact. Several authors, including Alston and Turner (1963), Birch (1963), Price (1963) and Alston and Irwin (1961), warned that it is best to use "secondary" plant substances for analysis. Secondary substances are defined as those for which no "practical" or physiological function has been found; they seem to be simply accumulated by-products, perhaps even waste products, of plant metabolism.

In a study of 19 species of nine genera from the Saxifragaceae, Kindl and Billek (1962) discovered a new organic acid and noted that it

was characteristic of the genus Astilbe. The authors suggested that a further study might have important taxonomic implications. They used thin-layer chromatography, which is essentially the same as paper chromatography except that a layer of non-reactive substance, such as calcium sulphate, is thinly coated on glass. This allows the use of paper-destructive indicators.

One group that has been very active in the field of paper chromatographic studies is Alston and Turner, and their students. Indeed their book, Alston and Turner (1963), is largely an outgrowth of their own work. In 1962, while working on the legume genus Baptisia, Alston and Turner showed, that what appeared to be random introgression of three species from a morphological standpoint, was distinguishable chemically as one species hybridizing with the other two and further that the hybrids of any two did not backcross to the third. At the same time, Alston and Simmons (1962) found that hybrids of two of the species did not contain species specific substances in the leaves, whereas a hybrid with a third species did. They reasoned that the hybrid which contained the compounds represented a cross between closer related species than the other case, even though morphologically the resemblance was stronger between the parents of the chemically deficient hybrid. In still another hybrid complex of Baptisia, Alston et al. (1962) regarded the chemical data as more reliable criteria for the establishment of phylogenetic affinities than morphological data. Earlier work of a more general nature indicated to Birdsong, Alston and Turner (1960) that the Papilionoideae tribe of legumes may have given rise to the tribes Mimosoideae and Caesalpinioideae. Members of the latter two were found to completely lack the substance, canavanine.

Bate-Smith (1958) found a correlation between certain leuco-anthocyanins (phenolic compounds) and the occurrence of lignification. Methoxycinnamic acid was associated with herbaceousness. In the genus Iris, he found that distribution of phenolic compounds closely followed the usually accepted taxonomic treatment. This was somewhat substantiated by Riley and Bryant (1961) who were able, in a preliminary study, to identify nine species of other genera in the Iridaceae by their chemical composition. In the Rosaceae, Bate-Smith (1961) found general agreement of the accepted taxonomic treatment of Potentilla and Prunus and their chemical constituents.

Dreiding (1961) studied the red pigments of the families belonging to the Centrospermae and determined that the possession of a nitrogenous compound (betacyanin) was fairly characteristic of the group. Only the Telygonaceae, for which no red pigment has been found, and the Caryophyllaceae do not have betacyanins. The latter is characterized by the possession of anthocyanin red pigments, commonly found in other groups of families. On the basis of this information, Harborne (1963) suggested that the Caryophyllaceae should be removed from the Centrospermae. He also mentioned that the presence of betacyanins helped to secure the Cactaceae its placement with this group of families.

According to Pecket and Selim (1962), the blueness of the flowers of Lathyrus was thought to be subject to modification by internal pH changes. They discovered on the other hand, from hybrid studies, that the blue anthocyanin pigment could be reinforced by the presence of a flavonol co-pigment. Przybylska and Nowacki (1961) analyzed the free amino acids in the same genus and concluded that a careful examination would help to eliminate the largely artificial classification of Lathyrus.

Paper chromatography has been used to study chemical components other than anthocyanins and other phenolic compounds. A preliminary study by Alston and Irwin (1961) on the free amino acids in a few species from different sections of the genus Cassia resulted in their suggestion that further work might be fruitful in casting light on phyletic relationships. Hillis and Orman (1962) studied correlations in the wood anatomy and chemical extracts of species of Nothofagus and concluded that chemical composition generally reflected accepted taxonomy. They proposed that chemical evidence alone would be insufficient to warrant a change in taxonomy, but when this kind of evidence could be correlated with data from other sources, then it ought to be considered.

Alston and Turner (1963) discussed the concept of "complementation", that is, when two chemically different entities hybridize, the effect is usually additive in the hybrid. Therefore, the parental chemicals should be recognizable in the offspring. Smith and Levin (1963) felt that they could adequately demonstrate this in the ferns they studied. Stebbins et al. (1963) were able to show morphological correlation in a case of complementation after it had been detected chemically, in a study of Viola. In species of Nicotiana, Smith and Abashian (1963) found nine alkaloids which seemed to be specific for the genus. They also found examples of complementation among hybrids, but they felt their data for the most part were too randomized to show evolutionary lines in the genus. Some workers were not able to find examples of complementation, even when it had been expected. Mikołajczyk and Nowacki (1961) believed the alkaloid constituents of Lupinus were more affected by environmental factors and perhaps modifying genes, than by direct genetic control. Clausen (1962, 1963) was able to detect the

backcross origins of only four hybrid birches, the others varied in such an extent as to cause some doubts of the usefulness of this technique for determining the hybrid origin of these plants.

Perhaps not all data obtained from phytochemistry are as useful in taxonomy as those mentioned above. Douglas et al. (1964) found two morphologically distinguishable species to be chemically identical in Heimia when their alkaloid compounds were compared. They admitted, however, that the morphological distinction is so small that it has been suggested that Heimia is in reality a monotypic genus. Alkaloids, in general, seem to lack usefulness as indicators of phylogeny, despite the fact that they are widely distributed. Perhaps it is this wide distribution that causes some of the difficulty. Rowson (1958) noted that the occurrence of identical alkaloids or groups of closely related alkaloids in widely separated plant taxa elicits a note of caution in the interpretation of these data. Still, Kupchan, Zimmerman and Afonso (1961), Raffauf (1962) and Preiniger et al. (1962) believed that the study of plant alkaloids could aid in the discrimination of species and the development of phyletic concepts.

Although Enslin and Rehm (1958) have used chromatography extensively in their study of the bitterness of cucurbits, they deny that the distribution of the bitter principles offer any taxonomic promise. Yet, in their data they indicated, without comment on possible evolutionary implications, that the genus Citrullus has bitter principles in the form of glycosides, the genus Cucumis has free bitter principles, while in Acanthosicyos the bitter principles are free in the fruit and combined in the root.

The preliminary results of Stafford (1959), on the distribution of

tartaric acid in angiosperm leaves, led to the later (1961) publication of a study of this compound in the Geraniaceae. These results could not be correlated with classical taxonomical treatments, nor was she able to show a correlation with morphological characters. Yet, she maintained that any revision of the family should take chemical data into consideration.

Gas Chromatography--The amount of research employing paper chromatography has increased in recent years and will no doubt be expanded further. However, still another type of chromatography is beginning to have some influence on this field. This is the technique of gas-liquid partition chromatography, otherwise known simply as gas chromatography. The theory for this device was mentioned by Martin and Synge (1941) in their paper explaining the work for which they later received the Nobel Prize. This theory was not put to use, however, until 1952, when James and Martin used an apparatus based on it. Basically the mechanics of this device (fully discussed by Keller, 1961) consist of passing vaporized test samples in a stream of gas over a stationary liquid phase which retains the vapor differentially and thus separates the components.

The gas chromatograph acquired essentially its present form when Ray (1954) added a thermal conductivity detector unit. This unit detects differences in the ability of various chemicals to conduct heat and registers these differences on a moving chart as peaks. The addition of the thermal detector increased the usefulness of the machine, especially for plant taxonomic purposes, since it increased the range of types of materials that could be accomodated.

Only three major groups of workers appear to have used this method of analysis extensively for taxonomic studies. Two of these are located

in New Zealand and the third is in Canada. In Canada, von Rudloff has analyzed the volatile oils of a few species and genera of Gymnosperms, including Thuja (1961, 1962 b), Picea (1962 a) and Juniperus (1963 b). In addition, one Compositae species, Tanacetum vulgare (1963 a), has been studied. These projects have been aimed primarily at the identification of the components of the oils, and taxonomic potentials are mostly co-incidental.

A more completely taxonomic work is found in that of Bannister and co-workers in New Zealand. Using the terpene fraction of Pinus oils, Bannister, Brewerton and McDonald (1959) tested artificial and natural hybrids and found a general correlation of the chemical and morphological data. In 1962, Williams and Bannister found characteristic chemical constituents of all species tested and noted chemical verification of what appears to be a varietal form of Pinus muricata, which had been suggested previously from morphological studies. However, although Pinus radiata from California is supposed to have been modified by introgression with P. attenuata, Bannister et al. (1962) could find no chemical support for the hypothesis. Several populations of Pinus radiata in New Zealand and California were studied chemically. A statistical analysis of the data showed a significant difference in the California populations, but not in the New Zealand ones. This supports Mirov's (1961) conclusions that geographic distribution of pine species could be correlated with their chemical content. Mirov pointed out that chemical information alone was insufficient for solving taxonomic problems, but that it could help in a number of cases.

The other research group in New Zealand worked primarily with the leaf waxes of a large number of New Zealand plants belonging to various

groups. These papers, Eglinton et al. (1962 a, b), Eglinton, Hamilton and Martin-Smith (1962), and Eglinton and Hamilton (1963), were preliminary studies but the authors expressed the idea that with further study, taxonomic clarification would result.

Gas chromatography is being used in another area closely allied with taxonomy, or at least having implications which could become important for taxonomy, and that is in food chemistry. The problem here is the detection of adulterants in the natural oils used in foods or in medicine. Swift (1961) was able to detect natural adulterants in orange juice from the crushed skins of the oranges. Slater (1963) used gas chromatography to detect un-natural dilutants in commercial lemon oil used by the candy, soft drink and related industries. Cocoa beans were studied by Bailey et al. (1962) who found they could detect differences in the volatile components of five different cultivars. Similarly, Smith and Levi (1961) were able to detect differences between peppermint oils of different species and also between the commercial oils of plants grown in different countries. In fact, they could determine the country of origin by observing the chromatogram of a particular oil. Differences were also demonstrated by Smith, Skakum and Levi (1963) in closely related spearmint plants.

An interesting outgrowth of this type of investigation was the work of Martin, Smith and Farmilo (1961) in which an attempt to determine the country of origin of Cannabis narcotics was made. They found differences in the fresh leaf oils of Canadian Cannabis and also in the dried extracts of oils from Egypt (called "hashish") and from India (called "charas"). Research of this nature should find adequate support from international police organizations.

General Considerations

Chemical taxonomy, from an overall point of view, is encouraging, even exciting. The enthusiasm for its study appears to be running high. Just how important a contribution chemistry will make to this field may depend on the follow-up work on several recently reported studies. These, for the most part, represent reports on studies in which the workers "became carried away" with their enthusiasm and randomly sampled a widely diverse group of plants and then sadly summarized the paper with the regret that they did not take, or could not have taken a closer look. Garrick and Habermann (1962) studied a single water soluble pigment in a large number of plants and could only conclude that it may have phylogenetic significance. In a similar study, Earle and Jones (1962) found similar results from a survey of ash, protein, oil, starch, alkaloid and tannin contents of 1,418 seed samples representing 113 plant families. The reviews of Kjaer, Paris, Plouvier, Price, Shorland and Sørensen in Swain (1963) universally plead for additional work on sulphur compounds, glycosides, aliphatic compounds, alkaloids, fatty acids and acetylenic compounds, respectively.

Asahima and Shibata (1954) did a comprehensive survey of the chemical constituents of lichens. They did not, however, alleviate all the taxonomic problems of lichens. Hale (1963) pointed out that different chemical strains are sometimes recognized as separate species, and sometimes not. Some lichenologists would reduce all chemical strains to "subspecies" and while this would create a certain uniformity of treatment, it would, at the same time, eliminate some rather well-known and acceptable species. Hale did not pretend to have the answer for these

problems, but suggested that taxonomists should at least consider all the chemical, morphological, physiological, ecological and distributional correlations possible.

Frustrating results are sometimes obtained from the study of too little material, as well as too much. Thus, Kessler and Soeder (1962), while separating mixed cultures of Chlorella based on chemical characters, were able to detect an unsuspected chemical intermediate between two of the species. They did not decide whether the new form was a hybrid, subspecies of one or the other, or a new species, but this led them to believe that a chemical taxonomic study of the entire section Euchlorella would be profitable. A recent preliminary study of a few species belonging to the tribes Asphodeleae and Aloineae (Liliaceae) by van Oudtshoorn (1964) resulted in the conclusion that chemical characters could be useful. Indications were that some recently proposed revisions of these tribes could be strengthened by the inclusion of a chemical study. Taxonomic problems in Coprosma (Rubiaceae) could be aided in their resolution, according to Taylor (1964), by the introduction of chemical data. This conclusion was based on his preliminary investigation of a supposed hybrid group.

One big and very real problem facing the chemical taxonomist involves the question of reliability of the information gained. Possibly one outstanding contribution of McNair's works stems from the caution in evidence in the writings of almost all present researchers. There is, by and large, a feeling that chemical data cannot be used alone, but that there must be a correlation with other equally convincing evidence. Heslop-Harrison (1963) has shown in tabular form the questions the chemical researcher must ask himself before he can begin to evaluate his

results. Unrelated plants may have identical chemicals, therefore the mere presence of a certain chemical cannot always be construed as evidence of affinity (Erdtman, 1963; Ibrahim, Towers and Gibbs, 1962; Price, 1963). Squalene, for instance, one of the components of shark liver oil, has now been reported from olive oil, carrot roots, a mold (Phycomyces blakesleeanus) and leaves of Acanthus, alfalfa, carrot, elderberry, lettuce and olive (Alam, Brossard and Mackinney, 1962). On the other hand, the different parts of a plant may contain different compounds (Fowden, 1962; Davies, Ashton and Borrill, 1962), and environmental, as well as growth factors, may affect the amount and kind of chemicals present. Davies, Ashton and Borrill (1962) found seasonal fluctuation in content of coumarin and related compounds in Anthoxanthum. Essential oil content fluctuated on a diurnal basis in Salvia but not in Pinus as indicated by Flück (1963). He also pointed out that as a result of these precautions, reports of an extremely exceptional lack of a compound should be critically treated until the techniques under which the information was gained are known. Mika (1962) found that alkaloid content in Blakeslee's Datura material appeared to be affected by environmental factors. Yet, Riley and Hopkins (1962) noted that chromatograms for different leaves on the same plant and at different times of the year were so nearly identical that any variation was considered unimportant, in the genus Haworthia.

This lack of uniformity from group to group lends credibility to the statements of Bate-Smith (1962, 1963), Hegnauer (1963 a), Erdtman (1963), Birch (1963) and Price (1963), that to get to the heart of the problem and fully understand it, we must know and understand something of the biosynthetic pathways involved in the formation of these substances.

This is undoubtedly a very desirable objective, but it limits us too, since it will be some future time before such a goal will be within reach. Meanwhile, taxonomists will continue to use what information is available in attempts (feeble though they may seem) to work out problems of classification and phylogeny. The limitations of chemical or perhaps morphological data were pointed out by Rangaswami (1962) who listed three examples consisting of plants, at one time considered separate species, which have now been reduced to synonyms but which are chemically distinct.

Genetic studies have long been used in determining phylogenetic affinities and several interesting ones from a chemical standpoint are extant. Goplen, Greenshields and Baenziger (1957) showed from crosses of "free-coumarin" clover with "bound-coumarin" plants that the bound condition was a simple recessive trait. When high-coumarin plants were crossed with coumarin-deficient plants, high-coumarin content was found to be incompletely dominant. Shimizu and Ikeda (1962) favored the reduction of two mint species to varietal rank of a third, based on morphological similarities and on the chemical-genetical information that their different oil composition was due to interaction of two non-allelic genes. The inheritance of anthocyanin pigments in Torenia are also produced by two sets of interacting genes (Endo, 1962). One appeared to be completely dominant, the other was incompletely dominant.

It is not unlikely that an occasional taxonomic unit may be delimited on the basis of chemistry alone, in spite of the current trend for caution and despite the plea of Hara (1962) that the taxonomy should not be changed unless there is sufficient morphological variation correlated with the other differences detected in phylogenetic studies. Further,

as pointed out above, only when biogenetic pathways have been discovered fully, will chemistry provide a major key to interpret the direction evolutionary forces have taken in the past and are taking at present. We cannot fully agree, therefore, with Sokal and Sneath (1963) and their statement (page 280) that numerical taxonomy alone "...will relate genotype to phenotype and will measure the degree, rate, and direction of evolution." We may use computers for working out correlations, but some of the data put into the machine, we believe, should originate from chemical plant taxonomy.

Before closing this section, mention should be made of several recent books on chemical taxonomy. The first to be published was that of Alston and Turner (1963). This is a book that must be read by all workers in this field. However, the numerous mistakes and printing errors in it somewhat limit its usefulness. Recently released copies have a list of 17 corrections, mostly of chemical structures, appended to it, and we have found almost 50 additional errors, mostly in citations of the research papers reviewed.

The symposium edited by Swain (1963) is a much more readable book and the fact that each contributer was well versed in his area makes the work much more useful than the preceding. Printing and citation errors seem to be almost totally absent from this work.

Finally, the series of volumes by Hegnauer (1962, 1963 b) promises to become a standard reference in the field. As he points out in his preface to volume one, almost no taxonomic treatments are to be found in chemistry laboratories, and only a little phytochemical literature is likely to find its way into the herbarium. If we may judge from the first two volumes, his work should help to alleviate this deficiency.

CHAPTER III

CYTOGEOGRAPHY OF BOTHRIOCHLOA, CAPILLIPEDIUM AND DICHANTHIUM

The genera Bothriochloa O. Kuntze, Capillipedium Stapf and Dichanthium Willemet were treated as part of Andropogon L. by Hackel (1889) and Hitchcock (1935). However, Stapf (1917) pointed out that Andropogon could more naturally be subdivided into a number of distinct genera. On the basis of morphological similarities, Gardner (1952) and Roberty (1960) combined Dichanthium and Bothriochloa, while Ohwi (1947) demonstrated that Capillipedium could be united with Bothriochloa. Genetically, Andropogon is isolated from the others and Capillipedium and Dichanthium are isolated from each other, but some members of Bothriochloa hybridize in nature with representatives of both Capillipedium and Dichanthium (Harlan et al., 1961).

This illustrates some of the taxonomic confusion characteristic of this group. Further complications are added by the fact that plants of the taxa included within these genera are highly variable cytologically and morphologically. A large number of them are polyploid and they exhibit varying degrees of apomixis. Heslop-Harrison (1963) indicated that in such cases, accurate delimitation of species is sometimes impossible. He suggested that the sexually reproducing forms and some of the strictly apomictic ones should be classified as species and the rest treated as an aggregate group. A study of chromosome number, hybridi-

zation range, gross morphology and distribution was made in an effort to better understand the relationships between these genera.

Material and Methods

Morphological studies are based on herbarium specimens of plants grown in a uniform nursery (Celarier and Harlan, 1956) and are filed with the Oklahoma State University. Artificial hybrids were produced by W. L. Richardson, using his technique (1958). For cytological studies, microsporocytes were stained with aceto-carmine.

Observations and Discussion

The genera Bothriochloa, Capillipedium and Dichanthium are characterized by sexually reproducing diploids and mostly-apomictic polyploids. Obligate apomictic species are usually more limited in their distribution range than are facultative apomicts. They are almost always less variable morphologically, also. Baker (1959) pointed out that obligate apomicts are usually characterized by a few distinct biotypes. These are, no doubt, the result of the accumulation of chance mutations that became fixed in clonal populations.

Morphologically variable races may be discerned in facultative apomicts, since they occasionally out breed. Occasional hybridization of the facultative apomicts, will result in a more continuous morphological variation than the distinct discontinuities characteristic of obligate apomicts. Hybrids could be produced with relative ease within and between some agamic complexes; between others, literally thousands of emasculations were necessary to obtain a single hybrid; while numerous attempted crosses, between some species produced no hybrids at all

(Harlan et al., 1961). Hybrid inviability and weakness were often encountered and, in some crosses, not even apomixis could overcome sterility.

Morphological and genetical affinities will be discussed separately for the sexual diploids, sexual polyploids and the agamic complexes.

Diploids--Diploids are divided in Table II into three groups; relict species, active species and members of agamic-complexes.

The relict species are narrowly endemic and confined to specialized ecological niches of the western Ghats, India (Harlan, 1963 a). Within these ecological niches each species characteristically exhibits very little morphological variation and is represented by a relatively large population, which never successfully invades adjacent regions. Survival is strictly correlated, therefore, with their specialized adaptations, and chances for their further development seem limited. The species Dichanthium armatum, D. maccannii and D. panchganiense, are closely allied morphologically but are completely isolated genetically (de Wet and Singh, 1964). Attempted crosses between them and other diploid species of Dichanthium, as well as with diploid members of Bothriochloa and Capillipedium were not successful, but they cross readily with the tetraploid apomict, D. annulatum. The relict Bothriochloa species, B. compressa, B. foulkesii, B. kuntzeana and B. longifolia, are only distantly related morphologically and are completely isolated from each other genetically. Attempted crosses between them and polyploid species were not successful (Harlan, Chheda and Richardson, 1962).

The active diploids (Table II) are widely distributed and are generally quite variable morphologically. The extremely variable Capilliped-ium huegelii extends throughout tropical and subtropical India and was divided into two species by Raizada (1951). Morphological variation,

TABLE II

DIPLOIDS AND THEIR DISTRIBUTION

Species	Distribution
a. RELICTS	
Bothriochloa	
B. compressa	Intermittent lakes, western Ghats, India
B. foulkesii	In shallow water courses, Nilgiris, India
B. longifolia	In shallow water courses, Mahabaleshwar, India
B. kuntzeana	Along streams, Poona, India
Dichanthium	
D. armatum	Rocky tablelands, Panchgani, India
D. maccannii	Rocky tablelands, Panchgani, India
D. panchganiense	Rocky tablelands, Panchgani, India
b. ACTIVE SPECIES	
Capillipedium	
C. huegelii	Tropical and subtropical India
Dichanthium	
D. humilius	Widely distributed in Australia
D. sericeum	Widely distributed in Australia
D. setosum	Tablelands of east-central Australia
D. superciliatum	Tropical northern Australia
c. MEMBERS OF AGAMIC-COMPLEXES	
Capillipedium	
C. assimile (2n, 4n)	India to Japan and southeast Asia
C. parviflorum (2n, 4n, 5n)	Tropical and subtropical Old World
Dichanthium	
D. annulatum (2n, 4n)	Tropical and subtropical Old World
D. aristatum (2n, 4n, 6n)	Tropical India to Australia
D. caricosum (2n, 4n)	Tropical India to southeast Asia

however, is continuous between the two, which suggests in actuality free gene exchange between morphologically different populations. The diploids Dichanthium humilius, D. sericeum, D. setosum and D. superciliatum are widely distributed in Australia. Morphologically, D. setosum is rather uniform and is confined to the tablelands of northern New South Wales and southern Queensland. The spikelets are well developed, with the pedicellate spikelet male and often awned, rather than neuter and awnless. It appears to be the most primitive of the four Australian diploids and it may represent the basic species which gave rise to the other three more widely distributed taxa. These, D. sericeum, D. humilius and D. superciliatum, grade into each other morphologically, with D. humilius apparently representing a less pilose form, and D. superciliatum an extremely robust variant of D. sericeum. Raceme number, a key character, varies from 1-15 in D. sericeum and from 12-40 in D. superciliatum. This suggested to de Wet and Harlan (1962) that introgressive hybridization must have played a role in the evolution of this species-complex. Attempts to artificially hybridize these species failed. This, however, may be due to difficulties in emasculating and pollinating the partially cleistogamous flowers.

Diploids forming part of agamic-complexes are as variable morphologically as their polyploid counterparts. Five such complexes, two in Capillipedium and three in Dichanthium (Table II), were identified. Diploid races of the tetraploids, Capillipedium assimile and C. parviflorum, are as widely distributed and morphologically variable as their tetraploid counterparts. They are isolated genetically at the diploid level, whereas at the polyploid level, although artificial hybrids have not yet been produced, the morphological data suggest some degree of

recombination of characters.

At the diploid level Dichanthium annulatum, D. aristatum and D. caricosum are genetically isolated from each other, however, they cross readily with their tetraploid counterparts to produce sterile triploids and apomictic pentaploids (de Wet and Richardson, 1963). At the tetraploid level, D. caricosum was crossed with both D. annulatum and D. aristatum. The last mentioned two species are genetically isolated from each other also at this level of ploidy. Diploid races resemble not only typical polyploid representatives of their species, but also artificially produced interspecific hybrids. Under experimental conditions, polyhaploids ($2n = 20$) are often obtained in the progeny of tetraploid interspecific hybrids (Harlan et al., 1961). Hybridization apparently is quite common in nature between tetraploid members of D. caricosum, and both D. aristatum and D. annulatum wherever these species are sympatric (Celarier, de Wet and Richardson, 1961). These observations led to the hypothesis that some of the naturally occurring diploids may actually represent fertile polyhaploids. Artificially produced polyhaploids are usually characterized by regular chromosome behavior due to genetically induced pairing (de Wet, Mehra and Borgaonkar, 1961), and some of these are actually fertile. Furthermore, morphologically typical diploid representatives of D. caricosum will not cross with either D. annulatum ($4n$) or D. aristatum ($4n$), whereas, slightly atypical diploid forms (D. caricosum which apparently include some genes of either D. annulatum or D. aristatum) cross readily to the tetraploid species that each resembles in some morphological traits.

Sexual polyploids--Strictly sexually reproducing polyploid species (Table III) were found to be limited to the genus Bothriochloa

TABLE III

SEXUALLY REPRODUCING POLYPLOIDS AND THEIR DISTRIBUTION

Species	2n	Distribution
Bothriochloa		
B. alta	120	Warmer parts of the Americas
B. ambigua	60	Tropical and subtropical Australia
B. barbinodis	180, 220	Warmer parts of the Americas
B. biloba	60	Subtropical southeastern Australia
B. decipiens	40	Tropical and subtropical Australia
B. edwardsiana	60	Southern United States and Mexico
B. erianthoides	60	Inland Queensland and New South Wales, Australia
B. exaristata	60	Warmer parts of the Americas
B. hybrida	120	Texas and Mexico
B. saccharoides	60, 80, 120	Warmer parts of the Americas
B. springfieldii	120	Warmer parts of the Americas
B. wrightii	120	Southern United States and Mexico

(de Wet, Borgaonkar and Richardson, 1963). They are confined geographically to Australia and the Americas and they range in chromosome number from 2n = 60, 80, 120, 180 to 2n = 220. Although allied morphologically, these polyploids are isolated genetically and behave cytologically like true allopolyploids. Borgaonkar and de Wet (1961) demonstrated an almost complete lack of chromosome homology, even between the morphologically closely allied B. exaristata (2n = 60) and B. saccharoides (2n = 120). The latter American species was also crossed with the Australian B. erianthoides and B. ambigua (Singh and de Wet, 1960; 1961) to produce completely sterile hybrids. The sexually reproducing polyploid species apparently represent relicts of a once widely distributed section of the genus Bothriochloa.

Apomictic polyploids--Apomictic polyploids are distributed throughout the tropics and subtropics of the Old World. They are mostly tetraploid, more rarely pentaploid or hexaploid and very rarely octaploid. Cytologically, apomictic polyploids behave like segmental allopolyploids as defined by Stebbins (1947). The chromosomes usually associate into bivalents, but some chromosomes occasionally fail to pair or else enter into multivalent formations.

Morphologically uniform apomicts (Table IV) are restricted in their distribution range. Thus, Bothriochloa woodrowii and B. concanensis are restrictively confined to dikes between rice paddies and rocks or sandy streams respectively (Harlan, 1963 a). The species Capillipedium kwashotense is also essentially an obligate apomict and limited in its distribution.

Other obligate apomicts are more widely distributed and are characterized by distinct biotypes. The Eurasian B. ischaemum and the tropical

TABLE IV

PREDOMINANTLY APOMICTIC SPECIES AND THEIR DISTRIBUTION

Species	2n	Distribution
a. MORPHOLOGICALLY UNIFORM		
Bothriochloa		
B. caucasica	40	Caucasus, Russia
B. concanensis	50	Mahabaleshwar, India
B. ischaemum	40, 50, 60	Tropical and subtropical Old World
B. pertusa	40, 60	Tropical and subtropical India, southeast Asia
B. radicans	40	Tropical and subtropical Africa
B. woodrowii	40	Maharashtra, India
Capillipedium		
C. kwashotense	40	Ryukyu Islands and Philippines
b. MORPHOLOGICALLY VARIABLE		
Bothriochloa		
B. glabra	40, 60	Tropical and subtropical Old World
B. grahamii	40, 60	Mainly Gangetic Plain, India
B. insculpta	50, 60	Tropical and subtropical Africa
B. intermedia	40, 60, 80	Tropical and subtropical Old World
B. odorata	40	Tropical India
Capillipedium		
C. assimile	20, 40	India to Japan and southeast Asia
C. parviflorum	20, 40	Tropical and subtropical Old World
C. spicigerum	40	Tropical and subtropical Australia
Dichanthium		
D. annulatum	20, 40	Tropical and subtropical Old World
D. aristatum	20, 40, 60	Tropical India to Australia
D. caricosum	20, 40	Tropical southeast Asia
D. fecundum	40	Tropical and subtropical Australia

Asian B. pertusa are mostly obligate apomicts. Thousands of attempted crosses, using these species as females, failed, but they can function as male parents. Celarier and Harlan (1958) demonstrated that B. ischaemum is characterized by two quite uniform varieties. Typical representatives of the species extend from the west coast of France to northeastern India. The Asiatic B. pertusa is even less variable morphologically than B. ischaemum. Two distinct varieties were recognized by de Wet and Higgins (1963), but the key morphological difference between them is probably controlled by a few closely linked genes. This species, B. pertusa, appears to be almost completely isolated genetically from other Bothriochloa species, although it is sympatric over its entire geographic range with the aggressive B. intermedia. The African B. insculpta and B. radicans are sympatric. The latter species is uniform morphologically while B. insculpta is more variable, yet both species are essentially obligate apomicts.

The variable apomicts (Table IV) form large interspecific complexes with each genus, and also unite the three genera into a single supercomplex. Celarier, de Wet and Richardson (1961) indicated that natural hybridization takes place between Dichanthium caricosum, and both D. annulatum and D. aristatum wherever these species are sympatric. It was proven conclusively (Harlan et al., 1961) that introgressive hybridization between Bothriochloa intermedia and Dichanthium annulatum had taken place to produce the species B. grahamii. The widely distributed Capillipedium parviflorum is extremely variable. It not only forms a hybrid-complex with C. assimile in India and Asia, but in Australia it has contributed towards the origin of B. glabra (de Wet, Borgaonkar and Chheda, 1961).

Taxonomical complexity, due to this introgression with other species,

characterizes Bothriochloa intermedia. Harlan and de Wet (1963 a) described this complex as a compilospecies. Special mechanisms to facilitate hybridization became established within this complex. The overall apomictic mode of reproduction is genetically dominant over sexuality. A sexual potential is maintained (de Wet and Borgaonkar, 1963), however, in the heterozygous condition, and fully sexual plants are often produced following hybridization between facultative apomicts. Harlan and de Wet (1963 b) demonstrated that sexual and asexual reproduction are not only in a state of equilibrium within the compilospecies as a whole, but are also nicely balanced the one against the other, within each individual. Apomixis and sexual reproduction are not genetical alternatives, but operate simultaneously and independently from each other. Seed production is controlled by sexual reproduction, nucellar apospory and parthenogenesis. When parthenogenesis operates in conjunction with nucellar apospory the plant produces seed apomictically. When it functions in cooperation with sexuality, polyhaploids are produced. When nucellar apospory and sexuality function together, cytologically unreduced gametes get fertilized, giving rise to polyploid-complexes. Harlan et al. (1961) indicated that hybridity within the compilospecies did not severely affect chromosome pairing. Chromosome association in hybrids is genetically controlled (de Wet, Mehra and Borgaonkar, 1961; Chheda and Harlan, 1962). Furthermore, Chheda, de Wet and Harlan (1961) demonstrated that desynaptic and aneuploid plants are eliminated in nature through selection. Functional gametes are therefore always produced even in the most apomictic biotypes.

The net result is a widely adapted population characterized by a more or less continuous morphological variation. At the extremes Both-

riochloa ischaemum, Capillipedium parviflorum and Dichanthium annulatum are still distinct members of otherwise genetically isolated genera. Within the compilospecies some populations are adapted to particular ecological niches and maintain some degree of morphological unity by means of apomixis. These are often referred to as species while some actively hybridizing populations defy classification even by classical taxonomists.

The eventual fate of species being plundered by B. intermedia must be complete absorption. Introgressive hybridization is making it increasingly easy for ever wider crosses to take place. The evolutionary potential of the compilospecies is tremendous. Its vast gene pool insures against extinction, and allows populations with superior gene pools to occupy a vast array of selected niches. Without genetical isolation, however, these microcenters of phylogenetic activity are continually being broken up. Complete genetical isolation will be difficult to achieve in a system designed primarily to increase heterozygosity. This will be unlikely to take place while the populations are facultative apomicts.

Essentially obligate apomictic populations, which may quite easily be obtained, provide one method of isolation. This type of isolation may have given rise to species, such as B. insculpta after hybridization between B. intermedia-like and B. radicans-like ancestors. Within the present day compilospecies a number of semi-isolated populations are obvious. These were treated as species, B. glabra, B. odorata and B. grahamii, by Bor (1960). Harlan and Chheda (1963) presented substantial evidence that the geographically isolated obligate apomictic B. caucasica represent hybrid derivatives between B. intermedia-like and C. parvi-

florum-like ancestors. This species was crossed with B. intermedia, but hybrid inviability and weakness insures genetic isolation between them. A combination of apomixis and of geographical and genetical isolation evidently is playing a role in speciation within the compilospecies.

Sexual reproduction may provide another method towards achieving genetical isolation. However, eliminating the dominant gene-complex controlling apomixis will not be easy. Sexual reproduction will greatly reduce the population size. Cytological irregularities will lead to the production of large numbers of aneuploid plants which will be eliminated (de Wet and Borgaonkar, 1963). Chromosome recombination between the various genomes present within partially isolated populations will eventually give rise to polyploids behaving cytologically like diploids. Different karyotypes may become established in different populations and this can eventually lead to the origin of distinct species.

Conclusions

1. The relict diploid species Bothriochloa compressa, B. foulkesii, B. longifolia and B. kuntzeana are only distantly related morphologically and are completely isolated from each other genetically. On the other hand Dichanthium armatum, D. maccannii and D. panchganiense are morphologically similar though genetically isolated.

2. Active diploids are quite variable and the species of Dichanthium have a tendency to overlap in morphological features. Included in this group are Capillipedium huegelii, Dichanthium humilius, D. sericeum, D. superciliatum and D. setosum.

3. Diploids forming part of agamospecies such as Capillipedium assimile, C. parviflorum, Dichanthium annulatum, D. aristatum and D.

caricosum, are isolated from each other at this ploidy level, but morphological evidence indicates some degree of recombination, possibly through the advent of polyhaploids of their tetraploid counterparts which are not so completely genetically isolated.

4. Sexual polyploid species occur only in the genus Bothriochloa and are limited in distribution to the Americas and Australia. Many appear to be morphologically allied but are genetically isolated.

5. Apomictic polyploid species may exhibit either obligate or facultative asexual reproduction. Those species of the former group are more apt to be morphologically uniform because of this mode of reproduction than those of the latter group. Introgressive products of the species of this group may represent stages of evolution, on the one hand in a divergent direction, and on the other hand in a convergent direction.

CHAPTER IV

ESSENTIAL OILS AS TAXONOMIC CRITERIA IN BOTHRIOCHLOA

Although the essential oils of several grasses have long been important commercially (Guenther, 1950) very little is known about their taxonomic importance. In some other plant groups the taxonomic significance of essential oils is well established. Baker and Smith (1902) studied the genus Eucalyptus. Von Rudloff (1961; 1962 a, b; 1963 b) studied various genera of the Gymnospermae. Eglinton et al. (1962 a, b), Eglinton, Hamilton and Martin-Smith (1962) and Eglinton and Hamilton (1963) demonstrated that essential oils could be used as taxonomic criteria in various components of the New Zealand flora. These studies represent chemical surveys, and the taxonomic implications were mostly inferred as possibilities for future investigations. More systematically oriented studies are those of Bannister, Brewerton and McDonald (1959), Bannister et al. (1962) and Williams and Bannister (1962) on the genus Pinus.

The intent of the present study was to determine whether chromatographic analysis of essential oils could be used in a taxonomic study of the morphologically difficult tribe Andropogoneae. The genus Bothriochloa was selected because it is comparatively well understood taxonomically (Harlan et al., 1961; Harlan and de Wet, 1963 a), and because a large collection of species was available for study.

Material and Methods

Plants were grown in a uniform nursery from seed collected in their native habitat. These collections were studied cytologically, morphologically and chemically. Herbarium specimens for each collection are filed with the Oklahoma State University.

Chemical studies are based on mature inflorescences. Sufficient material was collected to fill a 500 ml jar. This was refluxed with water for 3 hours and the oils were extracted with ether. The oil samples obtained were studied chromatographically.

The chromatograph used was a Perkin-Elmer Vapor Fractometer equipped with a 10 ft. x $\frac{1}{4}$ in. stainless steel column. The column was packed with 15% succinate polyester of butanediol as the stationary liquid phase on Chromosorb W. Operating temperature was 200° $\pm$ 2° C; helium flow was maintained at 120 ml/minute; a dual chamber thermistor thermal conductivity cell was used as the detector unit; and the recorder range was set at X2. A Leeds and Northrup 7-inch strip chart recorder having 0 to 1.0 mv range, 1 second pen response and a speed of 30 inches per hour was used. The sample size was 2 microliters and 30 minutes was required for full elution of the sample's components.

The chromatograms obtained were checked visually for proper corresponding peak alignment, and readings were made of the retention time and peak height. These two dimensions, when multiplied, give an estimation of relative amounts of each chemical. The values, thus obtained, were calculated as percent-of-total and plotted on polygonal graphs to form a more easily visualized chemical profile characteristic of a particular plant or species.

Observations

One hundred and forty collections, belonging to 17 of the Bothriochloa species recognized by Blake (1944), Chippindall (1955) and Bor (1960), were studied. The chemical components of collections indicating the range of variation within each species are graphically presented in Plates III, IV, V as percentage of total. Twenty-one different peaks could be identified on the strip charts. Species differ from each other in the presence and absence of certain peaks, and in the height of individual peaks.

Bothriochloa barbinodis (Lag.) Herter--This is a morphologically variable species (Gould, 1957), but quite uniform chemically. Plants with $2n = 180$ and $2n = 220$ chromosomes resemble each other almost in detail chemically (Plate III, Fig. 1, 2).

Bothriochloa caucasica (Trin.) C. E. Hubbard--Cytogenetical evidence presented by Harlan and Chheda (1963) indicated that this species is more closely allied to the genus Capillipedium than it is to Bothriochloa. Morphological and genetical studies demonstrated that this species probably represents an apomictic intergeneric hybrid derivative between these two genera. Chemically it falls within the range of variation characterizing the other species of Bothriochloa (Plate II, Fig. 3).

Bothriochloa compressa (Hook. f.) Henrard--This is an extremely pungent grass resembling B. woodrowii in gross morphological traits, but is a diploid ($2n = 20$) rather than a tetraploid. Chemically these two species are quite distinct (Plate III, Fig. 4).

Bothriochloa decipiens (Hack.) C. E. Hubbard--Two varieties were

LEGEND TO PLATE III

Selected chemical profiles.

Figure 1. *B. barbinodis* (2n = 180) Mexico

Figure 2. *B. barbinodis* (2n = 220) Texas

Figure 3. *B. caucasica* (2n = 40) Russia

Figure 4. *B. compressa* (2n = 20) India

Figure 5. *B. decipiens* (2n = 40) Australia

Figure 6. *B. decipiens* (2n = 40) Australia

Figure 7. *B. ensiformis* (2n = 40) India

Figure 8. *B. exaristata* (2n = 60) Brazil

Figure 9. *B. glabra* (2n = 40) India

Figure 10. *B. glabra* (2n = 40) Indonesia

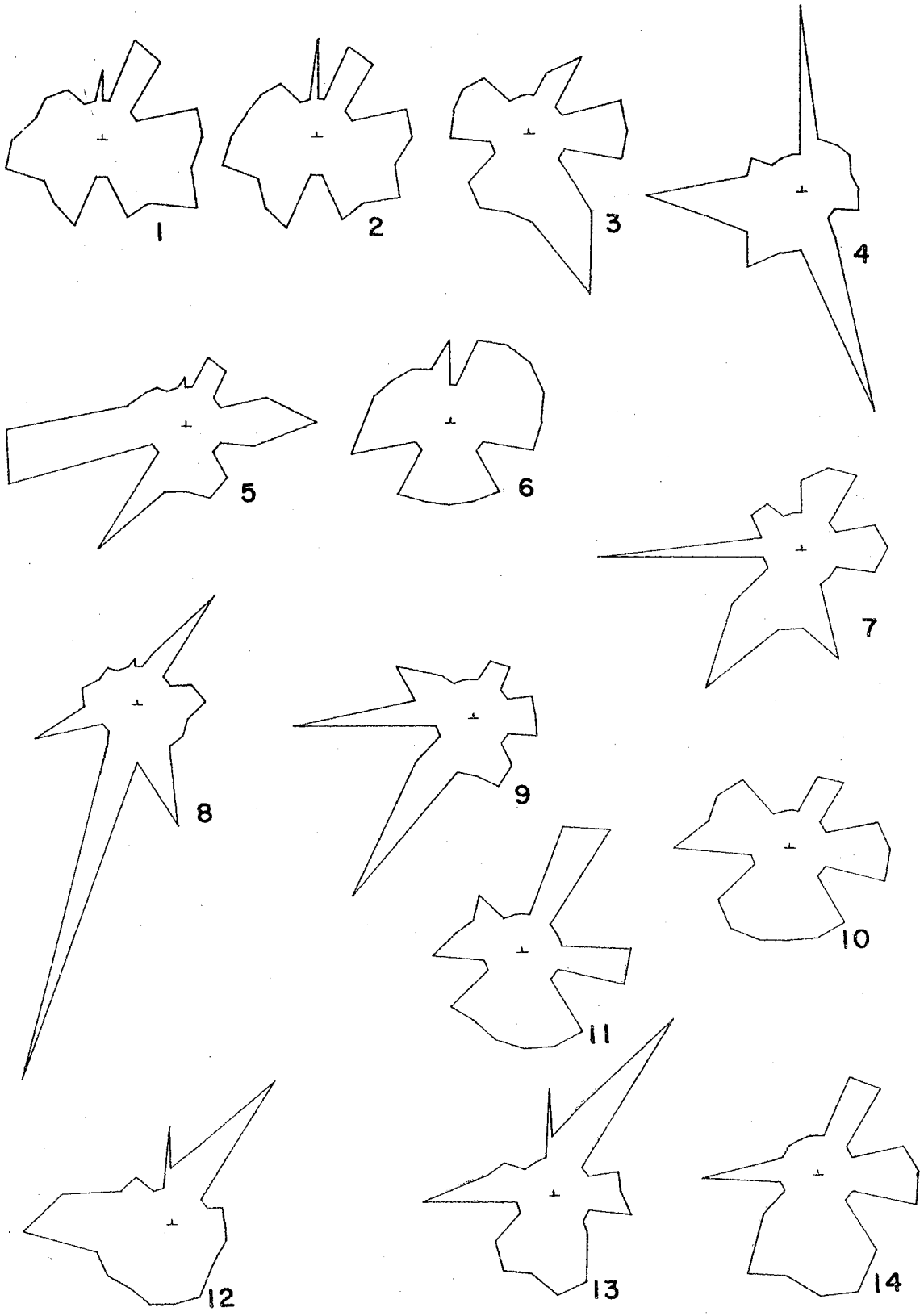
Figure 11. *B. glabra* (2n = 40) Rhodesia

Figure 12. *B. glabra* (2n = 60) Rhodesia

Figure 13. *B. glabra* (2n = 40) South Africa

Figure 14. *B. glabra* (2n = 40) South Africa

PLATE III



described by Hubbard (1934). They differ from each other primarily in that B. decipiens var. cloncurrensis is more robust than typical representatives of the species. The robust variety was studied chemically and found to be extremely variable. Two basic types were recognized, and they differ from each other in respect to the absence or presence of one chemical, and also in quantity of almost all chemicals (Plate III, Fig. 5, 6).

Bothriochloa ensiformis (Hook. f.) Henrard--This species resembles typical Indian representatives of B. intermedia in morphological characters except that the leaves are distinctly broader. These two species are quite distinct chemically (Plate III, Fig. 7).

Bothriochloa exaristata (Nash) Henrard--Morphologically this species is closely allied to B. saccharoides, but is easily distinguishable from it by the absence of well developed awns. Borgaonkar and de Wet (1961) demonstrated an almost complete absence of chromosome homology between the genomes of B. exaristata ($2n = 60$) and B. saccharoides ($2n = 60$). They also differ from each other chemically in many respects (Plate III, Fig. 8).

Bothriochloa glabra (Roxb.) A. Camus--This species is recognized in the sense of Henrard (1940) and Bor (1960) to include plants with strongly divided panicle branches arranged along an elongated primary axis. This includes a morphologically variable group of plants. Harlan and de Wet (1963 b) demonstrated that this species probably originated as apomictic hybrid derivatives between Bothriochloa intermedia and Capillipedium parviflorum. This species is quite distinct from B. intermedia and is also chemically variable (Plate III, Fig. 9, 10, 11, 12, 13, 14).

Bothriochloa grahamii (Haines) Bor--Harlan and de Wet (1963 a)

conclusively demonstrated that this species represents an apomictic intergeneric hybrid between Bothriochloa intermedia and Dichanthium annulatum. This species is both chemically and morphologically somewhat variable (Plate IV, Fig. 15, 16, 17, 18).

Harlan (1963 b) demonstrated that this facultative apomictic species hybridizes in northern West Pakistan with the Eurasian B. ischaemum. As could be expected these natural hybrids are extremely variable morphologically and chemically. Two representative collections are graphically presented in Plate IV, Fig. 19, 20.

Bothriochloa insculpta (Hochst.) A. Camus--This species is essentially an obligate apomict, and characterized by $2n = 50$ and $2n = 60$ chromosome races. Hexaploids ($2n = 60$) are of two morphological types. These are extremely robust, suberect plants with divided inflorescence branches, arranged along a somewhat elongated primary axis and slender, rambling plants with subdigitately arranged racemes. The pentaploids ($2n = 50$) connect these two morphological extremes. Morphological evidence suggests that the robust, suberect hexaploids combine the complete genome of the African B. glabra ($2n = 40$) and the haploid genome of B. radicans ($2n = 40$). The more slender plants, in contrast, probably combine the complete genome of B. radicans and the haploid genome of B. glabra. The morphologically variable pentaploids may represent backcrosses to the two original parents. Plate IV, Fig. 21 represents a chromatogram of the robust type and Plate IV, Fig. 22 that of slender ramblers. They are obviously different chemically, and also different from both the assumed parents. The pentaploids are as variable chemically as they are morphologically (Plate IV, Fig. 23, 24).

Bothriochloa intermedia (R. Br.) A. Camus--This variable species is

LEGEND TO PLATE IV

Selected chemical profiles.

Figure 15. *B. grahamii* (2n = 40) India

Figure 16. *B. grahamii* (2n = 40) Pakistan

Figure 17. *B. grahamii* (2n = 40) Pakistan

Figure 18. *B. grahamii* (2n = 40) India

Figure 19. Natural hybrid of *B. grahamii* x *B. ischaemum* (2n = 40) Pakistan

Figure 20. Natural hybrid of *B. grahamii* x *B. ischaemum* (2n = 40) Pakistan

Figure 21. *B. insculpta* (2n = 60) South Africa

Figure 22. *B. insculpta* (2n = 60) South Africa

Figure 23. *B. insculpta* (2n = 50) Rhodesia

Figure 24. *B. insculpta* (2n = 50) South Africa

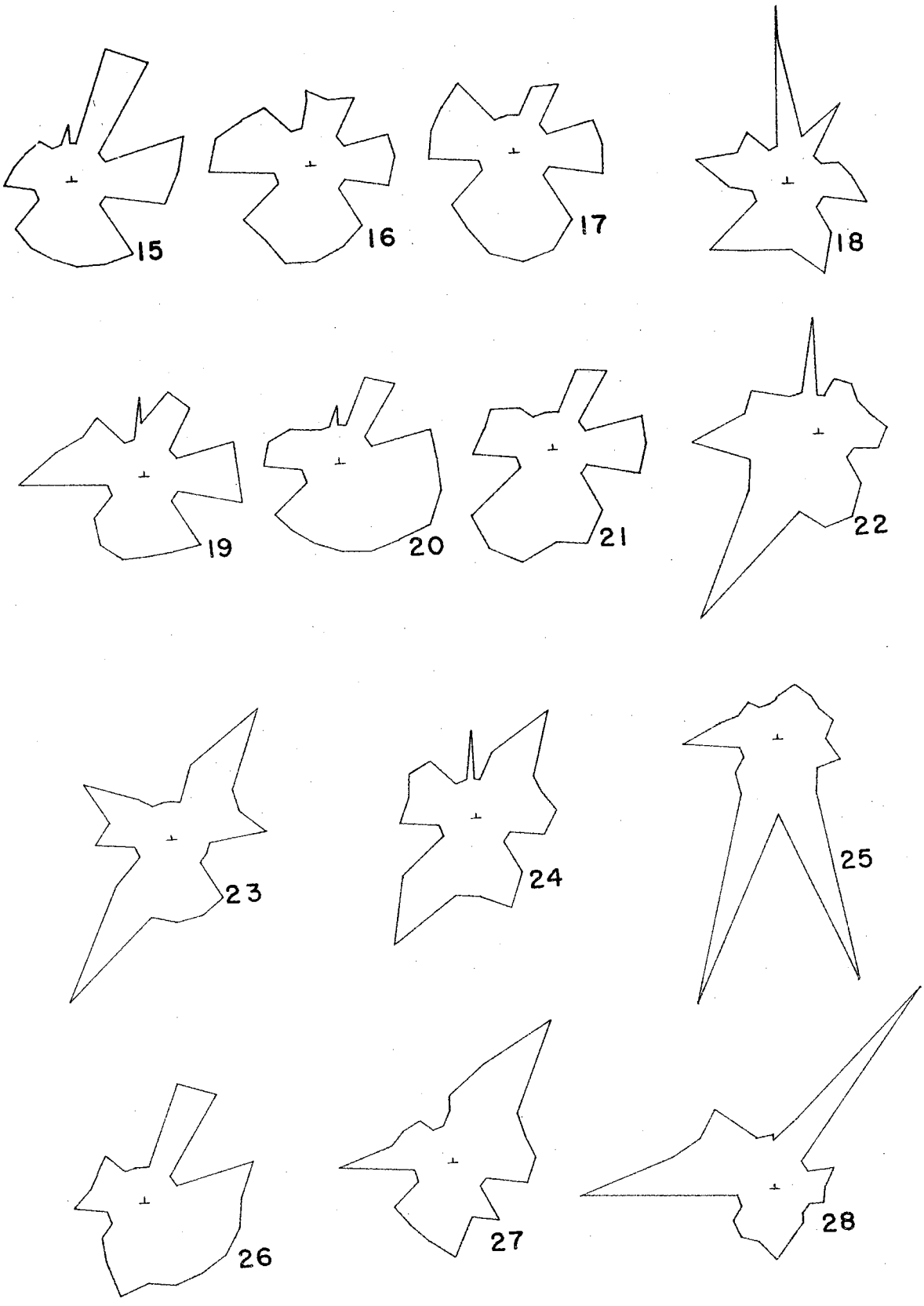
Figure 25. *B. intermedia* (2n = 40) India

Figure 26. *B. intermedia* (2n = 40) India

Figure 27. *B. intermedia* (2n = 40) India

Figure 28. *B. intermedia* (2n = 40) India

PLATE IV



recognized in the sense of Bor (1960) to include plants with simple or moderately divided panicle branches arranged along an elongated primary axis. The plants are extremely variable chemically, and the chromatograms represented by Plate IV, Fig. 25, 26, 27, 28 were selected at random from 16 collections studied.

Bothriochloa ischaemum (L.) Keng--This species was subdivided into two varieties by Celarier and Harlan (1958). The collections studied all represent typical B. ischaemum, and although this is a morphologically rather uniform variety it is extremely variable chemically. Celarier (1957) demonstrated three chromosome races, $2n = 40$, 50, and 60, in B. ischaemum var. ischaemum. Tetraploid collections are represented by Plate V, Fig. 29, 30, 31, 32; Plate V, Fig. 33 is of a pentaploid, and hexaploids were not studied.

Bothriochloa kuntzeana (Hack.) Henrard--This sexually reproducing diploid ($2n = 20$) is morphologically allied to B. longifolia ($2n = 20$). Hybrids between these two species, however, are lethal in the seedling stage. The two collections studied are chemically alike.

Bothriochloa longifolia (Hack.) Bor--The chromatograms of this diploid species and that of B. kuntzeana are compared in Plate V, Fig. 34, 35. These two species are not only genetically but also chemically quite distinct.

Bothriochloa pertusa (L.) A. Camus--This is an essentially obligate apomictic tetraploid species, and rather uniform morphologically. Cytogenetical and morphological data presented by de Wet and Higgins (1963; 1964) indicated that this species probably occupies an isolated phylogenetic position in Bothriochloa. It is also quite distinct chemically from the other species studied (Plate V, Fig. 36).

LEGEND TO PLATE V

Selected chemical profiles.

Figure 29. *B. ischaemum* (2n = 40) Austria

Figure 30. *B. ischaemum* (2n = 40) Turkey

Figure 31. *B. ischaemum* (2n = 40) Pakistan

Figure 32. *B. ischaemum* (2n = 40) Pakistan

Figure 33. *B. ischaemum* (2n = 50) Pakistan

Figure 34. *B. kuntzeana* (2n = 20) India

Figure 35. *B. longifolia* (2n = 20) India

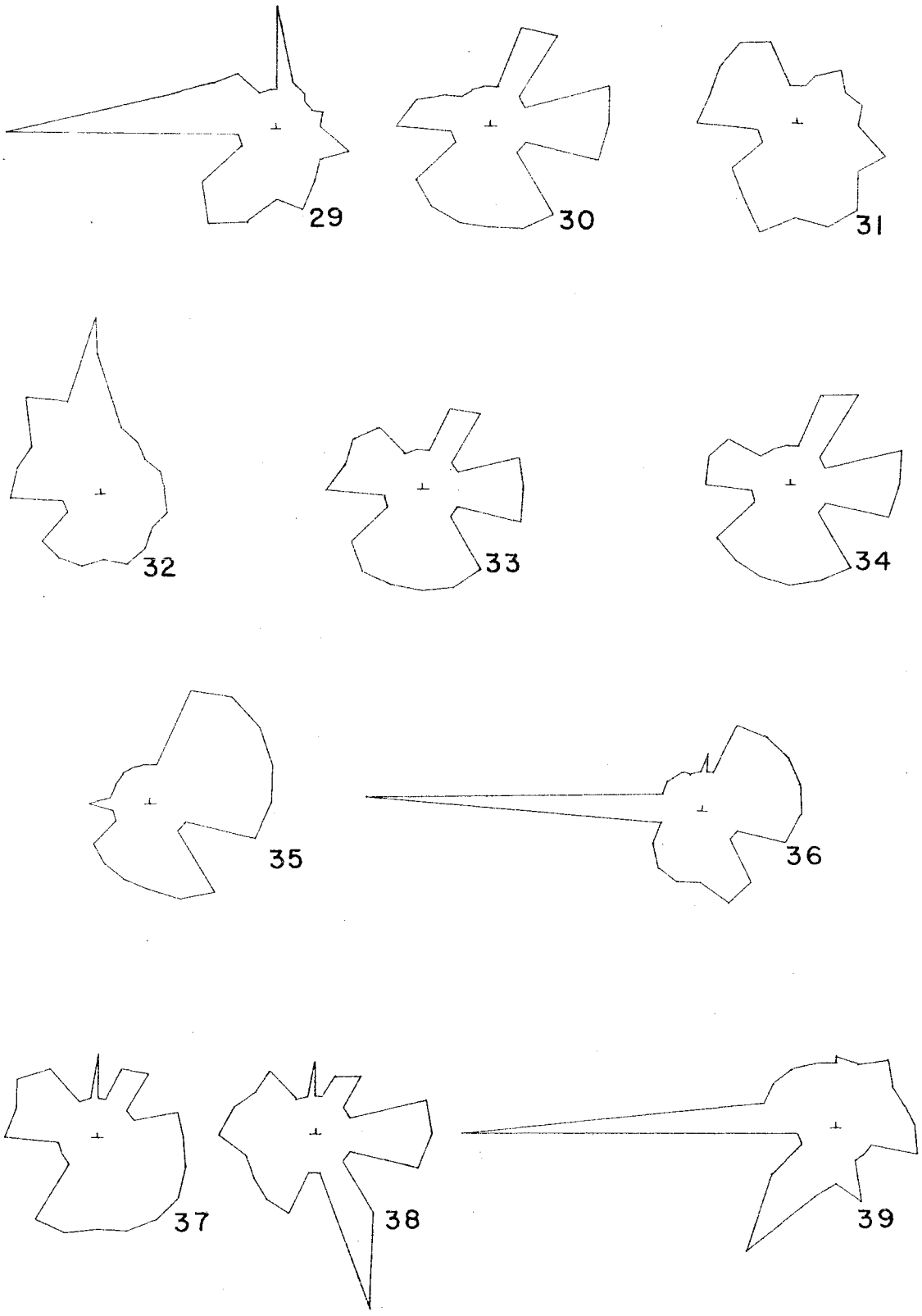
Figure 36. *B. pertusa* (2n = 40) India

Figure 37. *B. radicans* (2n = 40) Rhodesia

Figure 38. *B. saccharoides* (2n = 60) Oklahoma

Figure 39. *B. woodrowii* (2n = 40) India

PLATE V



Bothriochloa radicans (Lehm.) A. Camus--This species is morphologically distinct from the other African representatives of Bothriochloa. The single collection studied is represented by Plate V, Fig. 37.

Bothriochloa saccharoides (Swartz) Rydberg--This species was studied by Gould (1956) who recognized two chromosome races which he described as varieties. The single collection studied represents typical B. saccharoides with $2n = 60$ chromosomes (Plate V, Fig. 38).

Bothriochloa woodrowii (Hook. f.) A. Camus--Harlan (1963 a) demonstrated that this tetraploid apomictic species, together with the diploids B. compressa, B. ensiformis and B. kuntzeana are very narrowly endemic and confined to specialized ecological niches in the Western Ghats of India. Chemically this species (Plate V, Fig. 39) is distinct from the diploids, and also obviously different from the more widely distributed apomictic polyploids.

Discussion

Species can be distinguished visually from each other by studying either the peaks on the chromatograms (Plate VI) or more readily by plotting the chemical components as percentage of total on a polygonal graph (Plates III, IV, V). From a taxonomic and phylogenetic point of view the absence or presence, and the quantity of each chemical are also important (Table V).

The chemical nature of the various components detected is not known. They are referred to by letters of the alphabet in the order they elute from the column. Most species are characterized by the absence of chemical A, or it is present in quantities less than 5% of the total. Only in B. compressa, and some collections of B. grahamii

TABLE V

CHEMICAL CHARACTERISTICS OF BOTHRIOCHLOA SPECIES

Species		Chemical Component* A	D	H	I	J	K	L	M	N	O	P	Q
B. barbinodis	(18n)	-	-	+	o	o	-	o	o	o	o	o	o
B. caucasica	(4n)	-	-	-	o	+	o	o	o	o	-	o	o
B. compressa	(2n)	+	o	-	o	+	o	o	o	o	o	o	o
B. decipiens	(4n)	-	-	-	o	o	o	o	o	-	o	+	o
B. ensiformis	(4n)	-	-	-	-	+	o	o	+	o	-	+	-
B. exaristata	(6n)	-	-	+	o	+	o	+	o	-	o	o	o
B. glabra	(4n)	-	-	-	o	o	o	o	+	o	o	+	o
B. grahamii	(4n)	o	-	-	o	o	o	o	o	o	-	+	o
B. insculpta	(4n)	-	-	-	o	o	o	o	+	o	-	o	o
B. intermedia	(4n)	o	-	o	o	o	o	o	o	o	-	+	o
B. ischaemum	(4n)	-	-	o	o	o	o	o	o	o	-	o	o
B. kuntzeana	(2n)	-	-	-	o	o	o	o	o	o	-	o	o
B. longifolia	(2n)	-	o	-	o	o	o	o	o	o	-	o	-
B. pertusa	(4n)	-	o	-	-	o	o	o	o	o	-	+	-
B. radicans	(4n)	-	-	+	o	o	o	o	o	-	-	o	o
B. saccharoides	(6n)	-	-	-	o	o	-	o	-	o	o	o	o
B. woodrowii	(4n)	o	o	-	-	o	o	o	o	o	-	+	o

* The symbols -, o, + represent comparative quantities of chemical present; - versus o indicates absence or traces against distinct quantities; o versus + indicates smaller versus larger quantities. Each chemical component is quantitatively compared only with itself; -, o, + does not indicate the same quantity in different chemical components.

LEGEND TO PLATE VI

Reproductions of selected chromatograms. Horizontal scale: One inch = 4 min. retention time.

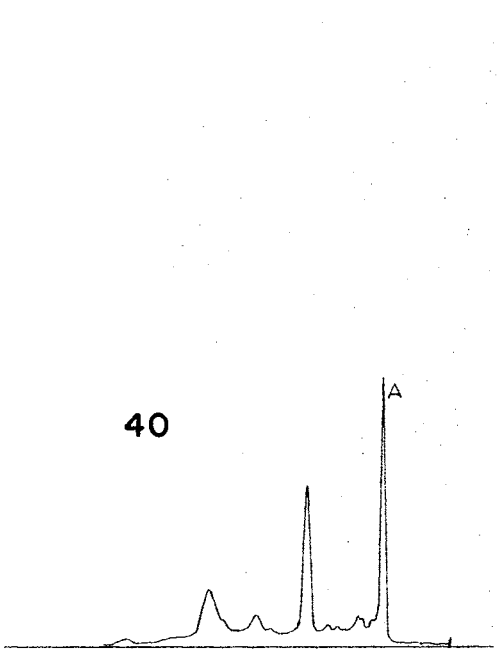
Figure 40. B. compressa, chromatogram represented in Plate II, Fig. 4. Note the prominence of peak A.

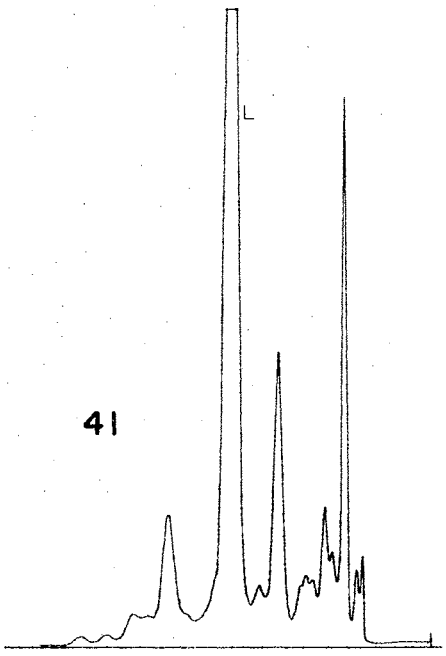
Figure 41. B. exaristata, chromatogram represented in Plate II, Fig. 8. Note the prominence of peak L.

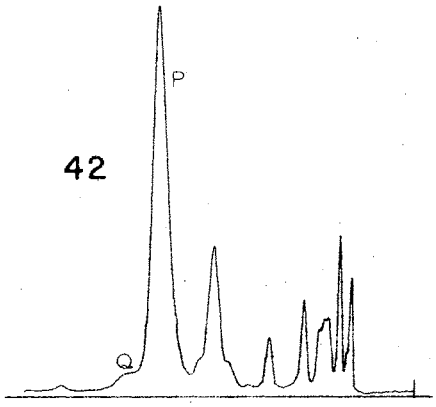
Figure 42. B. woodrowii, chromatogram represented in Plate IV, Fig. 39. Note the prominence of peak P and the presence of peak Q.

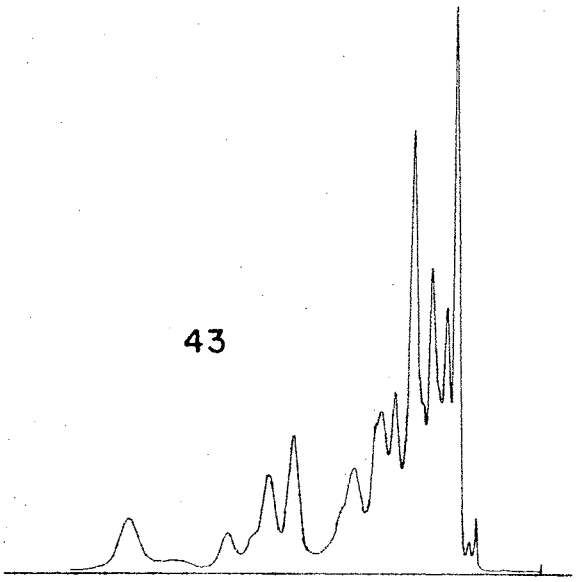
Figure 43. B. barbinodis, chromatogram represented in Plate II, Fig. 1.

PLATE VI









and B. intermedia did this chemical produce a distinct peak.

Chemicals B and C are present in all the collections studied, and their quantities are variable even in morphologically uniform species. However, distinct peaks at position B and C are characteristic of African representatives of B. glabra; whereas Indian and Southeast Asian plants have only small quantities of these chemicals. Chemicals B and C can also be used to distinguish between tropical Indian representatives of B. intermedia and plants collected from the Himalayan foothills. The latter are usually characterized by a small quantity of B but a distinct peak at C, and the more tropical plants by a large quantity of B and a small quantity of C.

Distinct peaks of chemical D characterize B. compressa, B. longifolia, B. pertusa and B. woodrowii. It is absent in some but distinctly present in other specimens of B. decipiens and often also characteristic of B. ischaemum. It is absent or present as traces in the other species studied. Chemicals E, F and G, although present in all the collections studied, are not very significant taxonomically. Their quantities appear to be closely correlated. When the percentage of E is high in a collection, chemicals F and G will also be characterized by distinct peaks. These three chemicals probably represent products of a single chemical that was broken down during distillation. The species B. compressa, B. decipiens, B. ensiformis, B. exaristata, B. radicans and B. woodrowii usually have about 50% less of these chemicals than the other species studied.

The presence of chemical H characterizes B. barbinodis, B. exaristata, B. radicans, and most of the collections studied belonging to B. intermedia and B. ischaemum. The absence of chemical I characterizes

B. ensiformis, B. pertusa and B. woodrowii. The other species studied are characterized by distinct peaks at position I.

Chemicals J, K, L and M are present, at least as traces, in all the collections studied. Chemical J forms less than 15% of the total constituents in all the species except B. caucasica, B. compressa, B. ensiformis and B. exaristata, where it is very prominent. Chemicals K, L and M each form about 10% of the total in most of the species studied; exceptions are B. barbinodis and B. saccharoides where K is present only as a trace, B. exaristata where L forms about 30% of the total, and B. insculpta with a distinct peak at the position M on the chromatogram. The quantity of chemical M varies considerably in different collections of B. glabra, but this species is mostly characterized by a rather distinct peak at position M.

The absence of chemical N characterizes B. decipiens, B. exaristata and B. radicans. In the other species studied this chemical is present in small quantities. Chemical O is mostly absent or present as traces, except in B. barbinodis, B. compressa, B. decipiens, B. exaristata, B. saccharoides and most collections of B. glabra.

Chemicals P and Q are present throughout the collections studied. Extremely large quantities of P characterize B. decipiens, B. ensiformis, B. pertusa, B. woodrowii and some collections classified with B. glabra, B. grahamii and B. intermedia. Traces of Q are present in B. ensiformis, B. longifolia and B. pertusa, whereas it forms a more distinct peak in the chromatograms of the remaining species studied. Chemicals R, S, T and U are either present as small quantities or often absent in the collections studied, and could not be used to distinguish species.

Morphologically distinct species are also distinct chemically.

Each species can usually be recognized by a distinct peak or a combination of chromatographic peaks (Plates III, IV, V, VI). Morphologically variable species are also variable chemically. Harlan and de Wet (1963 a) demonstrated that B. intermedia, B. glabra and B. grahamii form a hybrid complex, and Harlan (1963 b) indicated introgressive hybridization between B. ischaemum and B. grahamii. For these reasons it is often almost impossible to classify collections with certainty into a particular species. Basically these species seem to be distinct chemically (Table V), and hybrid derivatives can usually be distinguished chemically. This species complex will be discussed in the next chapter.

The absence or presence of volatile oils in these grasses have no obvious selective advantage. Plants extremely pungent to the human taste and smell are just as commonly eaten by animals in their native habitat as essentially odorless and tasteless plants. Most of the chemicals present in the obviously pungent species are also present in the non-pungent species, but in other quantity combinations. For these reasons chemical composition may be a more reliable character than gross morphology, in determining phylogenetic affinities.

The diploid species B. kuntzeana and B. longifolia are only slightly pungent while B. compressa is very aromatic. Similarly, some polyploid species are very aromatic while others are not. Evidently, pungent and non-pungent species contributed to the origin of the polyploids. The present day diploids are genetically only distantly related to the polyploids and chemical affinities between diploids and particular polyploids could not be demonstrated.

The New World species seem to represent relicts of a once widely distributed section of Bothriochloa. They are allied to the sexually

reproducing Australian diploids and are, as a group, morphologically distinct from the Old World apomictic polyploids. Chemically, however, they do not form a distinct group. Variation between the three New World species studied chemically is as great as between New and Old World species. Chemical data can, however, serve to confirm affinities between species. The morphologically similar species B. saccharoides and B. exaristata differ very conspicuously from each other chemically. Borgaonkar and de Wet (1961) also indicated that they are only distantly related genetically. Similarly, the morphologically allied diploids B. kuntzeana and B. longifolia are distinct genetically and chemically.

From a taxonomic point of view the chemical data can be used to separate species. Chemical characteristics are only as good as gross morphological characters. However, chemical data combined with morphological and cytogenetical observations were found useful in the classification of morphologically difficult species.

Conclusions

1. Gas chromatographic analysis of the essential oils revealed twenty-one separable chemicals in the genus Bothriochloa.

2. The presence or absence of these peaks on the chromatograms and the relative amounts of these chemicals are considered taxonomically important.

3. Species are unique chemically, except those apomicts that form species complexes.

4. Chemical data are closely correlated with data from morphological and cytogenetical studies

5. Chemical evaluation of the essential oils of these grasses,

therefore, appears to be a valid source of information in clarifying or confirming systematic relationships.

CHAPTER V

CHEMICAL TAXONOMY OF THE COMPILOSPECIES BOTHRIOCHLOA INTERMEDIA

The concept of Bothriochloa intermedia as recognized by Harlan and de Wet (1963 a) includes a diverse group of plants originally described as Andropogon intermedius by Brown (1810). The type specimen (Brown 6184, BM) appropriately consists of four different plants indicating some of the range of variation and complexity of this taxon.

Hackel (1889) recognized numerous varieties under Andropogon intermedius. Camus (1931), when transferring members of Andropogon subgenus Amphilophis to Bothriochloa, recognized three species with elongated inflorescences. Plants with non-divided panicle branches were retained in B. intermedia (R. Br.) A. Camus, while plants with divided panicle branches were distributed among B. odorata (Lisboa) A. Camus and B. glabra (Roxb.) A. Camus on the basis of pungency. Henrard (1940) subdivided B. glabra into two subspecies on the basis of lower glume indentations, and Ohwi (1942) described the non-pitted type as B. haenkei (Presl) Ohwi.

Blake (1944) and Vickery (1961) correctly included plants with simple and divided panicle branches, as well as pitted and non-pitted spikelets, in B. intermedia. This species is widely distributed in the tropics and subtropics of the Old World. Evidence presented by Harlan and de Wet (1963 a) demonstrated that B. intermedia hybridizes in nature with

Capillipedium parviflorum and Dichanthium annulatum. Hybrid derivatives of introgression between B. intermedia and D. annulatum were given specific rank by Bor (1960) who included them in B. grahamii (Haines) Bor. Harlan (1963 b) indicated that hybridization is taking place in northern West Pakistan between B. grahamii and B. ischaemum. Harlan and de Wet (1963 a) referred to this hybrid complex as a compilospecies.

The hybrid nature of this compilospecies makes its taxonomy extremely difficult. The origin of some natural populations could be determined from comparative cytological and morphological studies, while others completely defied classification. One hundred and fifty populations were studied chemically, cytologically and morphologically in an effort to trace their origin.

Material and Methods

Plants were grown in a uniform nursery from seed samples collected over the complete range of geographic distribution characteristic of B. intermedia. Herbarium specimens are filed with the Oklahoma State University. Chromosome numbers were determined from developing microspore mother cells stained with aceto-carmine.

For chemical analysis inflorescences were reflux-distilled for 3 hours, and the oil obtained was extracted with ether. Two microliters of essentially pure oil were analyzed by means of a Perkin-Elmer Vapor Fractometer. The column used was packed with 15% succinate polyester of butanediol on Chromosorb W. A dual chamber thermistor thermal conductivity cell was used as the detector unit and the results were recorded on a Speedomax strip chart recorder.

Observations

The widely distributed compilospecies B. intermedia is even more variable chemically than morphologically. It extends throughout the tropical and subtropical regions of the Old World. Over its entire range of distribution, extending from southern Africa to Australia, most plants are characterized by more or less strongly divided panicle branches. These plants could be included in B. glabra (Roxb.) A. Camus as recognized by Bor (1960). They are essentially obligate apomicts and tetraploid ($2n = 40$) or rarely hexaploid ($2n = 60$). Three distinct chemical races were recognized (Table VI; Plates VII, VIII, Fig. 7, 8, 9). These are closely correlated with geographic distribution. African populations, Indian and Southeast Asian populations, and Australian populations are chemically distinct. Morphologically they differ from each other only in minor detail.

Plants with simple panicle branches, or with only the lower ones divided, were collected in India, Southeast Asia and Australia. These conform with the concept of B. intermedia (R. Br.) A. Camus as recognized by Bor (1960). They are strictly tetraploid and facultative apomicts. Two chemical races were recognized (Table VI; Plate VII, Fig. 5, 6). Typically tropical plants, extending from India to Australia, are distinctly different chemically from morphologically similar plants collected in northern India.

Assumed natural hybrids between B. intermedia and Dichanthium annulatum (R. Br.) A. Camus (Plate VIII, Fig. 12) have occasionally been collected in Africa, India, Southeast Asia and Australia (Harlan and de Wet, 1963 b). In India, particularly along the Gangetic plain, these assumed

TABLE VI

CHEMICAL COMPONENTS OF THE COMPILOSPECIES BOTHRIOCHLOA INTERMEDIA

COMPARED WITH B. ISCHAEMUM AND CAPILLIPEDIUM PARVIFLORUM

Species and Origin of chemical types	Fig.	Taxonomically significant chemicals as % of total									
		A	B	C	F	G	J	L	M	P	U
Bothriochloa											
B. intermedia											
N. West Pakistan	2	2	8*	3	6	6	7	9	9	24*	0
Tropical Africa	7	5	14*	21*	4	8*	3	3	3	4	18*
India to Australia	5	4	16*	5	4	3	4	3	6	40*	0
Northern India	6	1	5	28*	2	2	2	2	3	20*	2
Gangetic plain, India	1	14	1	17*	3	5	8	6	5	13*	18
Tropical Australia	8	0	4	11*	5	5	6	7	8	13*	20*
India to S. E. Asia	9	0	2	3	3	3	4	2	23*	18*	0
B. ischaemum											
N. West Pakistan	4	0	3	6*	5	6	6	7	7	10*	8
B. intermedia (Fig. 1) x B. ischaemum (Fig. 4)	3	2	6*	4	7	7	7	8	8	18*	0
Capillipedium											
C. parviflorum											
Africa to Australia ssp. capilliflorum	10	17*	2	1	8*	28*	14*	9*	1	2	13*
India to Australia	11	1	1	1	3	18*	37*	14*	3	3	0
C. spicigerum											
Australia	12	1	2	4*	8*	2	27*	6	5	7	5

* Indicates prominent peaks marked on the chromatograms Fig. 1-12.

LEGEND TO PLATE VII

Reproductions of selected chromatograms. Horizontal scale: One inch = 4 min. retention time.

Figure 1. *B. grahamii*, India.

Figure 2. Assumed natural hybrid, *B. grahamii* x *B. ischaemum*, Pakistan.

Figure 3. Artificial hybrid, *B. grahamii* x *B. ischaemum*.

Figure 4. *B. ischaemum*, Pakistan.

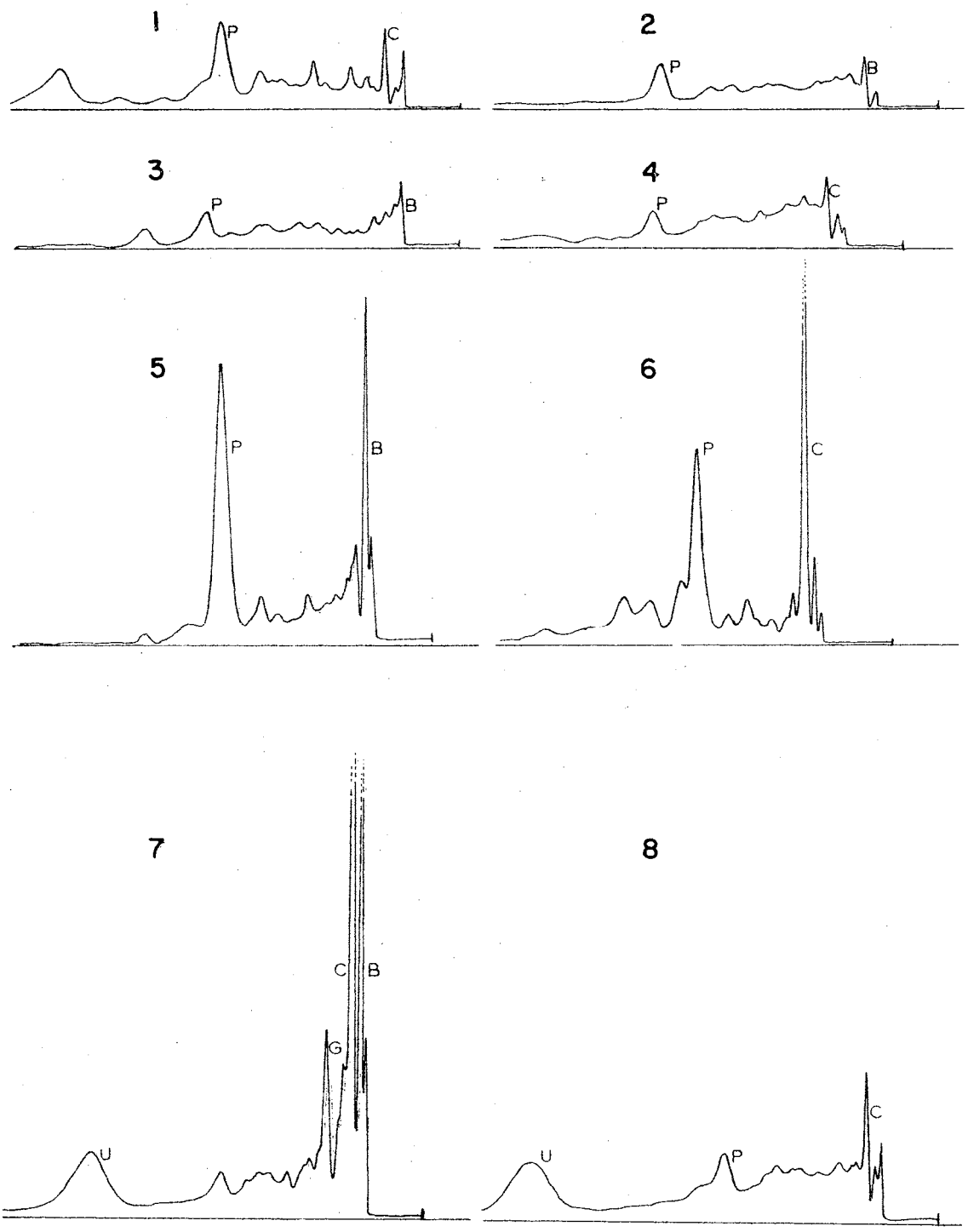
Figure 5. *B. intermedia*, India.

Figure 6. Assumed natural hybrid, *B. intermedia* x *B. ischaemum*, India.

Figure 7. *B. glabra*, Africa.

Figure 8. *B. glabra*, Australia.

PLATE VII



LEGEND TO PLATE VIII

Reproductions of selected chromatograms. Horizontal scale: One inch = 4 min. retention time.

Figure 9. *B. glabra*, India.

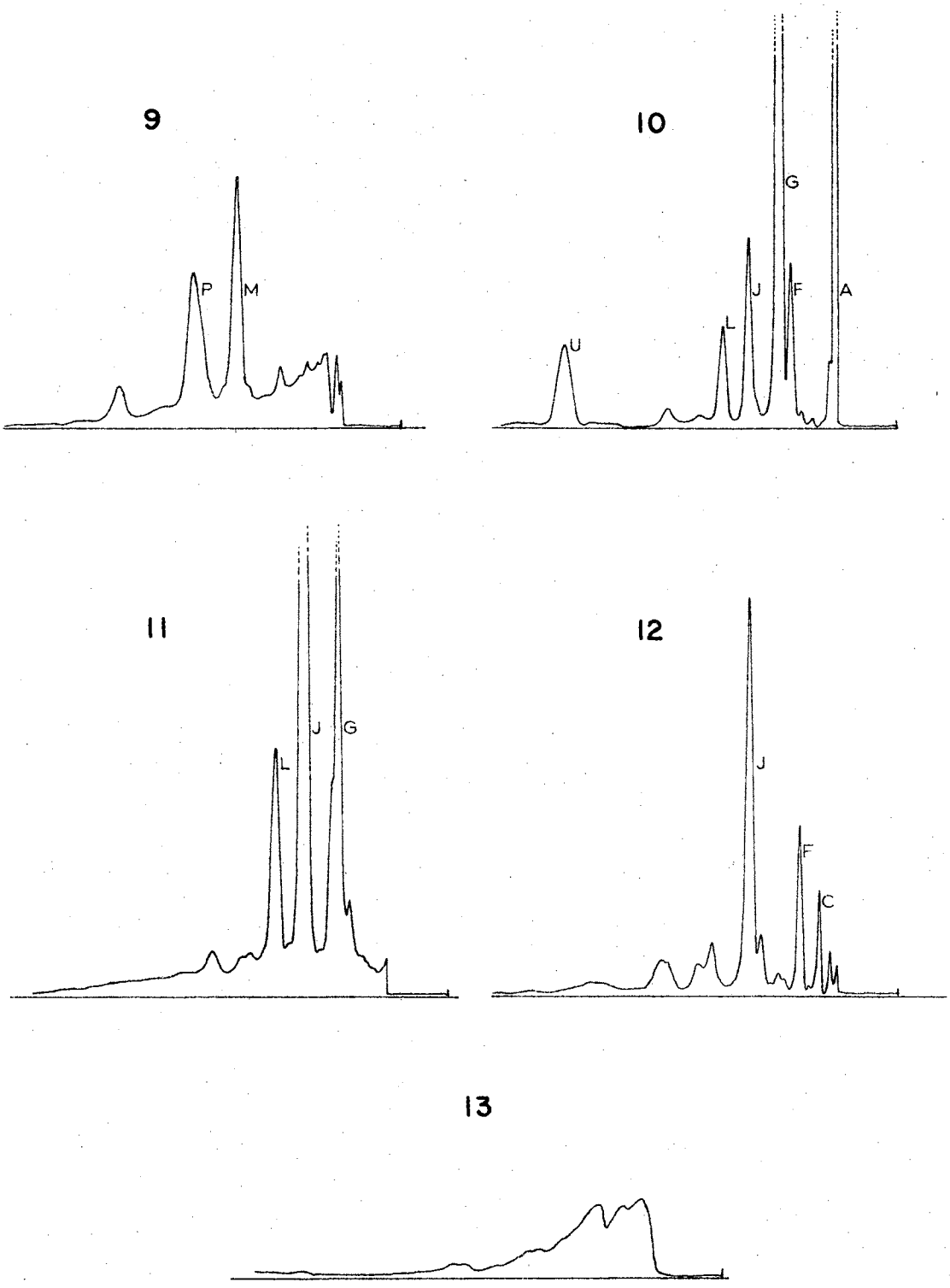
Figure 10. *C. parviflorum*, Africa.

Figure 11. *C. parviflorum* subsp. *capilliflorum*, India.

Figure 12. *C. spicigerum*, Australia.

Figure 13. *D. annulatum*, Israel.

PLATE VIII



intergeneric hybrids are common, and were recognized as B. grahamii (Haines) Bor. They differ chemically and morphologically from other Indian races of B. intermedia (Table VI), but they are chemically somewhat similar to typical representatives of this species from northern India.

Harlan (1963 b) demonstrated that B. grahamii hybridizes with the Eurasian species B. ischaemum (L.) Keng in northern West Pakistan. These assumed hybrids are chemically distinct from their parental species (Table VI; Plate VII, Fig. 2). Artificially produced hybrids between these species (Plate VII, Fig. 3) resemble the assumed natural hybrids both chemically and morphologically.

The related Capillipedium parviflorum (R. Br.) Stapf, C. parviflorum subsp. capilliflorum (Steud.) Henr. and C. spicigerum S. T. Blake (Table VI; Plate VIII, Fig. 10, 11, 12) were also studied. Harlan and de Wet (1963 a) suggested that hybridization between B. intermedia and C. parviflorum contributed towards the morphological variability characteristic of the compilospecies. Chemically the genera Bothriochloa and Capillipedium are quite distinct.

Discussion

Plants with simple, or essentially undivided, panicle branches are strictly tetraploid and behave cytologically more nearly like true allopolyploids than any other morphological race of B. intermedia. For this reason, these widely distributed tropical plants (Plate VII; Fig. 5) may represent relicts of the original tetraploid B. intermedia. Hybridization between this basic B. intermedia and related species presumably gave rise to the genetically aggressive compilospecies.

Artificial hybrids between basic B. intermedia and either Dichanthium annulatum or Capillipedium parviflorum are extremely difficult to produce. Literally thousands of attempts were necessary to produce a single hybrid. These hybrids resemble the assumed natural hybrids in detail morphologically, but they were not studied chemically. The only artificially produced hybrid studied chemically was one between the Gangetic plain race of B. intermedia (B. grahamii) and B. ischaemum.

Morphologically and chemically these artificially produced hybrids resembled the assumed natural hybrids in detail. They are chemically distinctly different from either parent (Table VI; Plate VII, Fig. 1, 2, 3, 4). In the hybrid, some chemicals are more or less intermediate in quantity between the larger and smaller amounts characteristic of the two parents. Some chemicals represented by small quantities in the hybrid, are present in one parent and absent in the other. Other chemicals are more abundant in the hybrid than in either parent, while some chemicals present in both parents are completely absent or present only as traces in the hybrid.

Observations on the chemical components of this artificially produced hybrid, indicated the futility of any attempt to trace the hybrid origin of populations belonging to this compilospecies by means of gas chromatography alone. They also demonstrate that comparative chemical studies can be used to distinguish hybrids from their parents and, when compared with artificially produced hybrids, to prove the hybrid origin of a natural population. Chemical comparisons may even be more reliable than comparative gross morphological studies. Morphologically all plants of the compilospecies having strongly divided panicle branches are assumed to represent hybrid derivatives between B. intermedia and

C. parviflorum. African, Asiatic and Australian plants, however, are distinctly different chemically. The more typical B. intermedia, although widely distributed, is basically of one chemical type. Therefore, either more than one species of Capillipedium was involved in the original hybridization, or else African, Asiatic and Australian races of C. parviflorum may differ from each other chemically. The species C. parviflorum is morphologically variable and the collection studied chemically is African in origin. The Indian collection, recognized as a different subspecies by Henrard (1940), is chemically also different from the African collection. Morphologically the different chemical, geographic races of the assumed natural hybrids between B. intermedia and C. parviflorum are sufficiently different to be recognizable. This is also true of C. parviflorum from different geographic regions. This would seem to indicate that hybridization took place between B. intermedia and C. parviflorum in different geographic regions to form local apomictic populations.

This is also true of natural hybridization between B. intermedia and D. annulatum. This cross only rarely takes place in Southeast Asia and Australia, but is a common weed in the extensively cultivated Gangetic plain of India. Chemical and morphological data suggested that hybridization takes place between D. annulatum and the northern, rather than the tropical, Indian race of B. intermedia. This chemical race from northern India may in itself represent a hybrid between tropical B. intermedia and the Eurasian B. ischaemum which are sympatric in northern Burma. This would also explain the ease with which hybrid derivatives between B. intermedia and D. annulatum cross with B. ischaemum in northern West Pakistan.

The genera Bothriochloa and Dichanthium are distinctly different chemically. The latter genus, and particularly D. annulatum is characterized by indistinct peaks (Plate VIII, Fig. 13). This is often also true of assumed natural hybrids between this species and B. intermedia.

The genera Bothriochloa and Capillipedium are also chemically distinct. Harlan and de Wet (1963 a) suggested that C. spicigerum represents a hybrid between B. intermedia from Australia and C. parviflorum. Chemically C. spicigerum is more closely allied to Capillipedium than it is to Bothriochloa.

The compilospecies incorporates genetic material of B. ischaemum, Dichanthium annulatum and Capillipedium parviflorum with that of B. intermedia. Morphologically and chemically the species contributing towards the variability of B. intermedia are quite distinct. The three genera, Bothriochloa, Capillipedium and Dichanthium, are each characterized by a distinct range of morphological and chemical variation. Should the compilospecies be removed, those species hybridizing with it would be completely isolated genetically. However, the species B. glabra, B. grahamii, B. haenkei and B. odorata classically recognized, represent morphological variants of hybrid derivatives involving B. intermedia, B. ischaemum, D. annulatum and C. parviflorum.

Conclusions

1. The aggressive nature of the compilospecies Bothriochloa intermedia is reflected in its chemical variability.

2. Each morphological type within this complex has a recognizable chemical type.

3. Assumed affinities can be detected chemically, but the origin

of natural hybrids are often difficult to determine on a strictly chemical basis.

4. Backcrossing of the hybrids in all directions probably accounts, in part, for this extreme chemical variability.

5. Another reason for the observed variability lies in the fact that additive chemical complementation apparently does not always operate in this group.

CHAPTER VI

CHEMICAL TAXONOMIC STUDIES IN THE BOTHRIOCHLOININAE

The grasses examined were grown in an essentially uniform garden at Stillwater, Oklahoma after the manner described by Celarier and Harlan (1956). Morphological studies of the features described by Harlan (1963 b) were made and the plants studied are on file at the Oklahoma State University. Cytological examinations of microsporocytes were made after staining with aceto-carmine. A gas chromatograph was used to study the specimens chemically. The resulting chromatograms were reproduced or an estimation of the relative amounts of the chemicals were plotted on polygonal graphs to form profiles characteristic of the plants or species studied.

Oil samples from 210 collections of 27 species belonging to the genera Bothriochloa, Capillipedium and Dichanthium were studied. This included collections from various ecological and geographical regions of some of the widely distributed species. All together twenty-one chromatographic peaks were detected in this study, but not all peaks were discernible in every chromatogram.

The genus Dichanthium--Under the experimental conditions used, the chromatograms of Dichanthium species, without exception, showed a high initial peak which tailed badly, often with a second major tailing-peak coming after the recorder pen had reached the halfway point in its downward travel. It would seem that these peaks, or more appropriately

"humps", are caused by chemicals that have a high polar affinity for the column substrate. The second is likely the isomeric form of the first, judging from the way it elutes from the column. The exact chemical nature of these compounds is unknown. All modifications of operating conditions in an effort to resolve these peaks proved futile. Some of these tailing peaks had superimposed upon them slight protrusions as a result of trace amounts of other chemicals belonging to the series of twenty-one mentioned above. A liberal interpretation of these chromatograms, that is, if we assume some or most of the 21 peaks are present but masked by the humps, would result in a profile that is oval-, elliptical- or circinate-shaped. This would tend to exaggerate the relatedness of the Dichanthium species. Therefore, the chromatographic characteristics of these species are summarized in Table VII.

From this table it will be noted that considerable variation exists in the genus. The species D. annulatum is especially variable, even within the two ecotypes. It is tempting to postulate that the one-hump, one-peak condition found in the tropical ecotype (9426) is the primative condition for this species, however our knowledge of the biogenesis of these substances or even the nature of them is too limited to warrant such an assumption. The plant (6897) appears to have some genes of the tetraploid D. caricosum influencing its morphology. This could be true of its chemistry also. Unfortunately however, only the diploid D. caricosum was available for chemical study. The tropical ecotype plant (x98) is an assumed autotetraploid, derived from an attempted cross of the diploid ($2n = 20$) plant (3242) and the tetraploid ($2n = 40$) tropical ecotype plant (5411). The original plant (3242) was not available for study, but on the basis of the chromatographic data it would appear that

TABLE VII

CHROMATOGRAPHIC CHARACTERISTICS OF SPECIES OF DICHANTHIUM

Species	2n	Collection number	No. of humps	Detectable superimposed peaks
D. annulatum var. *annulatum*				
Tropical ecotype	40	9426	1	U
	40	6897	2	E, F, J, L, P
	40	(x98)	2	A, B, C, E, F, J, L, P, U
Mediterranean ecotype	40	8894c	1	P
	40	8972c	1	E, G, J, P, U
	40	8853	1	E, F, G, J, L, N, P, U
	40	8874b	2	E, F, I, J, L, M, P, R
D. annulatum var. *papillosum*	60	9692	2	B, J, L, P, U
D. aristatum	40	9038	2	E, J, U
D. caricosum	20	8452b	2	A, E, F, J, L, P, R
D. humilius	20	7534	2	A, B, D, E, F, G, J, L
D. maccannii	20	9049	2	A, D, E, F, J, L, M, P, S, U
D. panchganiense	20	9040	1	B, D, E, G, J, L, P, R, S, U
D. sericeum	20	8137	2	none detectable
D. setosum	20	8138	2	E, P
D. superciliatum	20	9345	2	E, N

this plant may actually be a hybrid resulting from the union of an unreduced female gamete and normal pollen.

The variation in numbers of detectable peaks found in the mediterranean ecotype of D. annulatum might be explained as what one would expect to find in the progeny of a hybrid, especially if the progeny were largely apomictic. The D. aristatum samples were remarkably uniform in their chromatograms, especially when we consider that at the tetraploid level this species forms agamic complexes with both D. annulatum and D. caricosum (Celarier, de Wet and Richardson, 1961).

Of the diploids in Table VII, the morphologically and geographically allied species D. maccannii and D. panchganiense appear also to be rather closely allied chemically. The only major difference is that D. maccannii shows two humps, whereas D. panchganiense has only the first one. In those chromatograms with two humps the first elutes from the column at about the position of peak C and the second appears at the position of peak G, therefore the occurrence of peak G in D. panchganiense is perhaps not significant. The occurrence of so many peaks in both these species at first seems to suggest affinity with Bothriochloa, however, in view of the relict nature of these two species, it is more likely a case where Bothriochloa and Dichanthium overlap due to common ancestry.

The more widely distributed active diploids D. superciliatum, D. humilius, D. sericeum and D. setosum all show the characteristic Dichanthium chromatograms. Of these, only D. humilius appears to have several traces of peaks in its chromatogram. The relatively few chemicals in the others is interesting in view of the hypothesis suggested by de Wet and Harlan (1962) that D. superciliatum, D. humilius and D. seri-

ceum may have originated from D. setosum.

The genus Capillipedium--In direct contrast to the genus Dichanthium which universally lacks well defined peaks, the Capillipedium species studied, consistently had more easily discerned chemical peaks. Tetraploid representatives of C. assimile were not available for study. It should be especially interesting to study the tetraploid, since the chromatogram of the C. assimile showed less chemical contrast than the tetraploids of other species of this genus.

The chromatograms of the three related taxa C. parviflorum, C. parviflorum subsp. capilliflorum and C. spicigerum showed basic similarities reflecting their affinities, yet each is distinct from the others. The sample from C. spicigerum appeared to be the most distinct of the three.

The genus Bothriochloa--Chemically, Capillipedium appears to be the most uniformly asymmetrical group while Dichanthium is the least and Bothriochloa holds an intermediate position between them. Some species of Bothriochloa had chromatograms which resembled those of Dichanthium, others resembled those of Capillipedium and still other species of Bothriochloa deviated from one extreme to the other. Such profiles cannot be taken as prima-facie evidence of introgression of Bothriochloa with the other two genera; however, when these profiles can be correlated with morphological and cytological data then the inferences cannot be ignored. Harlan et al. (1961) emphasized that one of the basic genomes of Bothriochloa as well as the basic Dichanthium genome is devoid of aromatic pungency. This may reflect the ancestral affinities of this genus with Dichanthium and as a corollary, the presence of pungency may be the result of ancestral affinities with Capillipedium. At any rate,

both conditions are well established in the genus Bothriochloa at the present time.

The diploid species of Bothriochloa studied included the relicts, B. compressa, B. longifolia and B. kuntzeana. The species B. compressa had a profile which bore a Capillipedium-like sharpness, however no species of the latter genus was found to have an exactly similar profile. It may be simply a case of parallel evolution from common ancestors. The other two species showed closer chemical affinities with Dichanthium. The species B. kuntzeana possessed the one-hump chromatogram, and traces of peaks E, G, J, N, P and R. The species B. longifolia had the two-hump type of chromatogram with peaks at B, D, E, J, K and P.

The Australian sexual polyploid B. decipiens showed a considerable amount of chemical variation. This could be expected of a sexually reproducing group. But, in contrast to this variability, some North American sexual polyploids were remarkably constant chemically. Specimens of B. barbinodis vary considerably, both morphologically and cytologically, but not so much chemically. The $2n = 180$ specimens from Texas and Mexico were slightly different, but the latter was chemically almost identical with the $2n = 220$ race from Texas. The species B. saccharoides and B. exaristata were chemically distinct from one another and from the other sexual polyploids, yet an overall basic similarity in the profiles of these sexual polyploids lends credence to the idea that these species represent relicts of a once widely distributed section of the genus Bothriochloa.

The obligate apomictic species B. concanensis and B. woodrowii, although closely associated ecologically, were found to be chemically

unique. A single very weak Dichanthium-like hump with traces of peaks B, E, G, P, S and U characterized B. concanensis. On the other hand, B. woodrowii may have had affinities with the more Capillipedium-like ancestors of Bothriochloa, in fact its profile had a strong resemblance to some specimens of typical B. intermedia. The B. pertusa var. bifoveolata had very little in common chemically with the two preceding species. It did possess the single slowly eluting hump, but the chemical peaks were very prominently registered upon it.

The hexaploid B. insculpta appeared to be quite variable, while the pentaploid form was very uniform chemically within its distribution range from Rhodesia to its southern limit in South Africa. It probably represents a widely-spread strictly-obligate apomict which is somehow related to the tetraploid B. intermedia.

Essentially, there were four moderately overlapping types of chromatograms found in the variable apomict B. ischaemum. Some had the two trailing humps reminiscent of Dichanthium with many trace peaks superimposed upon them. However, since some specimens possessing this type of profile are from Russia, and are presumed to have been isolated from Dichanthium for quite some time, it is more likely that these represent the non-aromatic Bothriochloa group. Others had a very flat chromatogram with good peaks which closely resembled specimens of B. grahamii. Another type of profile, which is probably the aromatic B. ischaemum used in the essential oil industry (Tucakov, 1957), resembled that of typical B. intermedia, while still others were more closely allied chemically with an apparent natural hybrid of B. intermedia and B. ischaemum.

The compilospecies B. intermedia is a highly variable taxon chemically as well as morphologically and cytogenetically. Six taxa and

morphological variants were recognized in this complex. Among these there was considerable overlap and in some instances almost continuous gradients from one extreme to another were recognizable. Thus, one entity may have chemical profiles approaching or almost duplicating profiles of other entities. These similarities are undoubtedly the result of the introgression that is taking place within the compilospecies itself.

The profile of B. ensiformis not only resembled some of typical B. intermedia, but also B. glabra. A type of typical B. intermedia was similar to the profiles of B. grahamii, B. glabra and B. ischaemum. Still another type of profile of typical B. intermedia showed affinities with Capillipedium parviflorum.

Two types of profiles, representing the two extremes of a gradient from the one to the other, were found in B. glabra. The chromatogram of one type appeared to be peaks superimposed on a single tailing hump, while the other was devoid of such a feature. In reviewing the chromatograms, perhaps because of the gradient involved, there appeared to be a striking amount of uniformity in this group. Another apparently chemically uniform group was found in B. grahamii. Intergradation of chromatograms was found to be characteristic of this group also. These chromatograms revealed that, by and large, B. grahamii lacked the high asymmetrical peaks of some of the other members of this complex.

Chemically more variable than the taxa above, was the apparently natural hybrid of B. grahamii and B. ischaemum. Besides profiles resembling the assumed parents, this hybrid had some profiles which resembled typical B. intermedia and others appeared to have affinities with Capillipedium, or at least Capillipedium-like B. intermedia.

Another apparently natural hybrid, (B. intermedia x B. ischaemum) was also found to be quite polytypic chemically. Specimens of this morphological entity chemically resembled typical B. intermedia, B. glabra, B. ischaemum, and the assumed hybrid B. grahamii x B. ischaemum. The highly polytypic nature of the compilospecies is indicative of its aggression and of the wide latitude of backcrossing it maintains.

LITERATURE CITED

Alam, S. Q., J. Brossard and G. Mackinney. 1962. Detection and estimation of squalene in leaves. Nature 194: 479-480.

Alston, R. E. and H. S. Irwin. 1961. The comparative extent of variation of free amino acids and certain "secondary" substances among Cassia species. Amer. Jour. Bot. 48: 35-39.

Alston, R. E., A. J. Mabry and B. L. Turner. 1963. Perspectives in chemotaxonomy. Science 142: 545-552.

Alston, R. E. and J. Simmons. 1962. A specific and predictable biochemical anomaly in interspecific hybrids of Baptisia viridis x B. leucantha. Nature 195: 825.

Alston, R. E. and B. L. Turner. 1962. New techniques in analysis of complex natural hybridization. Proc. Natl. Acad. Sci. 48: 130-137.

Alston, R. E. and B. L. Turner. 1963. Biochemical Systematics. Prentice-Hall, Inc., Englewood Cliffs, N. J.

Alston, R. E., B. L. Turner, R. N. Lester and D. Horne. 1962. Chromatographic validation of two morphologically similar hybrids of different origins. Science 137: 1048-1050.

Asahima, Y. and S. Shibata. 1954. Chemistry of Lichen Substances. Japan. Soc. for Promotion of Sci. Ueno, Tokyo, Japan.

Bailey, S. D., D. G. Mitchell, M. L. Bazinet and C. Weurman. 1962. Studies on the volatile components of different varieties of cocoa beans. Jour. Food Sci. 27: 165-170.

Baker, H. G. 1959. Reproductive methods as factors in speciation in flowering plants. Cold Spring Harbor Symp. Quant. Biol. 24: 177-191.

Baker, R. T. and H. G. Smith. 1902. A Research on the Eucalypts, especially in Regard to their Essential Oils. 1st ed. Sydney, Australia.

Bannister, M. H., H. V. Brewerton and I. R. C. McDonald. 1959. Vapour-phase chromatography in a study of hybridism in Pinus. Svensk Papperstidning 62: 567-573.

Bannister, M. H., A. L. Williams, I. R. C. McDonald and M. B. Forde. 1962. Variation of turpentine composition in five population samples of Pinus radiata. New Zealand Jour. Sci. 5: 486-495.

Bate-Smith, E. C. 1958. Plant phenolics as taxonomic guides. Proc. Linn. Soc. London 169: 198-211.

Bate-Smith, E. C. 1961. Chromatography and taxonomy in the Rosaceae, with special reference to Potentilla and Prunus. Jour. Linn. Soc. London (Bot.) 58: 39-54.

Bate-Smith, E. C. 1962. The phenolic constituents of plants and their taxonomic significance. I. Dicotyledons. Jour. Linn. Soc. London (Bot.) 58: 95-173.

Bate-Smith, E. C. 1963. Usefulness of chemistry in plant taxonomy as illustrated by the flavonoid constituents. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 127-139.

Bernhard, R. A. and R. Wrolstad. 1963. The essential oil of Schinus molle: The terpene hydrocarbon fraction. Jour. Food Sci. 28: 59-63.

Birch, A. J. 1963. Biosynthetic pathways. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 141-166.

Birdsong, B. A., R. E. Alston and B. L. Turner. 1960. Distribution of Canavanine in the family Leguminosae as related to phyletic groupings. Canad. Jour. Bot. 38: 499-505.

Blackman, F. F. 1921. The biochemistry of carbohydrate production in the higher plants from the point of view of systematic relationship. New Phytol. 20: 2-9.

Blake, S. T. 1944. Monographic studies in the Australian Andropogoneae. I. Univ. Queensl. Papers 2(3): 1-62.

Block, R. J., E. L. Durrum and G. Zweig. 1958. A Manual of Paper Chromatography and Paper Electrophoresis. Academic Press Inc., N. Y.

Blundstone, H. A. W. 1963. Paper chromatography of organic acids. Nature 197: 377.

Bonner, J. 1963. The future welfare of botany. AIBS Bull. 13(1): 20-21.

Bor, N. L. 1960. The Grasses of Burma, Ceylon, India and Pakistan. Pergamon Press, London.

Borgaonkar, D. S. and J. M. J. de Wet. 1961. Interspecific hybrids in Bothriochloa. III. Relationships in some American species. Proc. Okla. Acad. Sci. 41: 10-13.

Brown, R. 1810. Prodromus Florae Novae Hollandiae: I. London.

Camus, A. 1931. Le genre Bothriochloa. Ann. Soc. Linn. Lyon 76: 162-165.

Carvalho, A. 1962. Variability of the niacin content in coffee. Nature 194: 1096.

Celarier, R. P. 1957. The cytogeography of Bothriochloa ischaemum complex. II. Chromosome behavior. Amer. Jour. Bot. 44: 729-738.

Celarier, R. P., J. M. J. de Wet and W. L. Richardson. 1961. Species relationships in Dichanthium. I. Hybrids between D. caricosum, D. aristatum and D. annulatum. Phyton (Argentina) 16: 63-67.

Celarier, R. P. and J. R. Harlan. 1955. Studies on Old World Bluestems. Okla. Agric. Expt. Sta. Tech. Bull. T-58: 1-32.

Celarier, R. P. and J. R. Harlan. 1956. An Andropogoneae garden in Oklahoma. Taxon 5: 183-186.

Celarier, R. P. and J. R. Harlan. 1958. The cytogeography of the Bothriochloa ischaemum complex. I. Taxonomy and geographic distribution. Jour. Linn. Soc. London (Bot.) 55: 755-760.

Chester, K. S. 1937. A critique of plant serology. Quart. Rev. Biol. 12: 19-46; 165-190; 294-321.

Chheda, H. R., J. M. J. de Wet and J. R. Harlan. 1961. Aneuploidy in Bothriochloa hybrids. Caryologia 14: 205-217.

Chheda, H. R. and J. R. Harlan. 1962. Mode of chromosome association in Bothriochloa hybrids. Caryologia 15: 461-476.

Chippindall, L. K. A. 1955. A guide to the identification of grasses in South Africa. (in: Meredith, D., Grasses and Pastures of South Africa) I: 1-527.

Clausen, K. E. 1962. Introgression in Minnesota birches. Proc. Forest Genet. Workshop (Macon, Ga., Oct. 25-27, 1962): 55-58.

Clausen, K. E. 1963. Characteristics of a hybrid birch and its parent species. Canad. Jour. Bot. 41: 441-458.

Dal Nogare, S. and R. S. Juvet, Jr. 1962. Gas-liquid Chromatography, Theory and Practice. Interscience Publishers, N. Y.

Davidson, J. F. and T. L. Thompson. 1956. Phytoserology versus genealogy in Zea mays. Madroño 13: 252-259.

Davies, E. G., W. M. Ashton and M. Borrill. 1962. Distribution of coumarin and related compounds during leaf growth and development in Anthoxanthum species. Jour. Brit. Grassland Soc. 17: 294-299.

Dent, J. W. and D. T. A. Aldrich. 1963. Soluble carbohydrate content of varieties of tetraploid Ryegrass. Nature 198: 905.

de Wet, J. M. J. and D. S. Borgaonkar. 1963. Aneuploidy and apomixis in Bothriochloa and Dichanthium. Bot. Gaz. 124: 437-440.

de Wet, J. M. J., D. S. Borgaonkar and H. R. Chheda. 1961. Intergeneric hybrids in the Bothriochloininae II. Bothriochloa and Capillipedium. Cytologia 26: 268-273.

de Wet, J. M. J., D. S. Borgaonkar and W. L. Richardson. 1963. Chromosome number and mode of reproduction in the Bothriochloininae. Caryologia 16: 47-55.

de Wet, J. M. J. and J. R. Harlan. 1962. Species relationships in Dichanthium. III. D. sericeum and its allies. Phyton (Argentina) 18: 11-14.

de Wet, J. M. J. and M. L. Higgins. 1963. Species relationships within the Bothriochloa pertusa complex. Phyton (Argentina) 20: 205-211.

de Wet, J. M. J. and M. L. Higgins. 1964. The cytology of the Bothriochloa pertusa complex. Cytologia 29: (in press).

de Wet, J. M. J., K. L. Mehra and D. S. Borgaonkar. 1961. Chromosome association in Dichanthium hybrids. Cytologia 26: 78-82.

de Wet, J. M. J. and W. L. Richardson. 1963. Morphological variation and species relationships in Dichanthium. Phyton (Argentina) 20: 19-28.

de Wet, J. M. J. and A. P. Singh. 1964. Species relationships in Dichanthium V. The diploid species. Caryologia 17: 153-160.

Douglas, B., J. L. Kirkpatrick, R. F. Raffauf, O. Ribeiro and J. A. Weisbach. 1964. Problems in Chemotaxonomy. II. The major alkaloids of the genus Heimia. Lloydia 27: 25-31.

Dreiding, A. S. 1961. The betacyanins, a class of red pigments in the Centrospermae. (in: Ollis, W. D., Recent Developments in the Chemistry of Natural Phenolic Compounds. Pergamon Press, N. Y.) pp. 194-211.

Earle, F. R. and Q. Jones. 1962. Analysis of seed samples from 113 plant families. Econ. Bot. 16: 221-250.

Eglinton, G., A. G. Gonzalez, R. J. Hamilton and R. A. Raphael. 1962 a. Hydrocarbon constituents of the wax coatings of plant leaves: A taxonomic survey. Phytochem. 1: 89-102.

Eglinton, G. and R. J. Hamilton. 1963. The distribution of alkanes. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 187-217.

Eglinton, G., R. J. Hamilton and M. Martin-Smith. 1962. The alkane constituents of some New Zealand plants and their possible taxonomic implications. Phytochem. 1: 137-145.

Eglinton, G., R. J. Hamilton, R. A. Raphael and A. G. Gonzalez. 1962 b. Hydrocarbon constituents of the wax coatings of plant leaves: A taxonomic survey. Nature 193: 739-742.

Endo, T. 1962. Inheritance of anthocyanin concentrations in flowers of Torenia fournieri. Japan. Jour. Genet. 37: 284-290.

Enslin, P. R. and S. Rehm. 1958. The distribution and biogenesis of the cucurbitacins in relation to the taxonomy of the Cucurbitaceae. Proc. Linn. Soc. London 169: 230-238.

Erdtman, H. 1963. Some aspects of chemotaxonomy. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 89-125.

Fairbrothers, D. E. and M. A. Johnson. 1961. The precipitation reaction as an indicator of relationship in some grasses. Recent Adv. in Bot. 1: 116-120.

Flück, H. 1963. Intrinsic and extrinsic factors affecting the production of natural products. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 167-186.

Flynn, T. 1964. Some thoughts on chromatographic anomalies and computer efficiencies. Gas Chromatography Newsletter (The Perkin-Elmer Corp., Norwalk, Conn.) 1(2): 4.

Fowden, L. 1962. The non-protein amino acids of plants. Endeavour 21: 35-42.

Gardner, C. A. 1952. Flora of Western Australia. I. (Pt. I): 297-344.

Garrick, L. S. and H. M. Habermann. 1962. Distribution of allagochrome in vascular plants. Amer. Jour. Bot. 49: 1078-1088.

Gibbs, R. D. 1954. Comparative chemistry and phylogeny of flowering plants. Trans. Roy. Soc. Canada III, 48: 1-47.

Gibbs, R. D. 1963. History of chemical taxonomy. (in: Swain, T., Chemical Plant Taxonomy, Academic Press Inc., London) pp. 41-88.

Gildemeister, E. and Fr. Hoffman. 1956. Die Ätherischen Öle. Vol. 4. Akademie-Verlag, Berlin.

Goplen, B. P., J. E. R. Greenshields and H. Baenziger. 1957. The inheritance of coumarin in sweet clover. Canad. Jour. Bot. 35: 583-593.

Gould, F. W. 1956. Chromosome counts and cytotaxonomic notes on grasses of the tribe Andropogoneae. Amer. Jour. Bot. 43: 395-404.

Gould, F. W. 1957. New North American Andropogons of subgenus Amphilophis and a key to those species occurring in the United States. Madroño 14: 18-29.

Guenther, E. 1950. The Essential Oils. Vol. IX. Individual Essential Oils of the Plant Families Gramineae, Lauraceae, Burseraceae, Myrtaceae, Umbelliferae and Geraniaceae. D. Van Nostrand Co., Inc., N. Y.

Gysels, H. 1963. New biochemical techniques applied to avian systematics. Experentia 29: 107-108.

Haag, H. B. 1941. Rafinesque's interests - A century later: Medicinal plants. Science 94: 403-406.

Hackel, E. 1889. Monographia Andropogonearum. (in: de Candolle, A., Monographiae Phanerogamarum 6., Paris).

Hagen, C. W., Jr. 1960. The role of paper chromatography in taxonomy. Proc. Indiana Acad. Sci. 70: 207. (abstr.)

Hale, M. E., Jr. 1963. Populations of chemical strains in the lichen Cetraria ciliaris. Brittonia 15: 126-133.

Hall, O. and B. L. Johnson. 1962. Electrophoretic analysis of the amphiploid of Stipa viridula x Oryzopsis hymenoides and its parental species. Hereditas 48: 530-535.

Hara, H. 1962. Racial differences in widespread species, with special reference to those common to Japan and North America. Amer. Jour. Bot. 49: 647-652.

Harborne, J. B. 1963. Distribution of anthocyanins in higher plants. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 359-388.

Harlan, J. R. 1963 a. Two kinds of gene centers in Bothriochloininae. Amer. Nat. 47: 91-98.

Harlan, J. R. 1963 b. Natural introgression between Bothriochloa ischaemum and B. intermedia in West Pakistan. Bot. Gaz. 124: 294-300.

Harlan, J. R. and H. R. Chheda. 1963. Studies on the origin of Caucasian Bluestem, Bothriochloa caucasica (Trin.) C. E. Hubbard. Crop Sci. 3: 37-39.

Harlan, J. R., H. R. Chheda and W. L. Richardson. 1962. Range of hybridization with Bothriochloa intermedia (R. Br.) A. Camus. Crop Sci. 2: 480-483.

Harlan, J. R. and J. M. J. de Wet. 1963 a. The Compilospecies concept. Evolution 17: 497-501.

Harlan, J. R. and J. M. J. de Wet. 1963 b. Role of apomixis in the evolution of the Bothriochloa-Dichanthium complex. Crop Sci. 3: 314-316.

Harlan, J. R., J. M. J. de Wet, W. L. Richardson and H. R. Chheda. 1961. Studies on Old World bluestems III. Okla. Agric. Expt. Sta. Tech. Bull. T-92: 1-30.

Harney, P. M. and W. F. Grant. 1963. Biochemical anomaly in flower extracts of interspecific hybrids between Lotus species. Science 142: 1061.

Harney, P. M. and W. F. Grant. 1964. The cytogenetics of Lotus (Leguminosae). VII. Segregation and recombination of chemical constituents in interspecific hybrids between species closely related to L. corniculatus L. Canad. Jour. Genet. Cytol. 6: 140-146.

Harz, C. O. 1880. Beiträge zur Systematik der Gramineen. Linnaea 43: 1-30.

Hegnauer, R. 1961. Taxonomic value of cyanogenesis in higher plants. Recent Adv. in Bot. 1: 82-86.

Hegnauer, R. 1962. Chemotaxonomie der Pflanzen. I. Thallophyten, Bryophyten, Pteridophyten and Gymnospermen. Birkhauser Verlag. Basel, Switzerland.

Hegnauer, R. 1963 a. The taxonomic significance of alkaloids. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 389-427.

Hegnauer, R. 1963 b. Chemotaxonomie der Pflanzen. II. Monocotyledoneae. Birkhauser Verlag. Basel, Switzerland.

Henrard, J. Th. 1940. Notes on the nomenclature of some grasses. I. Blumea 3: 411-480.

Heslop-Harrison, J. 1963. Species concepts: Theoretical and practical aspects. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 17-40.

Hillis, W. E. and H. R. Orman. 1962. The extractives of New Zealand Nothofagus species. Jour. Linn. Soc. London (Bot.) 58: 175-184.

Hitchcock, A. S. 1935. Manual of the Grasses of the United States. (1st ed.) USDA Misc. Publ. No. 200. Washington

Hubbard, C. E. 1934. Gramineae Australiensis. II. Kew Bull. 1934: 444-451.

Ibrahim, R. K., G. H. N. Towers and R. D. Gibbs. 1962. Syringic and sinapic acids as indicators of differences between major groups of vascular plants. Jour. Linn. Soc. London (Bot.) 58: 223-230.

Issenberg, P. and E. L. Wick. 1963. Volatile components of Bananas. Jour. Agric. Food Chem. 11: 2-8.

Jahnsen, V. J. 1962. Complexity of hop oil. Nature 196: 474-475.

James, A. T. and A. J. P. Martin. 1952. Gas-liquid partition chromatography: the separation and microestimation of fatty acids from formic acid to dodecanoic acid. Jour. Biochem. 50: 679-690.

Keller, R. A. 1961. Gas chromatography. Sci. Amer. 205(4): 58-67.

Kessler, E. and C. J. Soeder. 1962. Biochemical contributions to the taxonomy of the genus Chlorella. Nature 194: 1096-1097.

Kindl, H. and G. Billek. 1962. 2-Hydroxyphenylacetic Acid, a new constituent of higher plants. Nature 194: 579.

Kjaer, A. 1963. The distribution of sulphur compounds. (in: Swain, T. Chemical Plant Taxonomy. Academic Press Inc., London) pp. 453-473.

Kloz, J. 1962. An investigation of the protein characters of four Phaseolus species with special reference to the question of their phylogenesis. Biol. Plant. (Praha) 4: 85-90.

Kloz, J. and V. Turková. 1963. Legumin, Vicilin and proteins similar to them in seeds of some species of the Viciaceae Family (a comparative serological study). Biol. Plant. (Praha) 5: 29-40.

Kupchan, S. M., J. H. Zimmerman and A. Afonso. 1961. The alkaloids and taxonomy of Veratrum and related genera. Lloydia 24: 1-26.

Laetsch, W. M. 1963. The welfare of botany re-examined. AIBS Bull. 13 (6): 21-22.

Löve, Á. and D. Löve. 1957. Drug content and polyploidy in Acorus. Proc. Genet. Soc. Canada 2: 14-17.

McNair, J. B. 1916. The poisonous principle of poison oak. Jour. Amer. Chem. Soc. 38: 1417-1421.

McNair, J. B. 1917. Fats from Rhus laurina and Rhus diversiloba. Bot. Gaz. 64: 330-336.

McNair, J. B. 1929. The taxonomic and climatic distribution of oils, fats, and waxes in plants. Amer. Jour. Bot. 16: 832-841.

McNair, J. B. 1930 a. Gum, tannin, and resin in relation to specificity, environment and function. Amer. Jour. Bot. 17: 187-196.

McNair, J. B. 1930 b. The taxonomic and climatic distribution of oil and starch in seeds in relation to the physical and chemical properties of both substances. Amer. Jour. Bot. 17: 662-668.

McNair, J. B. 1931. Some properties of plant waxes in relation to climate of habitat. Amer. Jour. Bot. 18: 518-525.

McNair, J. B. 1932. Some properties of plant substances in relation to climate of habitat--Volatile oils, saponins, cyanogenetic glucosides, and carbohydrates. Amer. Jour. Bot. 19: 168-193.

McNair, J. B. 1934. The evolutionary status of plant families in relation to some chemical properties. Amer. Jour. Bot. 21: 427-452.

McNair, J. B. 1935 a. The taxonomic and climatic distribution of alkaloids. Bull. Torr. Bot. Club 62: 219-226.

McNair, J. B. 1935 b. Angiosperm phylogeny on a chemical basis. Bull. Torr. Bot. Club 62: 515-532.

McNair, J. B. 1941. Energy and evolution. Phytologia 2: 33-50.

McNair, J. B. 1942. Some chemical properties of Eucalyptus in relation to their evolutionary status. Madroño 6: 181-190.

McNair, J. B. 1945. Plant fats in relation to environment and evolution. Bot. Rev. 11: 1-59.

Martin, A. J. P. and R. L. M. Synge. 1941. A new form of chromatogram employing two liquid phases. Jour. Biochem. 35: 1358-1368.

Martin, L., D. M. Smith and C. G. Farmilo. 1961. Essential oil from fresh Cannabis sativa and its use in identification. Nature 191: 774-776.

Masłowski, P. 1962. Comparative biochemical studies of proteins in different varieties of maize (Corn). Genet. Polon. 3: 275-284.

Mika, E. S. 1962. Selected aspects on the effect of environment and heredity on the chemical composition of seed plants. Lloydia 25: 291-295.

Mikołajczyk, J. and E. Nowacki. 1961. Inheritance of alkaloid content in white Lupin (Lupinus albus L.) Genet. Polon. 2: 55-64.

Mirov, N. T. 1961. Biochemical geography of the genus Pinus. Recent Adv. in Bot. 1: 72-77.

Mirov, N. T. 1963. Chemistry and plant taxonomy. Lloydia 26: 117-124.

Nowacki, E. 1963. Inheritance and biosynthesis of alkaloids in Lupin. Genet. Polon. 4: 161-202.

Ohwi, J. 1942. Gramina japonica: III, IV. Act. Phytotax. Geobot. 11: 27-56; 145-193.

Ohwi, J. 1947. New or noteworthy grasses from Asia. Bull. Tokyo Sci. Mus. n. 18.

Paris, R. 1963. The distribution of plant glycosides. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 337-358.

Parry, E. J. 1921. The Chemistry of Essential Oils and Artificial Perfumes. Vol. I. Scott, Greenwood & Son, London.

Pecket, R. C. 1960 a. Phenolic constituents of leaves and flowers in the genus Lathyrus. (in: Pridham, J. B., Phenolics in Health and Disease. Pergamon Press, N. Y.) pp. 119-126.

Pecket, R. C. 1960 b. The nature of the variation in flower colour in the genus Lathyrus. New Phytol. 59: 138-144.

Pecket, R. C. and A. R. A. A. Selim. 1962. Blueness of flowers of Lathyrus hybrids. Nature 195: 620.

Plouvier, V. 1963. The distribution of aliphatic polyols and cyclitols. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 313-336.

Preiniger, Vl., P. Vácha, B. Šula and F. Šantavý. 1962. Isolierung der Alkaloide aus einigen Papaver-Arten. 5. Planta Medica 10: 124-133.

Price, J. R. 1963. The distribution of alkaloids in the Rutaceae. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 429-452.

Przybylska, J. and E. Nowacki. 1961. An attempt to apply a chromatographic analysis of free amino-acids in the taxonomy of the genus Lathyrus. Genet. Polon. 2: 39-53.

Raffauf, R. F. 1962. Mass screening of plants for alkaloids. Lloydia 25: 255-256.

Raizada, M. B. 1951. The genus Capillipedium in India. Indian For. Rec. n. s. (Bot.) 4:4; 73-81.

Rangaswami, S. 1962. Application of chemical analysis to some cases of synonymity in botanical nomenclature. Current Sci. 31: 495-496.

Ray, N. H. 1954. Gas chromatography. I. The separation and estimation of volatile organic compounds by gas-liquid partition chromatography. Jour. Appl. Chem. 4: 21-25.

Reichert, E. T. 1919. A biochemic basis for the study of problems of taxonomy, heredity, evolution, etc. with special reference to the starches and hemoglobins of varieties, species and genera. 2 pts. Carnegie Inst. Publ., No. 270.

Richardson, W. L. 1958. A technique of emasculating small grass florets. Indian Jour. Genet. Plant Breed. 18: 69-73.

Riley, H. P. and T. R. Bryant. 1961. The separation of nine species of the Iridaceae by paper chromatography. Amer. Jour. Bot. 48: 133-136.

Riley, H. P. and J. D. Hopkins. 1962. Paper chromatographic studies in the Aloineae. II. The Rigidae and Retusae sections of Haworthia. (Abstract). Internatl. Conf. on Taxonomic Biochemistry, Physiology, and Serology: 51.

Riley, H. P. and C. J. Isbell. 1963. Paper chromatographic studies in the Aloineae I. Preliminary observations on some species of Haworthia. Jour. So. Afr. Bot. 29: 59-73.

Roberty, G. 1960. Monographia Systematique des Andropogonées du globe. Boissiera 9: 1-455.

Rowson, J. M. 1958. Alkaloids and plant taxonomy. Proc. Linn. Soc. London 169: 212-216.

Shetler, S. G. 1963. Botany--A passing phase? AIBS Bull. 13(6): 23-25.

Shimizu, S. and N. Ikeda. 1962. Studies on the essential oils of interspecific hybrids in the genus Mentha VIII. The essential oils of Mentha spicata L. with 36 or 48 somatic chromosomes. Agric. Biol. Chem. 26: 543-545.

Shorland, F. B. 1963. The distribution of fatty acids in plant lipids. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London). pp. 253-311.

Shulgin, A. T. 1963. Composition of the Myristicin fraction from Oil of Nutmeg. Nature 197: 379.

Singh, A. P. and J. M. J. de Wet. 1960. Interspecific hybrids in Bothriochloa. I. Relationships between B. ambigua and B. ischaemum. Phyton (Argentina) 15: 159-162.

Singh, A. P. and J. M. J. de Wet. 1961. Interspecific hybrids in Bothriochloa. II. Relationships between some American and Australian species. Proc. Okla. Acad. Sci. 41: 35-38.

Slater, C. A. 1963. Citrus Essential Oils. III. Evaluation of Sicilian natural lemon oils. Jour. Sci. Food Agric. 14: 58-64.

Smith, C. E., Jr. 1964. How dead? How soon? BioScience 14(1): 15-16.

Smith, D. M. and L. Levi. 1961. Treatment of compositional data for the characterization of essential oils. Determination of geographical origins of peppermint oils by gas chromatographic analysis. Jour. Agric. Food Chem. 9: 230-244.

Smith, D. M. and D. A. Levin. 1963. A chromatographic study of reticulate evolution in the Appalachian Asplenium complex. Amer. Jour. Bot. 50: 952-959.

Smith, D. M., W. Skakum and L. Levi. 1963. Essential Oils, determination of botanical and geographical origin of spearmint oils by gas chromatographic and ultraviolet analysis. Jour. Agric. Food Chem. 11: 268-276.

Smith, H. H. and D. V. Abashian. 1963. Chromatographic investigations on the alkaloid content of Nicotiana species and interspecific combinations. Amer. Jour. Bot. 50: 435-446.

Sokal, R. R. and P. H. A. Sneath. 1963. Principles of Numerical Taxonomy. W. H. Freeman and Co., San Francisco.

Sørensen, N. A. 1963. Chemical taxonomy of acetylenic compounds. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 219-252.

Stafford, H. A. 1959. Distribution of tartaric acid in the leaves of certain angiosperms. Amer. Jour. Bot. 46: 347-352.

Stafford, H. A. 1961. Distribution of tartaric acid in the Geraniaceae. Amer. Jour. Bot. 48: 699-701.

Stapf, O. 1917. Gramineae. (in: Prain, D., Flora Tropical Africa 9, London) pp. 1-768.

Stebbins, G. L. 1947. Types of polyploids; their classification and significance. Adv. Genet. 1: 403-429.

Stebbins, G. L. 1950. Variation and Evolution in Plants. Columbia Univ. Press, N. Y.

Stebbins, G. L., B. L. Harvey, E. L. Cox, J. N. Rutger, G. Jelencovic and E. Yagil. 1963. Identification of the ancestry of an amphiploid Viola with the aid of paper chromatography. Amer. Jour. Bot. 50: 830-839.

Swain, T. 1963. Chemical Plant Taxonomy. Academic Press Inc., London.

Swift, L. J. 1961. Determination of Linaloöl and α-Terpineol in Florida orange products. Jour. Agric. Food Chem. 9: 298-301.

Taylor, A. O. 1964. A biochemical approach to some taxonomic problems in the genus Coprosma. New Phytol. 63: 135-139.

Tswett, M. 1906. Adsorptionsanalyse und chromatographische Methode. Anwendung auf die Chemie des Chlorophylls. Ber. deutsch. bot. Ges. 24: 384-393.

Tucakov, J. 1957. Contibution a l'etude de quelques plantes aromatiques et huiles essentielles Yougoslaves. Materiae Veg. 2: 184-218.

van Oudtshoorn, M. C. B. van Rheede. 1964. Chemotaxonomic investigations in Asphodeleae and Aloineae (Liliaceae). Phytochem. 3: 383-390.

Vickery, J. W. 1961. Flora of New South Wales. I. Gramineae. Contr. N. S. W. Natl. Herb. 19: 1-124.

Vincent, W. A. 1962. Detection of Diols on paper chromatograms. Nature 196: 771.

von Rudloff, E. 1961. Gas-liquid chromatography of terpenes. IV. The analysis of the volatile oil of the leaves of eastern white cedar. Canad. Jour. Chem. 39: 1200-1206.

von Rudloff, E. 1962 a. Gas-liquid chromatography of terpenes. V. The volatile oils of the leaves of black, white and Colorado spruce. Tappi 45: 181-184.

von Rudloff, E. 1962 b. Gas-liquid chromatography of the terpenes. VI. The volatile oil of Thuja plicata Donn. Phytochem. 1: 195-202.

von Rudloff, E. 1963 a. Gas-liquid chromatography of terpenes. VIII. The volatile oil of Tanacetum vulgare L. Canad. Jour. Chem. 41: 1737-1743.

von Rudloff, E. 1963 b. Gas-liquid chromatography of terpenes. IX. The volatile oil of the leaves of Juniperus sabina L. Canad. Jour. Chem. 41: 2876-2881.

Walters, S. M. 1963. Methods of classical plant taxonomy. (in: Swain, T., Chemical Plant Taxonomy. Academic Press Inc., London) pp. 1-15.

Weevers, T. 1943. The relation between taxonomy and chemistry of plants. Blumea 5: 412-422.

Wheldale, M. 1911. The chemical differentiation of species. Biochem. Jour. 5: 445-456.

Willaman, J. J. and B. G. Schubert. 1961. Alkaloid-bearing plants and their contained alkaloids. USDA Tech. Bull. 1234.

Willaman, J. J. and H. L. Li. 1963. General relationships among plants and their alkaloids. Econ. Bot. 17: 180-185.

Williams, A. L. and M. H. Bannister. 1962. Composition of gum turpentines from twenty-two species of pines grown in New Zealand. Jour. Pharm. Sci. 51: 970-975.

Zalkow, L. H., V. B. Zalkow and D. R. Brannon. 1963. Constitution of Intermedeol. Chem. and Ind. (London) 1963: 38.

Zalkow, V. B., A. M. Shaligram and L. H. Zalkow. 1964. Constitution and synthesis of Neo-Intermedeol. Chem. and Ind. (London) 1964: 194-195.

Zuck, R. K. 1964. Molecular botany--A new anti-intellectualism? Bio-Science 14(2): 13-14.

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