A CHEMOTAXONOMIC STUDY OF THE BULRUSHES*

BY

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INTRODUCTION

During the last decade comparative phytochemistry has been widely employed in the resolution of problems in systematic botany. An important generalization that has emerged from the biochemical studies of hybrids is the concept of complementation of the constituents of the parental species in the interspecific hybrids (Smith and Levin, 1963), i,e. the detectable chemical substances of the parental species are found together in their hybrids. A comparison of the phytochemical data of various species and putative hybrids often yields important information as to presumptive relationships. In groups of closely related species suspected hybridity may be verified and parents of the hybrid may be determined.

The purpose of this study is to correlate the evidence for interrelationships among a closely related complex of three species of *Scirpus* (bulrushes) and a suspected hybrid from the data of comparative phytochemistry and to compare these results with those obtained from known morphological and cytological information.

For reasons of simplicity of technique and ease of detection a group of naturally occurring compounds, known under the general term "phenolics", have been chosen. These compounds, although encompassing a wide range of chemical structure, are characterized by their possession of a phenolic ring as the base of their structure and fluorescent properties in UV-light (Robinson, 1963).

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MATERIALS AND METHODS

Mature inflorescences containing seeds of *Scirpus acutus, S. validus, S. heterochaetus, S. atrovirens*, and a putative hybrid of *S. acutus* and *S. validus* were provided by Dr. Larry Harms, Department of Botany, NDSU, from field collected material.

Each inflorescence, and in one case a rhizome, was ground to powder in a mortar and pestal. The powder was placed in a vial and barely covered with 1% HCl in 80% methanol elutant (1:3 v/v). After 24 hours in the dark, micropipettes were used to deliver a spot on a point 3½″ from edge of 18½″ x 22½″ Whatman #1 chomatography paper. Spotting was continued until a 0.5 ml amount of the elutant had been delivered. After allowing sufficient time for drying of the spot, the paper was placed in a chromatocab in which the atmosphere had been previously saturated with the solvent discussed below.

Each glass tray containing the folded ends of two chromatography sheets was filled with a solvent of acetic acid and water (1:5 v/v), and the solvent allowed to move down the aper for approximately 8 hours. At the end of the run, the papers were removed, air-dried, and again placed in the solvent trays of the chromatocab, the edge at a right angle to the edge first used. The trays were then filled with a solvent of n-butanol, acetic acid and water (8:2:3 v/v), and the solvent allowed to move down the paper for approximately 12 hours. After removal and drying, the papers were examined under UV-light, the separated compounds encircled, and their fluorescent characteristics noted. The separated compounds were then reexamined under UV-light in the presence of ammonia vapor and the colors noted (see table I).

The above described procedure had been carried out twice on the same material.

RESULTS

A diagrammatic summary of the separated phenolic compounds is shown in Fig. 1. The master sheet (Fig. 1) represents a composite of all spots found in the four species of *Scirpus* and the putative hybrid under investigation. Spots occurring in the same position with the same fluorescent properties were considered to be identical and each spot on the master sheet was assigned a number with 20 different spots found in the various samples. The designation of color is represented by abbreviated symbols (YG/YB, etc.) which indicate the color in UV-light alone and UV-lingt under ammonia vapor, respectively. A color key is given in the explanation of Table I. A summary of the occurence and characteristics of the 20 spots in each of the samples is shown in Table I. The designation O for variable spots indicates a spot which is present in some, absent in others of the same species, or if present in such low concentrations as to make its identification impractical in some instances.

The information from Table I was used to compile the diagrams of Figs. 2-6. Each circle is divided into 20 sections representing the 20 spots present in a composite of the five samples, and the sections are darkened for each spot present in the indicated taxa. One may visully interpret the degree of chemical similarity between the patterns of each taxon by these diagrams.

A method of quantifying the degree of similarity between the patterns of the taxa was devised

Fig. 1. Diagrammatic Representation of Two-Dimensional Paper Chromatogram (Composite for the five species)

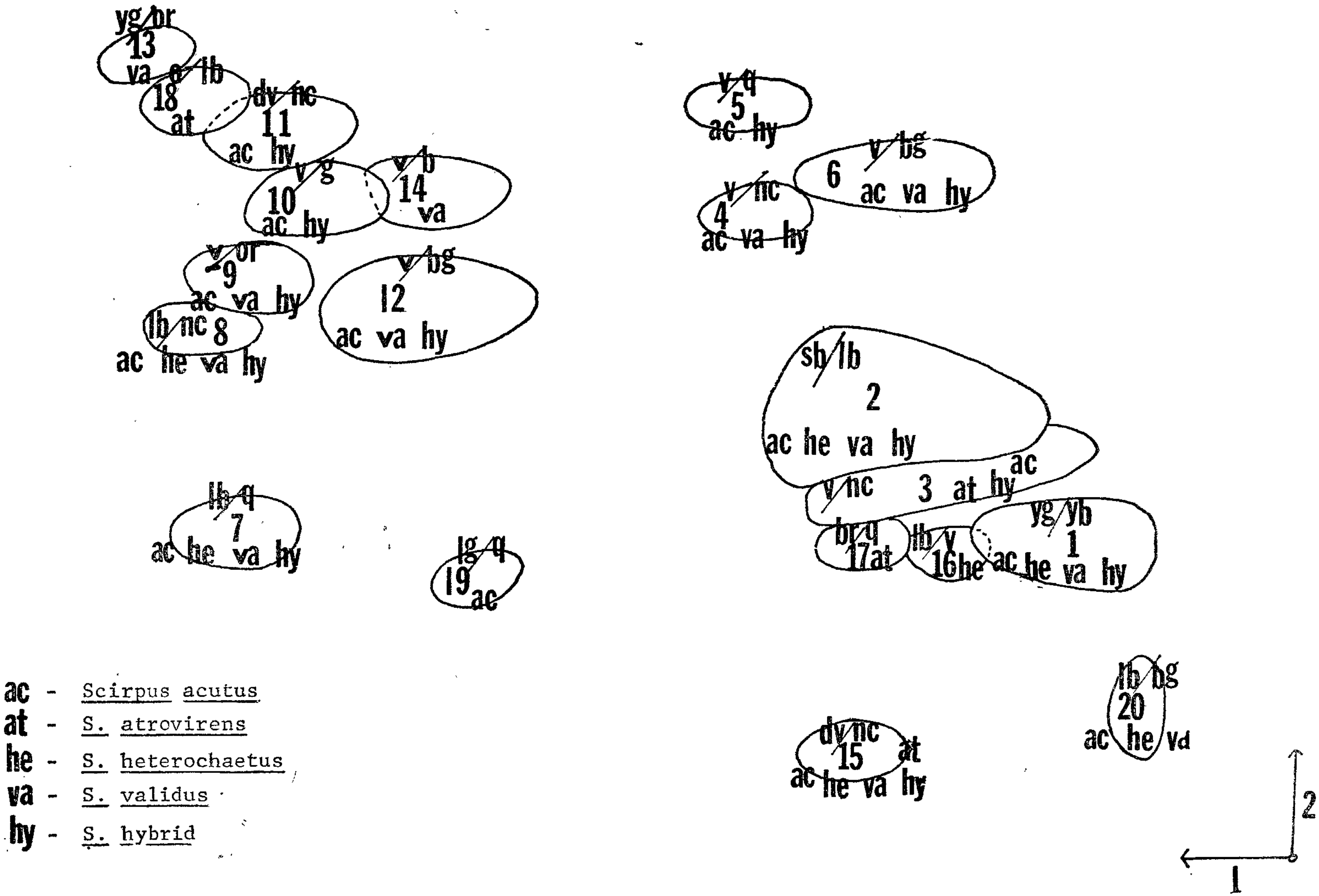

Table I. Occurrence and Characteristics of Separated Phenolics

No. of spot	color under UV/UV+am	ac	at	he-i	he-r	va	hy
1	YG/YB	X		X	X	X	X
2	SB/LB	X			X	X	X
3	V/NC	X	X	X			X
4	V/NC	X				X	X
5	V/Q	X					X
6	V/BG	X				X	X
7	LB/Q	X		X	X	X	X
8	LB/NC	X		X	X	X	X
9	Y/OR	X				X	X
10	V/G	X					X
11	DV/NC	X					O
12	V/BG	X				X	X
13	YG/OR					X	
14	V/B					X	
15	DV/NC	O	X	X	X	X	X
16	LB/V			X	X		
17	BR/Q		X				
18	O/LB		X				
19	LG/Q	O					
20	LB/Q	O		O	O	O	

Color Key

V-violet	SB-sky blue
Y-yellow	D-dark
G-green	L-light
B-blue	Q-quench
BR-brown	NC-no change
Or-orange	

X—constant spot, O—variable spot,
ac—*S. acutus*, at—*S. atrovirens*,
he-i—Inflorescence of *S. heterochaetus*,
he-r—rhizome of *S. heterochaetus*,
va—*S. validus*, hy—hybrid,
UV—ultraviolet light, UV+am—UV+ammonia.

(Harms, personal communication) by the calculation of a degree of chemical affinity (DCA) The DCA was calculated by assigning a +1 value if the two taxa being compared had a spot in common and −1 if the spot was present in one, absent in the other. An algebraic sum of the spots yields a value which is taken to indicate the degree of similarity between the patterns: the higher the positive value, the greater is the similarity. The data obtained from this analysis are plotted in Fig. 7 with an arbitrary value of 1 mm being assigned to the bars interconnecting the five taxa for each value of +1 or −1. Darkened bars are positive values and undarkened ones are negative values.

DISCUSSION

From visual comparison of Figs. 2-6 it seems that *S. acutus* and the putative hybrid are the most similar in their chromatographic patterns since they differ in only two spots, 19 and 20. This conclusion may be further verified from the information in Fig. 7 with the two taxa showing a DCA number more than twice as high as that for any of the other taxa compared (+14). In numerous previous phytochemical comparisons of hybrids and their parents (e,g. Alston and Turner, 1959, 1962, 1963; Alston, Lester, and Horne, 1962; Kawatani and Asakina, 1959; Mirov, 1956; Turner and Alston, 1959; Williams, 1955) a general conclusion has been forwarded: 1) hybrids normally possess a summation of the species specific components (spots) of the parental species which has been termed complementation by Alston and Turner (1959), and 2) hybrids usually possess some components present in neither parent. The putative hybrid in this study fulfills neither of these observations and on phytochemical grounds should probably be assigned to *Scirpus acutus*. However, the morphological and chromosomal features, which are not discussed in this paper, give strong evidence of its hybrid nature and the possibility of its being a backcross to *Scirpus acutus* is suggesed (Harms, personal comminucation).

At the other extreme is *S. atrovirens* which has only one (No. 15) of four spots in common with all of the other three taxa. This species was included as a crude control of the experiment. It is in a completely unrelated group of *Scirpus* (leafy type). Its dissimilarity morphologically is no less so on phytochemical grounds as all DCA values (Fig. 7) are negative; however, spot No. 5 may be interpreted as characteristic for the genus since it appears in all the species.

It is not possible to predict the degree of relationship of the three species, *S. acutus*, *S. validus* and *S. heterochaetus* on the basis of their chromatographic patterns as there are few significant differences. Each has a number of species specific spots as well as a preponderance of spots present in all three indicative of their close chemical relationship. For example, spot No. 1 is present in all three species and could be considered to be specific for the group (leafless *Scirpus*); whereas spot Nos. 5, 10, and 11 are found only in *S. acutus*, 16 only in *S. heterochaetus*, 13 and 14 only in *S. validus* and are therefore species specific spots. The remainder of the spots are either present in two of the species and absent in the third or are variable spots which are sometimes present and on replicate runs failed to appear. The presence of these variable spots has been noted in a number of previous studies (cf. Alston and Turner, 1959, 1962, 1963) and are explained as phenolics whose concentrations are dependent upon environmental conditions;

hence, it is not that they are absent but rather that their presence may be in such low concentrations as to be below the detection point of UV-light fluorescence.

A comparison of the occurrence of the phenolic compounds in the aerial and underground parts of the plant was made by utilizing a rhizome of *S. heterochaetus* in addition to the seeds. As shown in Table I, there was not a single difference between the phenolic compounds in the inflorescence and that in the rhizome. Since both rhizomes and seeds are comprised largely of storage materials and it has been shown that phenolics are often storage products (Robinson, 1963), it is not surprising that they possess the same phenolic constituents.

On the basis of morphological and cytological comparisons, *Scirpue acutus* and *S. heterochaetus* are closly related, both with a 2N=38, comparatively rigid culms, large spikelets, and similar rhizome features. Correspondingly, the two are strictly North American with no known cytological counterparts in Eurasia (Gleason and Cronquist, 1963). However, *Scirpus validus* is cytologically distinct from *S. acutus* and *S. heterochaetus* with 2N=42 and many morphological features which separate it from the other two taxa. It has definite European affinities where a complex of species with 2N=42 have been reported (Darlington and Wylie, 1956). A comparison between the cytomorphological affinities and the phytochemical affinities presented in this paper (Fig.7) show that the phytochemical information does not corroborate the information of cytomorphology. For instance, in Fig. 7, *S. heterochatus* shows the greatest relationship with *S. validus.* Obviously comparative phytochemistry can not be taken alone to solve problems in systematics.

In the investigation on taxonomic affinities with Iridaceae, the patterns among phenolic compounds were generally consistant, hence they were reliable indicators (Riley and Bryant, 1961). Also, Alston and Irwin (1961) showed in their study of *Cassia* that the patterns of variation of phenonlic compounds promise a greater potential for taxonomic work than does free amino acid analysis. Thus, the failure to obtain a parallel relationship from the present phytochemical study and the classical cytomorphogical information for the tested species of *Scirpus* does not necessarily void the new chemotaxonomic approach. Moreover, there has been general agreement that a taxonomic system will be "most natural" that is based on the utilization of the greatest possible number of similarities and differences. In actual practice, the taxonomist is limited in the number of characters he can consider, and this raises a number of different reasons, and taxa that are very similar in easily accessible characters may be rather different from each other in cryptic characters. Taxa may be very similar to each other because they have branched very recently, or because they have had a atagnant or parallel evolution since their branching, or they have converged. Thus, conspicuous and cryptic characters sometimes do not coincide and classification which neglects this possibility may have low predictive value (Mayr, 1964). However, there has been warning that chemical characters are often affected by environmental factors, such as temperature, mineral deficiencies. They should not be regarded as *a priori* more than other characters (Davis and Hoywood, 1963). Certainly the lack of correlation between the present phytochemical analysis of *Scirpus* and the known cytomorphological affinities suggests a need of caution in generalizing on the use of phytochemical analysis for determining phylogenetic relationships.

SUMMARY

The phenolic constituents in the seeds of three closely allied species of *Scirpus* known under the common name bulrushes (*S. acutus S. validus,* and *S. heterochaetus*) and a putative hybrid between two of the species were separated by using paper chromatography. An unrelated species, *S. atrovirens,* was also included for purpose of comparison. A total of 20 different phenolic compounds were located in the related taxa by means of fluoresent characteristics in UV-light and their relative positions on the paper. An attempt to objectively compare the degree of similarity in chromatographic patterns was made by means of circular diagrams and calculations of a numerical relationship. *S. acutus* and the putative hybrid were found to be so similar that a tentative conclusion was reached that the hybrid was either a backcross derivative or perhaps not a hybrid. As expected, *S. atrovirens* showed almost no similarity to the other four taxa possessing only one spot in common, this same spot appearing in all the taxa. *S. acutus, S. validus,* and *S. heterochaetus* show nearly identical inter-similarity with each possessing one or more species specific spots, the remaining spots present in two and absent in the third or of a variable nature. The phenolic constituents of the seeds and rhizomes of *S. heterochaetus* were found to be identical. A comparison between the degree of chemical relationship and the known cytomorphological affinities of the taxa is made with the result that little correlation exists, which may suggest a need of caution in generalizing on the use of phytochemical analysis for determining phylogenetic relationships.

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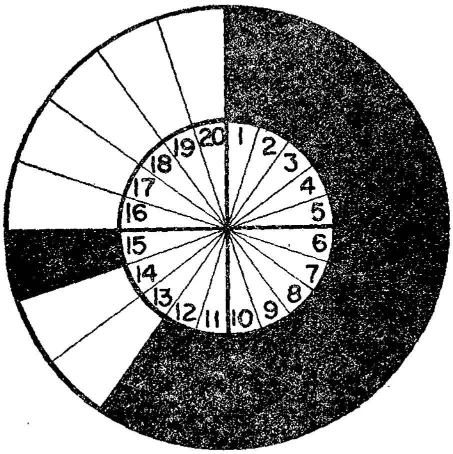


Fig. 2. Polygon diagram of *Scirpus acutus* showing presence or absence of spots on chromatographs.

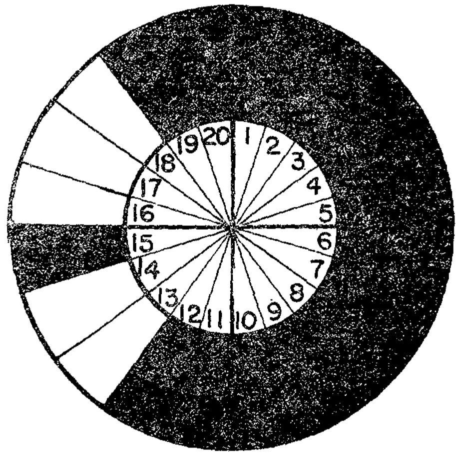


Fig. 3. Polygon diagram of the putative hybrid.

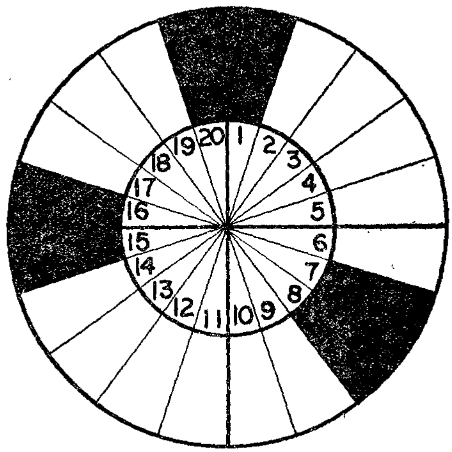


Fig. 4. Polygon diagram of *Scirpus heterochaetus*.

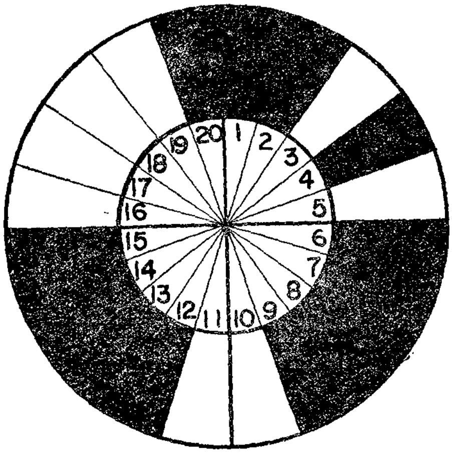


Fig. 5. Polygon diagram of *Scirpus validus*.

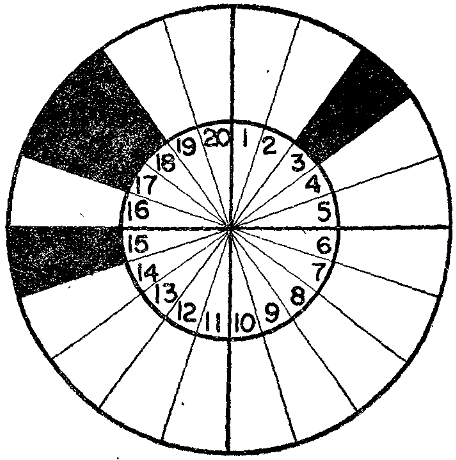
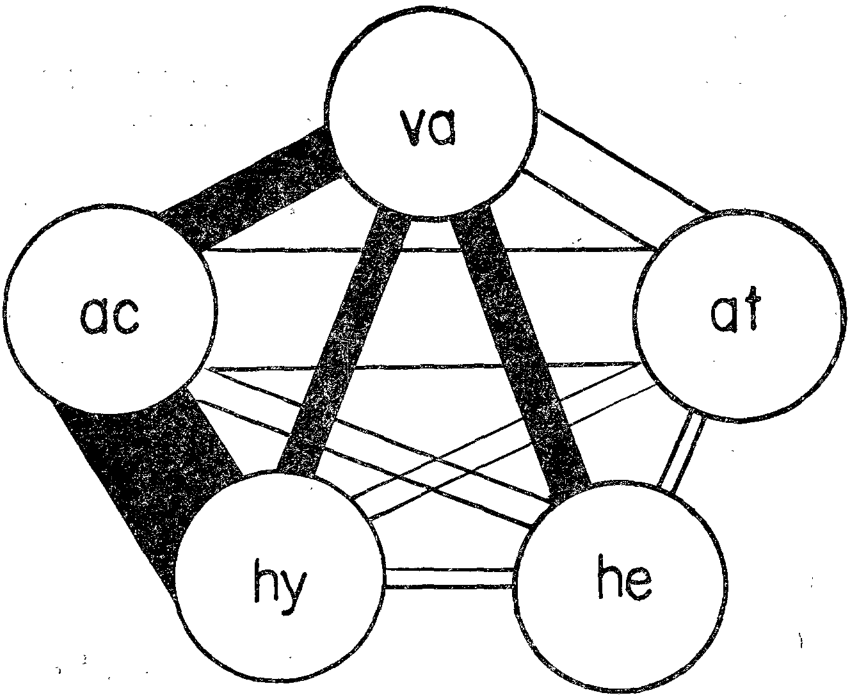


Fig. 6. Polygon diagram of *Scirpus atrovirens*.



ac - S. acutus va - S. validus
at - S. atrovirens hy - S. hybrid
he - S. heterochaetus

Fig. 7. Relationship of *Scirpus* species based on DCA. Positive values are indicated by black bars and negative values by white bars. Each +1 or −1 value = 1 mm.

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藨草屬 (Scirpus) 植物之化學分類 (Chemotaxonomy) 研究

宋 世 謹

本實驗係以濾紙色層分離法 (paper chromatography) 比較藨草植物 (bulrushes) 中所含之芳香族化合物 (phenolic compounds)，作種間關係的探討。使用材料包括*Scirpus acutus, S. validus, S. heterochaetus* 與 *S. atrovirens* 等四種及一*S. acutus* 與 *S. validus* 之可疑雜種。

含種子成熟花序，或根部，乾燥後，研成粉末，以80% 甲醇液 (1%HCl) 抽出其中之芳香族化合物，然後以濾紙色層分離法展開 (圖 1)。比較結果以*S. acutus* 與此可疑雜種之親緣關係最爲接近。在二十個芳香族化合物中，僅有二個相異化合物，併同細胞形態學方面之資料，此一可疑種或係第一子代雜種與 *S. acutus* 之返交種。*S. atrorens* 屬於有葉型 (leafy type)，與其餘三種屬無葉型 (leafless type) 者不同，其芳香族化合物僅有一個亦見於其餘三者。但在 *S. acutus, S. validus* 與 *S. heterochaetus* 三種之間，按細胞形態學之特徵 *S. acutus* 與 *S. heterochaetus* 之親緣較密切，但由本實驗所獲之結果顯示 *S. acutus* 與 *S. validus* 之關係更爲接近，是則隱蔽之生物化學特徵與明顯之細胞形態學特徵不能盡相吻合，因此植物化學 (phytochemistry) 方法雖可有助於親緣關係之研判，但不能作爲絕對之證據。

此外，在 *S. heterochaetus,* 以成熟花序與根部所作之試驗，顯示二者所含之芳香族化合物成份完全相同。