BIOSYSTEMATIC STUDIES OF FORMOSAN *SALVIA*[1]

JIUNN-TZONG WU[2] and TSENG-CHIENG HUANG[3]

Abstract: A chromatographic survey, qualitative analysis and quantitative measurements of the carotenoids of the Formosan *Salvia* were undertaken in order to obtain information which could be used to supplement the data from chromosome, pollen viability, morphology and ecological studies. Conclusions were derived from the chemotaxonomic technique and other approaches. The results agree in all respects with the concepts of the interspecific hybridization (Anderson, 1954) and reticulate evolution relationships or participation theory (Hayata, 1919) found between each entity of *Salvia*. Several dispositions are suggested, the most striking being the recognition of some populations as being hybrids. Of these, some populations of *S. hayatana* may have been derived from *S. hayatana* × *arisanensis* and *S. hayatana* × *hualiensis*, and *S. keitaoensis* from *S. hualiensis* × *arisanensis*. *S. japonica* might have originated from the hybridization of S. *japonica* subsp. *taipingshanensis* with a *hayatana*-like ancestor. The speciation pathway, by hybridization and chromosome doubling, and the reticulate relationships in Formosan *Salvia* is established.

INTRODUCTION

In the past ten years a rather large number of reports have been published in which chemical data have been applied to the analysis of interspecific relationships. The use of paper chromatography, for example, of certain biochemical constituents has been used to study interspecific hybridization (Alston *et al*, 1961, 63, 65; Riley & Bryant, 1961; Stebbins *et al*, 1963; Smith & Bevin, 1963 and Cornu & Paynot, 1969) and has been extended to investigate the problems of polyploidy. Since the evaluation of chromatographic patterns is not believed to be absolutely reliable, the qualitative and quantitative analyses of the carotenoids have been introduced in this paper, and are used in conjunction with other ordinary approaches, such as morphology, palynology, anatomy, cytology and geographical distribution, to compensate for this limitation.

The interspecific hybridization of two species of *Salvia* was studied by Epling (1947), Anderson & Anderson (1954) and Grant & Grant (1964). The Formosan *Salvia* have long been realized as having so many morphological intermediate populations that previous investigators have been puzzled by them. Hayata (1919), Stibal (1935), Murata (1952) and Yamazaki (1969) recognized seven species and two varieties from Taiwan. But with the exception of *S. nipponica* var. *formosana* and *S. plebia*, the others showed a complicated interrelationship that did not agree with the treatments of previous authors. The purpose of this paper is to survey, by all possible approaches, the interspecific relationships of all the Formosan *Salvia* complex with the exception of the two mentioned taxa.

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(2) 吳俊宗, Graduate Student of Botany, NTU.

(3) 黃增泉, Professor of Botany, NTU.

The taxa names used in this paper are the revised names based on the results of our studies. The descriptions of each taxon will be published later this year in the forthcoming Flora of Taiwan.

MATERIALS AND METHODS

Fresh plants were used in this study for analytical testing. Collections were made along mountain trails and in forested areas during the period between 1971 and 1973. Plants were collected from 47 localities (Table 1), each locality was considered a separate population, all specimens are deposited in the Herbarium of the Department of Botany, National Taiwan University (TAI). Some plants were planted in the greenhouse to provide for a longer time of observation, the records from greenhouse plants were compared with the field characteristics of each species.

Morphologial Study: The materials used for the morphological studies were both from fresh and herbarium specimens. Both the reproductive and vegetative features were recorded and various qualitative and quantitative characteristics were analyzed.

Anatomy of Leaves and Petioles: All specimens were killed in FAA, dehydrated in a *n*-butyl alcohol series (Sass, 1958), embedded in the paraffin and sectioned at 10 μ with a rotary microtome. The sections were stained with safranin O and fast green and mounted with Canada balsam.

Palynological Study: For pollen study, two kinds of treatment were used. (a) *Pollen morphologial study:* Pollen was collected from mature anthers of living flowers in each population. Pollen samples were subjected to Erdtman's acetolysis method (1952). Measurements were made within three days to eliminate the possibility of error introduced by differential swelling. At least fifty grains were measured from each population. (b) *Pollen viability:* Each mature anther removed from a living flower was smeared and mounted in lactophenol-aniline blue on a slide. Pollen viability was determined on the basis of the first 500 grains per plant observed, and eight to ten plants were studied for each population.

Chromosome Study of Pollen Mother Cells: Fresh flower buds from each population were fixed in ferric acetate saturated alcoholic propionic acid solution (3:1 *v/v*) for 24 hours. Materials were then transfered to 70% alcohol and stored under refrigeration. The contents of the anthers were squashed by using the acetocarmine stain.

Chromatographic Study: Whole fresh plants were sliced and extracted in 1% HCl (conc.) in methanol in the dark, at room temperature overnight. After reextraction with petroleum ether, at least 20 applications of the condensed extracts were spotted with a micropipette on Whatman No. 3 MM paper and developed in two-dimensions by the descending method. The first solvent system consisted of BAW (*n*-butanol: acetic acid: water = 4: 1: 5) and the second consisted of AcHCl (acetic acid: HCl(2N): water = 15: 3: 82). Dried chromatograms were studied in long-wave ultraviolet light and in ultraviolet light in the presence of ammonia vapor. On the basis of color and position, the R_f value for each spot was given a number for convenience in identification. Spots which were readily recognizable by their color and position on the chromatograms and those which infrequently occured and were usually too faint to be accurately characterized were also scored.

Qualitative and Quantitative Analyses of Carotenoids: (a) *Analysis of absorption spectra:* 0.10 g of freshly collected petals was extracted in a benzene-methanol

Table 1. Collection localities of *Salvia*.

Species	Population no.	Locality
S. *arisanensis*	33	Tsuyuanshan, Taitung
	36	Hohuanshan, Nantou
	40	Alishan, Chiayi
	42	Nanhutashan, Ilan
	46	Taslinshan, Taitung
	47	Hsuehshan, Taichung
S. hayatana (I)	1, 2	Hsiaokoto, Taipei
	3, 4	Kankou, Taipei
	5	Kueishan, Taipei
	21, 22	Kuantaochi, Nantou
	23	Shanping, Kaohsiung
	28	Kaopo, Taoyuan
	34	Wulai, Taipei
	41	Shihting, Taipei
	44	Fuhsingshan, Taoyuan
S. hayatana (II)	6, 7	Sinbaiyang, Hualien
	8	Husinshan, Hualien
	20	Tapachienshan, Hsinchu
	27	Chitou, Nantou
	29	Dachian, Taichung
	37	Lushan, Nantou
	38	Alishan, Chiayi
S. hualiensis	9	Fuhsingshan, Hualien
	10, 30	Losau, Hualien
	11	Tunmen, Hualien
	12	Lunchien, Hualien
	31	Liwushan, Hualien
	32	Tailuko, Hualien
S. japonica	24, 25	Tatunshan, Taipei
S. japonica subsp. *taipingshanensis*	13, 14, 15	Taipingshan, Ilan
	39	Nanhutashan, Ilan
	43	Hsuehshan, Taichung
S. japonica subsp. *taipingshanensis* var. *filicifolia*	16	Taipingshan, Ilan
	35	Peichatienshan, Taoyuan
	42	Nanhutashan, Ilan
	45	Fuhsingshan, Taoyuan
	46	Kuanwu, Hsinchu
S. keitaoensis	26	Chitou, Nantou
S. scapiformis	17	Sientunhu, Keelung
	19	Shihting, Taipei

(1:4 *v/v*) solution in the dark at room temperature for two hours. After filtration, sample solutions were measured with a spectrophotometer in the range of 330–900 nm for the absorption analysis. (b) *Quantitative analysis of β-carotene:* Setting the wavelength at 436 nm to measure the absorbance of crude carotenoid solution prepared from above, the β-carotene was quantified by the formula:

$$C = \frac{\text{absorbance} \times 454}{196 \times L \times W}$$

where C=concentration of β-carotene (ppm), L=cell length in cm and W=gram sample per *ml* of final dilution.

Flowering Time and Geographical Distribution: Flowering time was recorded based on the time of the field collections and herbarium data. The geographical distribution was indicated on a map for each population.

RESULTS

Morphological Study: Since some Formosan *Salvia* have a wide morphological variation in their populations, they are difficult to distinguish at a glance. Close inspection and survey of all characteristics are necessary in order to make a yardstick for measuring the populations.

The following characters were used: (a) color of corolla and color of anther wall; (b) exsertion or insertion of the stamens; (c) pubescences on inside and outside of the calyx; (d) hairs on the leaf surface; (e) type of leaf form; (f) presence or absence of hairs on the petiole; and (g) the position of petiole.

The most distinctive taxonomic characteristics for the separation of Formosan *Salvia* are those of floral morphology. The gross differences in floral and vegetative characters exhibited by all species (or subspecies) are shown in Table 2. The quantitative measurements of the floral parts are shown by their mean and standard deviation in Fig. 1. The proportional characteristics of floral parts are shown by diagrams of the length of filament versus length of style (Fig. 2), and with the width of the lower part of the lower lip (E) over the width of the upper part of the lower lip (D) versus width of upper lip (Fig. 3).

Most species are easily identified by the charcteristics of corolla and pubescence inside the calyx, except *S. hayatana* (II) and *S. keitaoensis*. Both of these species had all their morphological characters intermediate between those of *S. hayatana* (I) and *S. arisanensis* and that of *S. hualiensis* and *S. arisanensis* respectively. In which, the *S. hayatana* (I) is the ancestral entity group and *S. hayatana* (II) is the derivative group according to our results. On both Figs. 2 and 3, all species (or subspecies) except *S. hayatana* (II) and *S. keitaoensis* conform to their definite specific species positions. But these two species show intermediate positions.

Some individuals of the population #36, *S. arisanensis*, differ from the others of the same species. Proportionally, they are distinctively larger in flower size (Fig. 2). Their flowers also differ in color pattern, the corolla is homogeneously purple all over instead of the normal type which has the purple restricted to the margins.

Anatomical Features: Many anatomical features of the leaves and petioles were studied and various qualitative characteristics were analysed. Those which have been selected for presentation here are believed to contain the features which best served as the anatomical criteria for differentiating species.

Table 2. Comparative morphology of Formosan *Salvia*.

Taxa & Populations \ Characters		Color		Pubescence on calyx						Stamen[5]		Leaf						Petiole			
		Corolla[1]	Anther wall[2]	Inside[3]			Outside[4]					Pubescence on surface[6]		Pinnate[7]				Pubescence[8]		Based on[9]	
				L	M	S	DG	SG	S	E	I	P	A	S	P	B	T	P	A	C	R
S. hayatana (I)	1, 2, 3, 34	W	Y			+		+	+	+			+			+			+		+
	4	W	Y			+		+	+	+			+			+		+	+		+
	5	W	Y			+		+	+	+			+		+				+		+
	23	W	Y			+			+	+			+		+			+	+		+
	21	W	Y			+			+	+		+	+			+			+		+
	28	W	Y			+		+	+	+			+		+				+		+
S. hayatana (II)	6, 7, 20	W	Y		+				+	+			+		+			+	+		+
	8	W	Y		+			+	+	+			+			+		+	+		+
	37	W	Y&P			+			+	+			+		+			+			+
	38	W+LP	Y			+			+	+			+		+				+		+
	29	W	Y		+				+	+			+		+			+	+		+
S. arisanensis	40, 33	LP	P	+			+			+		+			+	+		+			+
	46, 36	LP	P	+					+	+		+				+		+			+
S. hualiensis	9, 10, 30	LP	Y	+					+		+		+		+				+		+
	11, 12, 32	LP	P	+					+		+		+		+			+	+		+
S. keitaoensis	26	LP	Y	+			+			+			+		+			+			+
S. japonica	24	LP	P	+			+			+		+	+		+			+		+	
	25	W	Y	+			+			+		+	+		+			+		+	
S. j. t.[10]	39, 13, 14, 15	DP	P	+			+			+		+			+	+		+		+	
S. j. t. f.[11]	16, 35, 45	DP	P	+			+			+		+					+	+		+	
S. scapiformis	17	LP	P			+			+	+			+	+					+		+
	19	LP	Y			+			+	+			+		+			+			+

1 corolla color: W-white; LP-light purple; DP-dark purple.
2 anther wall: Y-yellow; P-purple.
3 pubescences inside the calyx: L-longer than 1.5 mm; M-0.5–1.5 mm; S-shorter than 0.5 mm.
4 pubescences outside the calyx; DG-densely glandular haired; SG-sparsely glandular haired; S-non-glandular haired.
5 stamen: E-exserted; I-inserted.
6 pubescences on leaf surface: P-present; A-absent.
7 pinnate: S-simple leaf; P-pinnate; B-bipinnate; T-tripinnate.
8 pubescences on petiole: P-present; A-absent.
9 petiole based on: C-cauline; R-radical.
10 *S. j. t.*: *S. japonica* subsp. ***taipingshanensis.***
11 *S. j. t. f.*: *S. japonica* subsp. ***taipingshanensis*** var. ***filicifolia.***

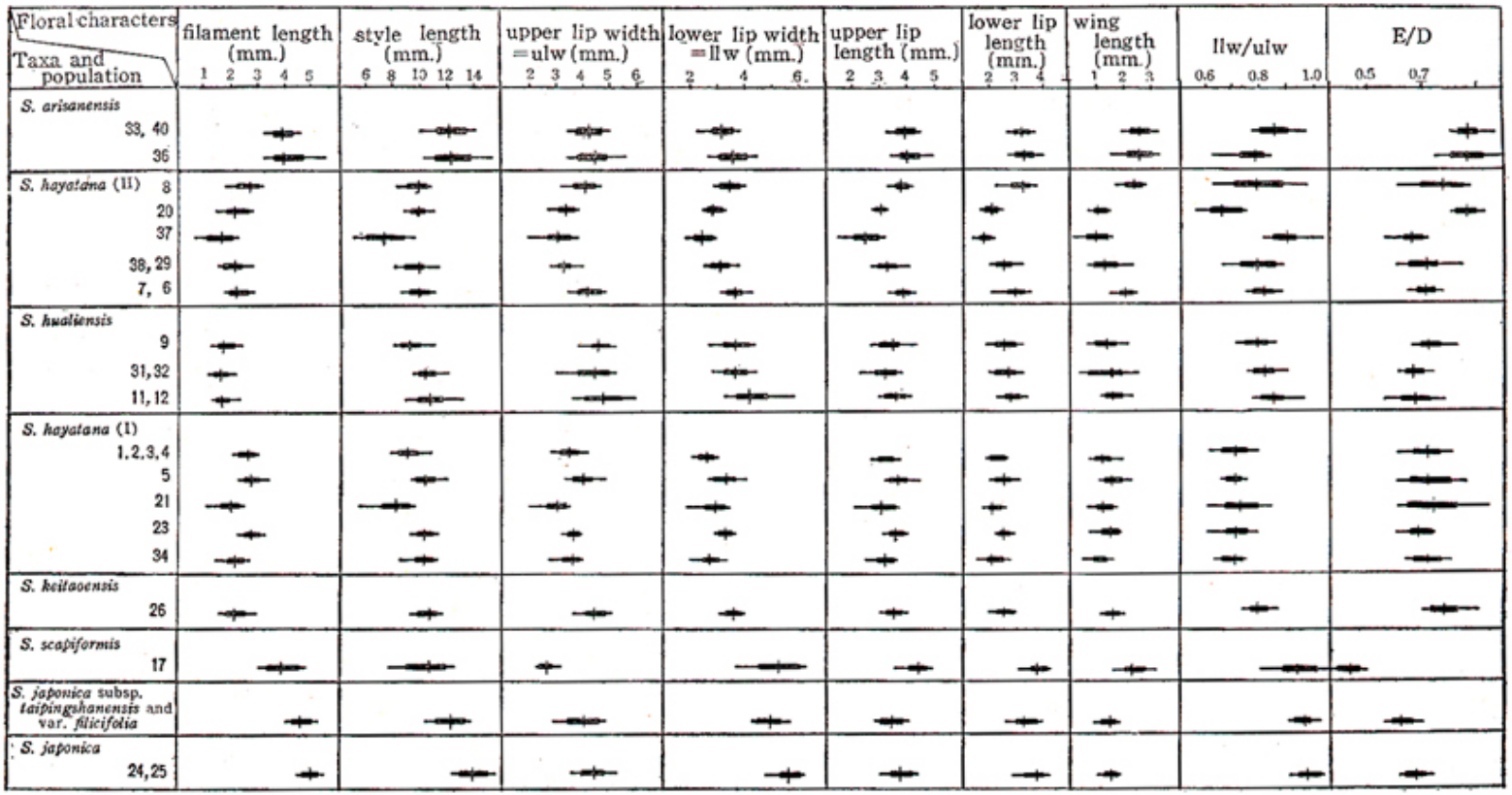

Fig. 1. Quantitative measurements of floral characteristics of ***Salvia*** with means and standard deviations

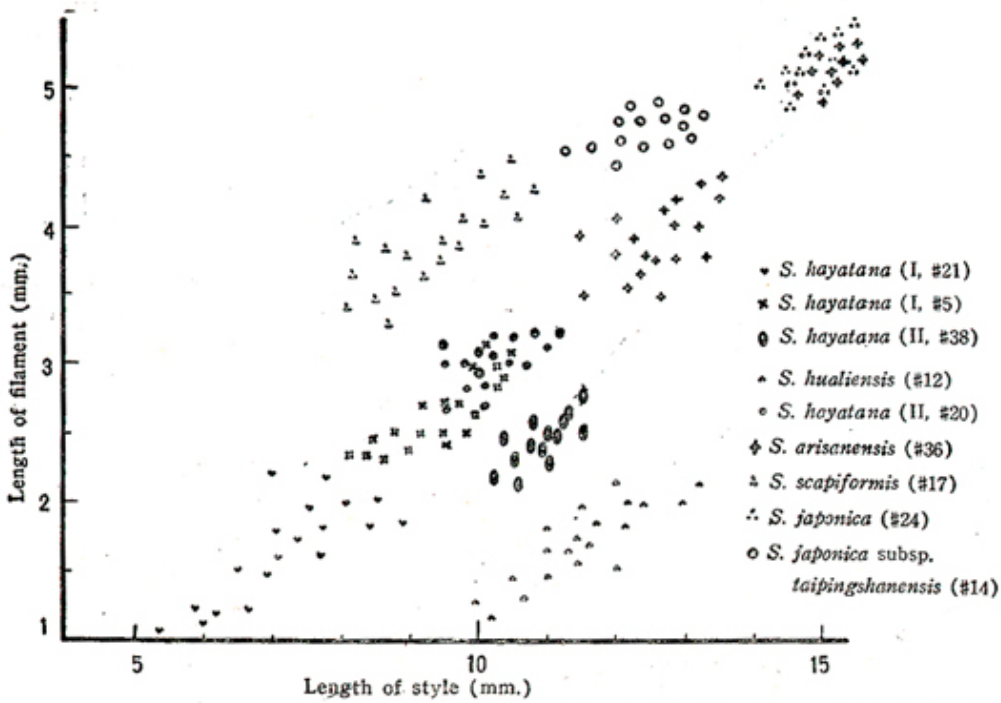

Fig. 2. Scattering diagram of length of filament vs. length of style of some taxa, showing the characteristics of the *S. japonica* and *S. arisanensis* (#36) and the intermediacy of *S. hayatana* (II).

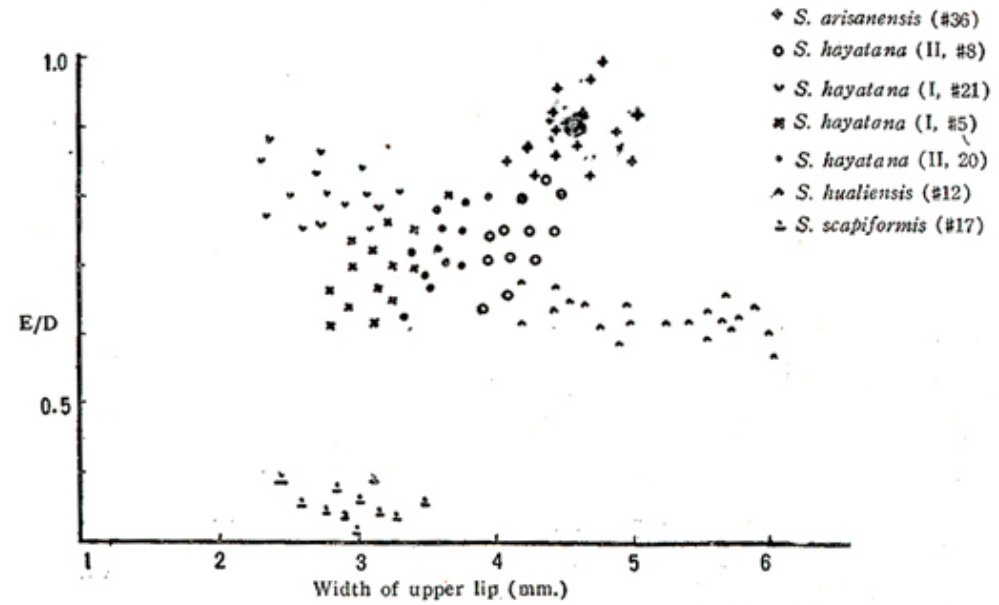

Fig. 3. Scattering diagram of width of upper lip vs. the ratio of width of lower part of lower lip over width of upper part of lower lip (E/D), showing the quantitative intermediacy of *S. hayatana* (II).

The characteristics of the sectioned leaf blades show no distinct differences among the species. The trichomes, as Webb (1964) indicated, differ in their incident frequency on both upper and lower surfaces and the length of trichomes present. The trichome characteristics vary from being bare on upper epidermis of *S. scapiformis*, *S. keitaoensis*, *S. hualiensis*, most *S. hayatana* (I) and some *S. hayatana* (II), through short and scarce trichomes on *S. japonica*, some of *S. hayatana* (II) and *S. arisanesis*, to puberulent and relatively abundant trichomes on some of *S. arisanensis*, *S. japonica* subsp. *taipingshanensis* and one population of *S. hayatana* (#21).

The vascular bundles of the petioles of most species, according to the type system described by Hrubý (1955), are of the types I, II and III (in Fig. 4 and Table 3). Of these, type I is the original and species with this type of vascular bundle are: *S. arisanensis*, *S. hayatana* (I), *S. hualiensis* and *S. scapiformis;* the type II and type III are derived from type I by different pathways. *S. japonica* belongs to the type IIa which was derived from the type II.

It was indicated by Hrubý (1961) that the type of the vascular bundle course in the leaf petiole is inherited independently of other morphological leaf characters and there is practically no correlation between vascular bundle type and any other morphological character. Formosan *Salvia* exemplifies this fact, and these provide a favorable basis for tracing the evolutional level of each taxa.

Palynological Characteristics: The general pollen features of *Salvia* (Fig. 6) have been characterized by Erdtman (1958) and Emboden (1964). The detail descriptions of each Formosan taxa has been given by Huang (1972).

Table 3. Types of vascular bundle courses in leaf petioles of *Salvia*.

Taxa and population number \ Types of vascular bundle course		I	II	III	IIa
S. arisanensis	33, 40		×#		
	36, 42	×			
S. hayatana (I)	1, 2, 3, 4, 5, 23, 34, 41	×#			
	28, 45			×	
	21	×*			
S. hayatana (II)	6, 7, 8	×			
	29			×	
	37, 38		×		
S. hualiensis	9, 10, 31, 32	×			
	11, 12	×*			
S. japonica	24, 25				×
S. japonica subsp. *taipingshanensis*	13, 15		×		
	14			×#	
S. japonica subsp. *taipingshanensis* var. *filicifolia*	16, 44		×#		
S. keitaoensis	26		×		
S. scapiformis	17	×#			

* a transitional type of type I to type III.

vascular bundle with sclerenchyma tissues between primary xylem and cambium layer.

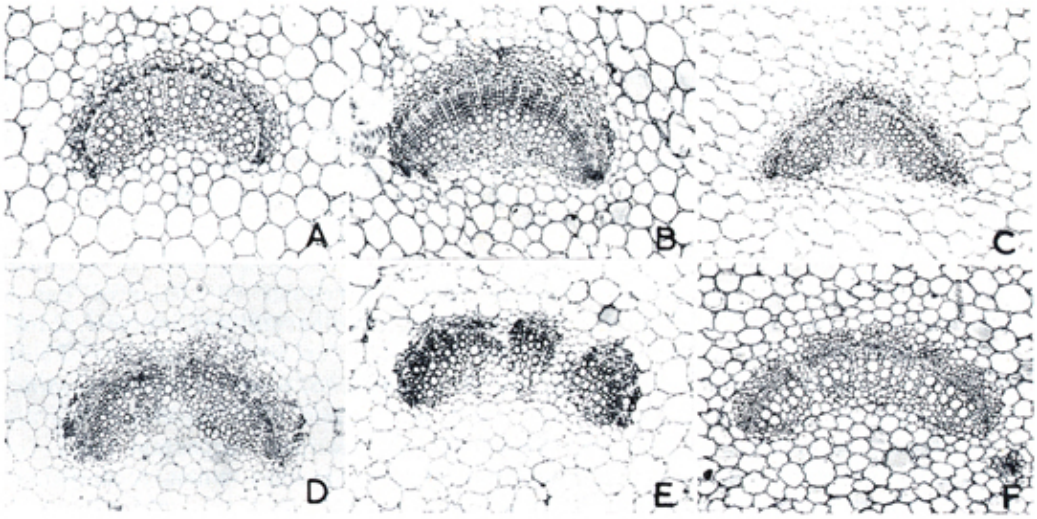

Fig. 4. The vascular bundle types of leaf petioles of *Salvia*. A-type I; B-type I with sclerenchyma tissue between primary xylem and phloem; C-a transitional type of I-III; D-type II; E-type III; F-type IIa. Characteristic species is illustrated in Table 3 All the types are cited from Hrubý (1955). (200×)

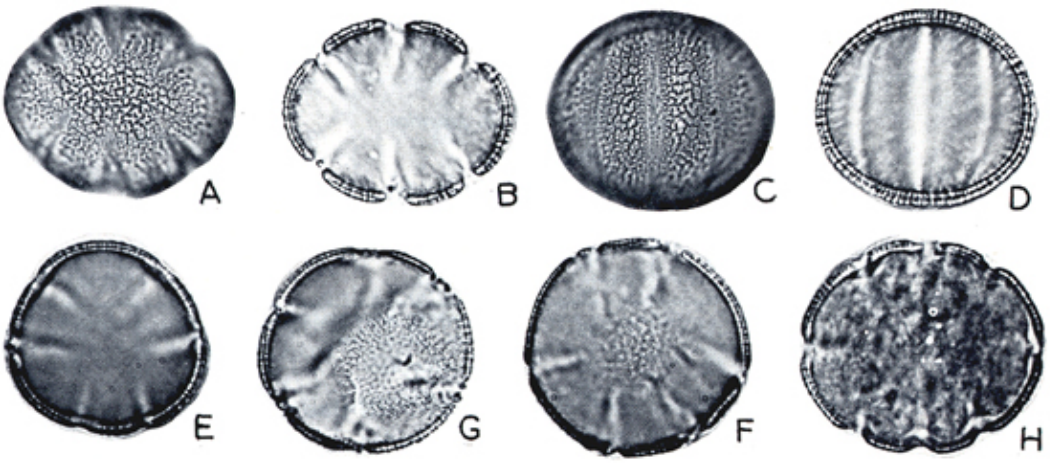

Fig. 5. The pollen grains of *Salvia*. A-D, normal pollen with A & B in polar view and C & D in equatorial view; E-H, abnormal pollen showing the aberrant colpi distribution and number. (1000×)

In a general sense, wide intraspecific variation in size and interspecific similarity in the sculpture of the reticulation makes it difficult to separate the species by their pollen. Methods using the ratio of length of P axis versus E axis, the sculpture features and the size relationships employed by Emboden (1969) for separating species and hybrids have failed to separate the Formosan *Salvia*. A prominent fact is the presence of some aberrant pollen (Fig. 5), occuring less than 10 percent and approximately of the same size as the normal, being always found in populations of *S. keitaoensis*, most *S. hayatana* (II) and some *S. arisanensis*, which is accompanied with lower viability of the pollen.

When pollen is stained with lactophenol-aniline blue, the darkly stained, spherical pollen is recognized as normal. Any pollen that is irregular in shape or has a shrunken or a non-staining protoplast is recognized as abnormal and inviable. Most species have the pollen viability as high as 90 percent or more (Table 4). Those entities with lower viability percentage as *S. japonica*, *S. japonica* subsp. *taipingshanensis* var. *filicifolia*, *S. keitaoensis* and most populations of *S. hayatana* (II) are considered to be genetically unstable. This is one of the most important facts for establishing genetical and evolutional statuses of each species when accompanied with chromosomal and chemical data.

Giant pollen was found on some slides of *S. japonica* and some populations of *S.*

Table 4. Pollen viability of Formosan *Salvia*.

Taxa	Population number	Percent of viability	Taxa	Population number	Percent of viability
S. arisanensis	33	93	*S. hualiensis*	9	80
	36	78		10	85
	40	94		11	95
	42	83		12	98
S. hayatana (I)	1	96		30	89
	2	98		31	91
	3	95		32	95
	4	97	*S. japonica*	24	29
	5	96		25	34
	21	97	*S. japonica* subsp. *taipingshanensis*	13	91
	23	92		14	88
	28	95		15	93
	34	96	*S. japonica* subsp. *taipingshanensis* var. *filicifolia*	16	83
	41	92		45	87
	45	98	*S. keitaoensis*	26	82
S. hayatana (II)	6	89	*S. scapiformis*	17	96
	7	82		19	43
	8	81			
	29	82			
	37	76			
	38	81			
	20	78			

arisanensis (#36 and #42) (Figs. 5 and 6) when treated with the acetolysis method as well as treated with the viability staining method. These usually differ from the normal in aperture number and/or aperture distribution. They are 7- or 8-colpate instead of the normal 6-colpate and the colpi are irregularly distributed on the amb view. It is suggested that the presence of giant pollen is probably caused by a genetic drift as indicated by chromosome and chemical informations.

Chromosome Study: It is difficult to use the general chromosome configurations of Formosan *Salvia* as a basis of species separation, for their chromosome are too small to be seen clearly. Only the basic chromsome number of PMCs of each species is scrutinized in this paper.

During the meiosis of PMCs, the diminution of chromosome number is invisible. According to Chaumen (1968), the chromocenters of *Salvia* in the last premeiotic interphase were found to be associated in group of two's, sometimes so closely that only the haploid number was seen. So the chromosome numbers observed in both prophase I and II are alike.

Most of the species have the chromosome number of $n=x=8$ (Table 5). Some of the *S. hayatana* (I) and (II), *S. hualiensis*, *S. japonica* subsp. *taipingshanensis* have two kinds of basic chromosome number, $n=x=8$ or 9, in the same anther. Some individuals of *S. japonica* and *S. arisanensis* (#36) are tetraploid with $n=2x=16$. Whether they are autotetraploid or allotetraploid cannot be known from viewing the chromosomes. In order to trace their possible origin, it is necessary to study their chemical constituents. A few microsporocytes of *S. japonica* are found to have an aberrant separation of chromosomes in the meiotic anaphase I (Fig. 7-3). This may be correlated with their surprisingly low pollen viability. This abnormal chromosome division may be caused by genetic unstability and correlated with the origin of this species.

Table 5. Chromosome numbers of Formosan *Salvia*

Taxa	x	Taxa	x
S. arisanensis	8	*S. japonica* subsp. *taipingshanensis*	8, 9
S. arisanensis #36*	16		
S. hayatana (I)°	8	*S. japonica* subsp. *taipingshanensis* var. *filicifolia*	8, 9
S. hayatana (II)°	8		
S. hualiensis°	8	*S. keitaoensis*	8
S. japonica	16-18	*S. scapiformis*	8

* Only a part of the population plants are found to be tetraploid.

° $n=7$ or 9 are sometimes found in a same anther in the populations.

Chromatographic Analysis: Each sample examined is scored in Table 6 for the presence or absence of each spot. Of these, plant-to-plant variations within populations are considered and repeated analyses of the same sample are made. As a tool of identification, this technique has its limitations. Nevertheless, chromatographic distinctiveness of these Formosan species is more or less established.

Examination of the Table 6 shows that although some differences exist between samples of the same species or subspecies, such differences are considerably less than those between different entities. Most of the differences between samples of

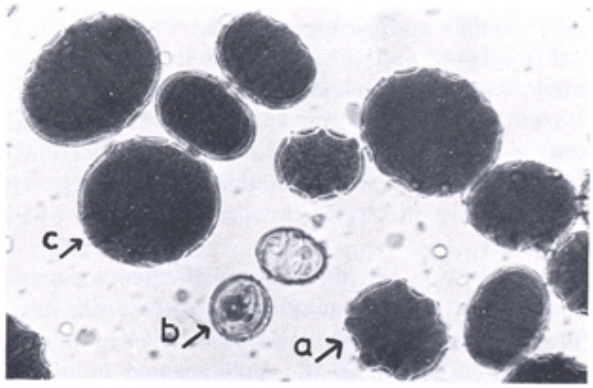

Fig. 6. The viable, non-viable and giant pollen. The viable pollen are darkly stained (a); the inviable pollen are shrunken and nonstaining (b); the giant pollen are abnormal in colpi number and distribution as well as in size (c). (400×)

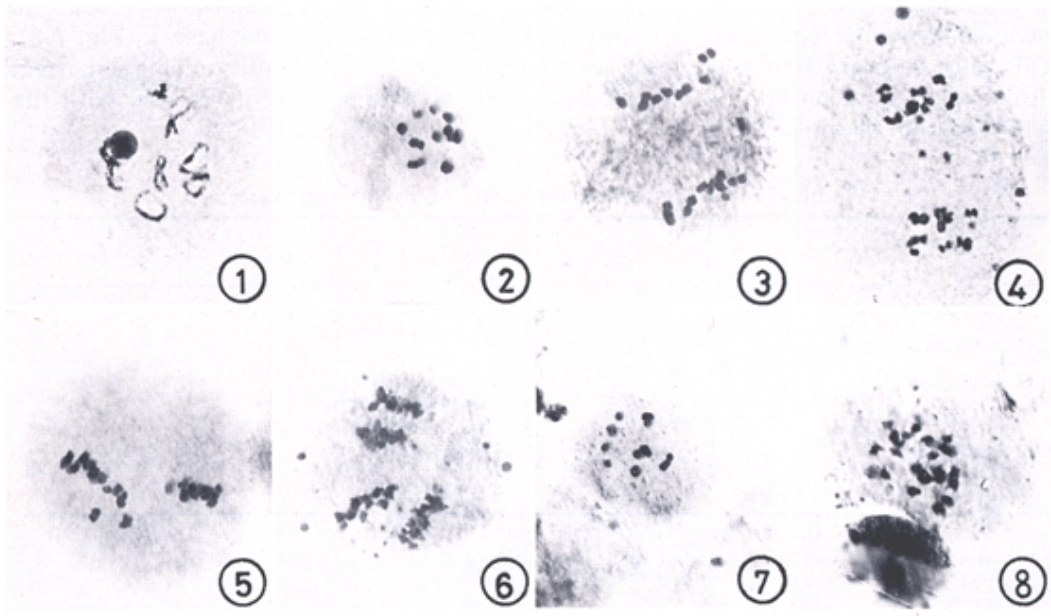

Fig. 7. Meiotic chromosomes of *Salvia* during meiosis and the tetraploid as well as diploid. 1-early Prophase I; 2-late Prophase I; 3-Anaphase I; 4-Telophase ı; 5-Metaphase II; 6-Anaphase II; 7-diploid; 8-tetraploid. (1000×). Their small size makes accurate counting very difficut.

Table 6. Characteristics of components on chromatograms of Formosan *Salvia*.

Taxa	Spot No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
R_f#	I	.63	.32	.47	.21	.53	.61	.81	.41	.32	.80	.67	.46	.63	.61	.34	.48	.55	.71	.68	.67	.73	.48	.44	.27	.59	.60	.48	.41	.51	.70
	II	.66	.06	.72	.67	.72	.80	.67	.42	.67	.58	.78	.58	.56	.74	.43	.85	.79	.70	.40	.50	.81	.58	.49	.48	.37	.46	.63	.72	.52	.50
Color†	A	—	YP	—	—	BG	BG	—	BP	—	YG	—	RP	—	—	RP	—	BG	—	Bl	BG	Bl	—	RP	RP	P	P	—	—	RP	BG
	B	BG	YP	BG	BG	BG	BG	Bl	—	BG	YG	YP	BP	YG	Bl	BP	BG	Bl	Br	Bl	G	BG	BG	Pi	Pi	Br	Br	BG	YG	Br	G
S. arisanensis	33, 40	+		+	+		+			+	+	+											±			+	+	+	+	+	
	36	+	+			+	+		+		+	+														+	+	+	+	+	
S. hayatana (II)	8	+		+	+		+			+	+	+			+				±		+					±	+	+	+		
	29						+				+	+							+	+	+						+	+	+		
	37		+	+	+		+		+							+		+	+		+		+	+		+	+		+		
	38		+	+				+									+	+	+	+			+			+	+	+	+	+	
	20						+	+										+	+	+	+	+	+	+	+						+
	27																	+	+		+	+		+				+	+		
	6							±		+	+	+	+			+	+	+	±					+	+	+	+	+	+	+	
	28										±	+						+	+	+	+			+		+	+	+		+	
S. keitaoensis	26				+					+						+		+	+		+	+			+	±	±	+	+	+	
S. hualiensis	9, 10										+	+		+	+	+		+	+		+			+							
	11, 12									±	+	+	+			+		+	+	±	+	+	+	+	+						
	31, 32									+	+	+				+		+	+		+		+	+	+						
S. hayatana (I)	23																	+	+	±		+		+				+			
	1																	+	+		+	+		+	+			+	+		
	2																	+	+			+		+				+			
	5																	+	+	+		+		+		+	+	+		±	+
	41																	+	+	+	+	+				+	+	+	+	+	
	34																		+	+	+	+	+	+	+				+	+	
	21																	+	±		±	+	+			+	+	+	+	±	
S. scapiformis	17						+				+									±	+	+						+	+		
S. japonica	24					+			+	+		+					+	+		+	+			+	+		+		+	+	+
*S. j. t.**	13, 14					+			+	+		+						+		+	+			+		+		+		+	
	15											+						+		+				+				+		+	
S. j. t. f.°	16									+		+						+		+				+	+			+	+	+	

Taxa	Spot No.	31	32	33	34	35	36	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61
R_f#	I	.36	.68	.94	.80	.51	.79	.69	.52	.18	.73	.65	.70	.60	.86	.91	.48	.26	.70	.75	.75	.78	.78	.56	.62	.68	.45	.56	.75	.62	0.63
	II	.44	.64	.00	.00	.50	.70	.30	.56	.43	.74	.85	.75	.56	.74	.78	.82	.69	.58	.01	.39	.49	.55	.59	.72	.79	.44	.70	.82	.78	0.80
Color†	A	RP	BG	RP	Br	—	—	—	BG	P	—	—	—	—	BP	BP	—	BG	—	—	Bl	Bl	Bl	YP	Bl	BG	Bl	Bl	—	—	—
	B	BP	GY	RP	Br	BG	YG	YP	BG	—	Bl	YP	Bl	YG	Bl	Bl	Bl	YG	YG	BG	—	G	YG	YP	YG	BG	Bl	Bl	Bl	BG	BG
S. arisanensis	33, 40														±	±		+	+	+	+	+	+	+	+	+	+	±	+	+	
	36								+											+	+	+	+	+	+	+	+		+	+	
S. hayatana (II)	8								±								+	+		+	+	+		+	+	+	+		+		
	29				+												+	+		+	+	+			+	+	+	+	+		
	37																+	+		+	+	+		+	+	+	+	+		+	
	38	+	+						±										+	+	+	+	+	+	+	+	+	+	+		
	20	+				+													+	+	+	+	+	+	+	+		+			
	27	+																	+	+	+	+	+	+	+	+		+	+		
	6	+																+	+		+	+	+	+	+	+			+		
	28	+		+	+		+								+	+			+	+	+	+	+	+	+	+			+		
S. keitaoensis	26	+		+	+												+	+	+		+	+	+	+	+	+	+		+	+	
S. hualiensis	9, 10																			+	+	+	+	+	+	+	+	+	+	+	
	11, 12	±		+														+		+	+	+	+	+	+	+	+	+		+	
	31, 32			+																±	+	+	+	+	+	+	+	+	+		
S. hayatana (I)	23	+	+					+											+	+	+	+	+	+	+	+	+	+			
	1	+	+	+	+		+	+										+	+	+	+	+	+	+	+	+	+				
	2	+	+	+	+			+												+	+	+	+	+	+	+					
	5	+	+		+		+											+		+	+	+	+	+	+	+		+		+	
	41		+	+	+														+	+	+	+	+	+	+	+	+			+	
	34	+	+		+			+										+	+	+	+	+		+	+	+	+		+		
	21	+	+	±															+	+	+	+	+	+	+	+	+			+	
S. scapiformis	17																			+	+	+		+	+	+				+	+
S. japonica	24		+	+	+	+				+				+			+		+	+	+	+			±	+					±
*S. j. t.**	13, 14	+		+			+		+		+	+	+	+	+	+		+	+	+	+	+	+		+	+	+		+	+	
	15			+			+		+		+	+	+		+	+		+	+	+	+	+			+	+	+		+	+	
S. j. t. f.°	16	+		+			+			+	+	+	+		+	+		+	+	+	+				+	+	+	+			

\# R_f: I-developed in solvent BAW; II-developed in solvent HCl-HAc-H_2O.
† Color: A-observed under UV light; B-observed under UV light with presence of NH_3. BG-blue green; Bl-light blue; BP-blue purple; Br-brown; G-green; GY-green yellow; P-purple; Pi-pink; YP-yellow pink; YG-yellow green.
* *S. j. t.*=*S. japonica* subsp. *taipingshanensis*.
° *S. j. t. f.*=*S. japonica* subsp. *taipingshanensis* var. *filicifolia*.

the same entity probably represent plant-to-plant variation within populations. Considering the resemblances of the different entities to each other, it shows that those of *S. hayatana* (I), *S. arisanensis*, *S. scapiformis* and *S. japonica* subsp. *taipingshanensis* have groups of species-specific substances. The number of distinguishing substances differs for these species. The other species e. g. *S. hayatana* (II), *S. keitaoensis* and *S. japonica* show intermediate constituents: *S. hayatana* (II) seems to have the combining constituents of *S. arisanensis* and *S. hayatana* (I); *S. keitaoensis* is intermediate between *S. arisanensis* and *S. hualiensis* and more resembles the latter; *S. japonica* illustrates a high similarity to its subspecies *taipingshanensis*, intensive study of its other characteristics displays it to be derived from this subspecies rather than from any other ancestor. *S. arisanensis* shows more or less close resemblance to *S. japonica* subsp. *taipingshanensis* in its chemical constituent as well as its morphological characteristics. Evolutionally, both entities are considered to be rather closely related.

Drawings of actual chromatograms of the six entities are presented in Figs. 8 and 9, each represents the morphological and chemical relationships of three ecologically related entities. Both show clearly that *S. hayatana* (II) is not only intermediate in morphology between *S. hayatana* (I) and *S. arisanensis* and between *S. hayatana* (I) and *S. hualiensis* but also in its chemical constituents.

Qualitative and Quantitative Analysis of Carotenoids:

(a) *Absorption spectra of carotenoids.* All species show a similar absorption near the ultraviolet region and have approximately the equal peak height at 355 nm. Most of the entities have distinctively specific absorption peaks between the range of 460–470 nm (Fig. 10). Upon this, *S. hayatana* (I) and *S. hualiensis* have the highest peak and *S. arisanensis* the lowest; *S. scapiformis* does not have a peak in this range; all other species are of moderate quality.

Quantitative analysis of β-carotene: When absorption characteristics are quantified as illustrated in Table 7, it is more prominent to characterize each species specifics than that of absorption peak. Both the *S. hayatana* (I) and *S. hualiensis* have the amounts of β-carotene as high as 30 ppm or more and that of *S. scapiformis* as low

Table 7. Quantitations of β-carotene in flower parts of *Salvia*

Taxa	Population number	Amount of β-carotene (ppm)	Taxa	Population number	Amount of β-carotene (ppm)
S. arisanensis	33	17.7	*S. hayatana* (II)	6	31.1
	36	20.4		8	29.5
	40	18.3		29	24.9
S. hayatana (I)	1	28.7		37	31.9
	3	31.5		38	25.2
	5	31.0	*S. hualiensis*	9	33.9
	21	36.7		11	34.4
	23	34.9		12	31.8
	28	32.1		31	39.8
	34	29.1		32	38.1
	41	30.4	*S. keitaoensis*	26	28.2
			S. scapiformis	17	14.6

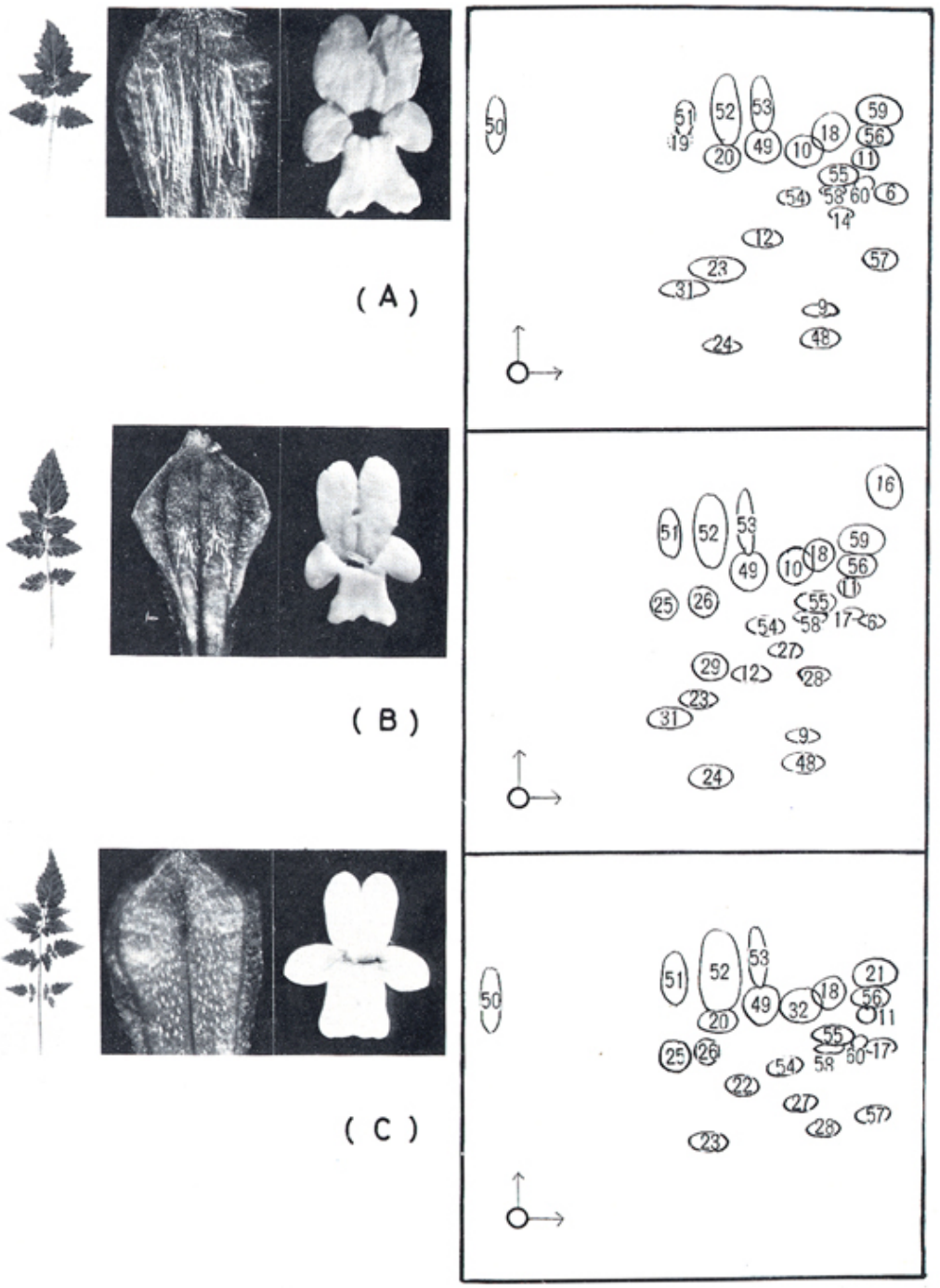

Fig. 8. Diagrammatic representations of composite chromatographic maps with some of their morphological characters. The properties of these chemical constituents are illustrated in Table 6. The morphological characteristics presented are leaves (1/3 ×), pubescences inside the calyx (5×) and floral parts from optical view (2.5 ×). (A)-*S. hualiensis* (#12) (B)-*S. hayatana* (II, #6). (C)-*S. hayatana* (I, #37).

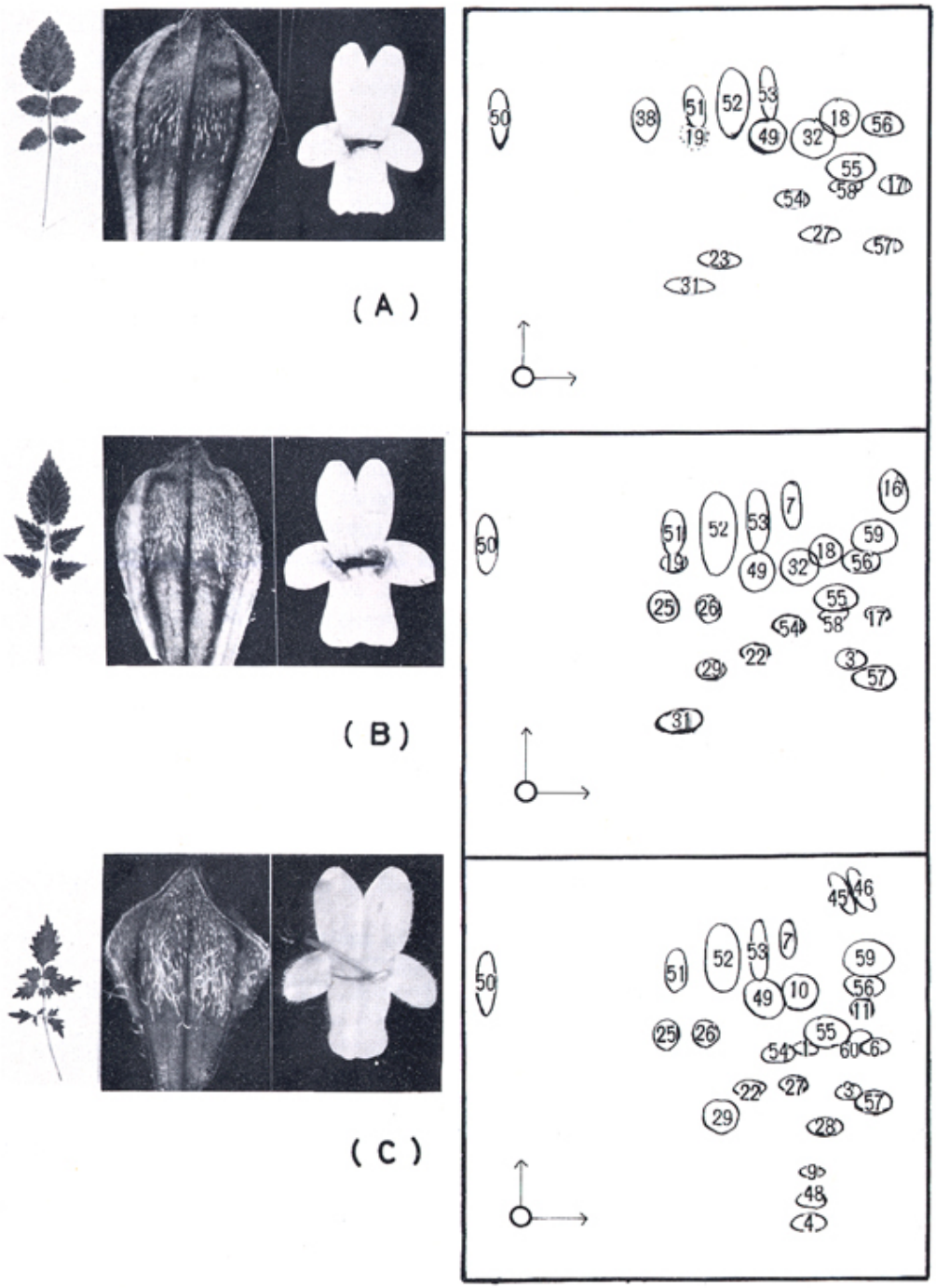

Fig. 9. Diagrammatic representations of composite chromatographic maps with some morphological characters. Each characteristic illustrated is explained as in Fig. 8. (A)-*S hayatana* (I, #23). (B)-*S. hayatana* (II, #38). (C)-*S. arisanensis* (#40).

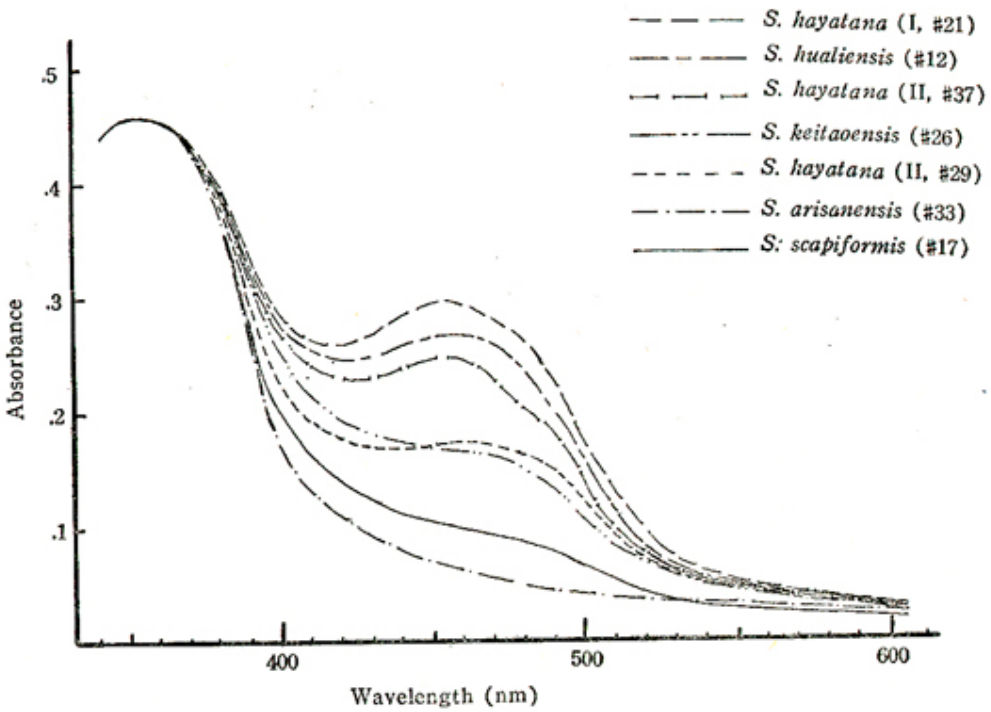

Fig. 10. Absorption spectra of carotenoids in floral parts of some species of *Salvia*.

as 15 ppm. This quantitation of β-carotene is of advantage in distinguishing entities with light purple flowers as *S. arisanensis, S. hualiensis, S. keitaoensis* and *S. scapiformis.* The quantitation of β-carotene shows that the amount of β-carotene in one population of *S. hayatana* II lies between that of *S. hayatana* I and *S. arisanensis,* and another population of *S. hayatana* II lies between that of *S. hayatana* I and *S. hualiensis;* and the anaunt of β-carotene in *S. keitaoensis* lies between that of *S. arisanensis* and *S. hualiensis.*

Table 8. Flowering times of Formosan *Salvia*.

month / taxa	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
S. arisanensis	×	×	×	×	×			×	×	×		
S. hayatana (I)	×	×	×	×	×			×	×	×	×	×
S. hayatana (II)	×	×	×	×	×		×	×	×	×	×	×
S. hualiensis	×	×	×	×	×		×	×	×	×	×	×
S. japonica				×	×	×	×	×	×	×	×	
S. japonica subsp. *taipingshanensis*						×	×	×	×	×	×	
S. japonica subsp. *taipingshanensis* var. *filicifolia*	×					×	×	×	×	×	×	×
S. keitaoensis		×	×	×				×	×	×	×	
S. scapiformis			×	×	×				×	×	×	

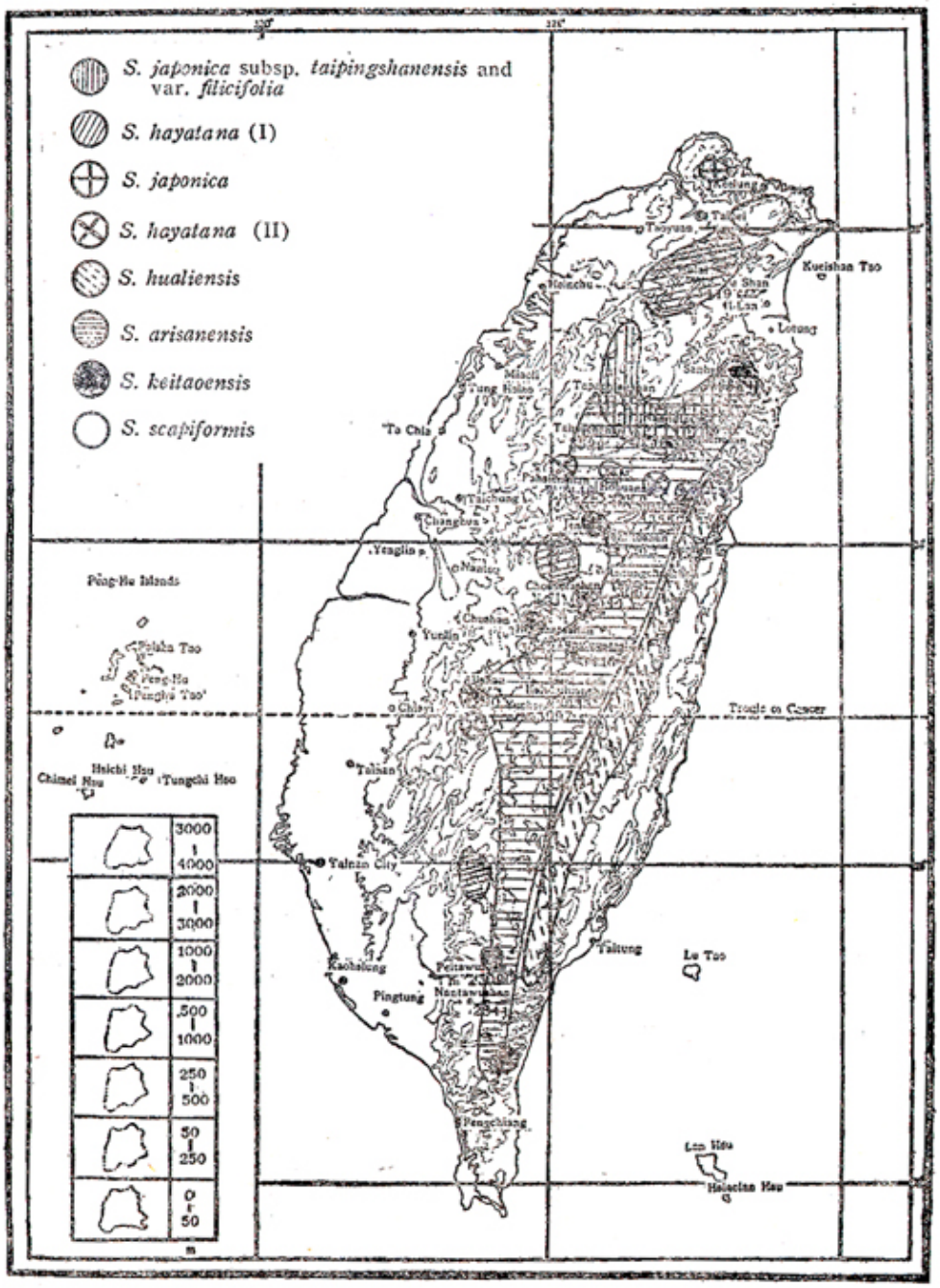

Fig. 11. Distribution map of Formosan *Salvia*.

Flowering Time and Ecological Study: All of the entities studied are insect pollinated annual herbs which die in the winter or die back to a certain extent and produce new growth in the spring. Some problems about the pollination of *Salvia* were well discussed by Visco & Capon (1970). Since, on the one hand, each population have a long flowering period and, on the other hand, they have a wide range of distribution and adaptability, most of the species show an overlap in their blooming time.

Although the exact times of initial blossoming and the duration of blooming varied with the locality and season, many entities are shown to blossom simultaneously over a long period of the year. Thus it is possible for any two species to hybridize if the ecological and other factors are favorable.

The geographical distribution of the Formosan *Salvia*, is indicated in Fig. 11, and it is found that all entities are endemic to the mountaineous ranges. They occured on the slopes, along the roadside or in the shady and moist spots along the mountain trails and on the forest floor. *S. arisanensis* is distributed widely in areas with altitudes ranging from 1800 to 3000 m all over the Central Mountain Range of Formosa. A great number of populations are found throughout this area. There is some variation in leaf morphology and pubescence between the northern and southern population groups.

Most of *S. hayatana* (I) is distributed in northern Formosa, which may be the original center of species. Some are spreaded on hill slopes surrounding the Puli Basin and some in Tawushan, in southern Formosa. It occupies the lowest altitude of the zones and is found below 800 m. *S. hayatana* (II) grows between *S. hayatana* (I) and *S. arisanensis* or *S. hualiensis* at altitudes from 600 to 1800 m. Each comes with a more or less small population.

S. hualiensis is restricted to the ranges on the eastern region along the hillsides of Hualien and Taitung and is found below the altitude of 1200 m. This species is kept apart from the others by the Central Mountain Range.

S. japonica is localized only on Tatunshan, north of Taipei. It is scattered almost all over that mountain. Subsp. *taipingshanensis* is distributed very abundantly on Tahsuehshan, Nanhutashan, Taipingshan and Peitsatienshan. Variety *filicifolia* of this subspecies is distributed in outlying areas where the subspecies is located. Based on morphological and ecological studies, subsp. *taipingshanensis* diverges from the original center in Taipingshan and differentiates in some areas in morphological characteristics to form its variety, *filicifolia*. Sympatric distribution of this subspecies with *S. arisanensis* is found in those districts of Tahsuehshan, Nanhutashan and Sauhsingshan, and with *S. hayatana* (I) is found in those areas near Peitsatienshan and Fusingshan.

Two populations of *S. keitaoensis* are seen, one in Chitou, Nantou, and the other in Wutai, Pingtung. Both are small populations.

S. scapiformis is localized near Keelung in northern Formosa, at altitudes no more than 500 m.

DISCUSSIONS

Chromatographic studies of anthocyanic constituents provide an available approach for investigating the interspecific relationships of each entity when coordinated with qualitative and quantitative analyses of carotenoids, pollen viability, chromosome characteristics and geographical distribution.

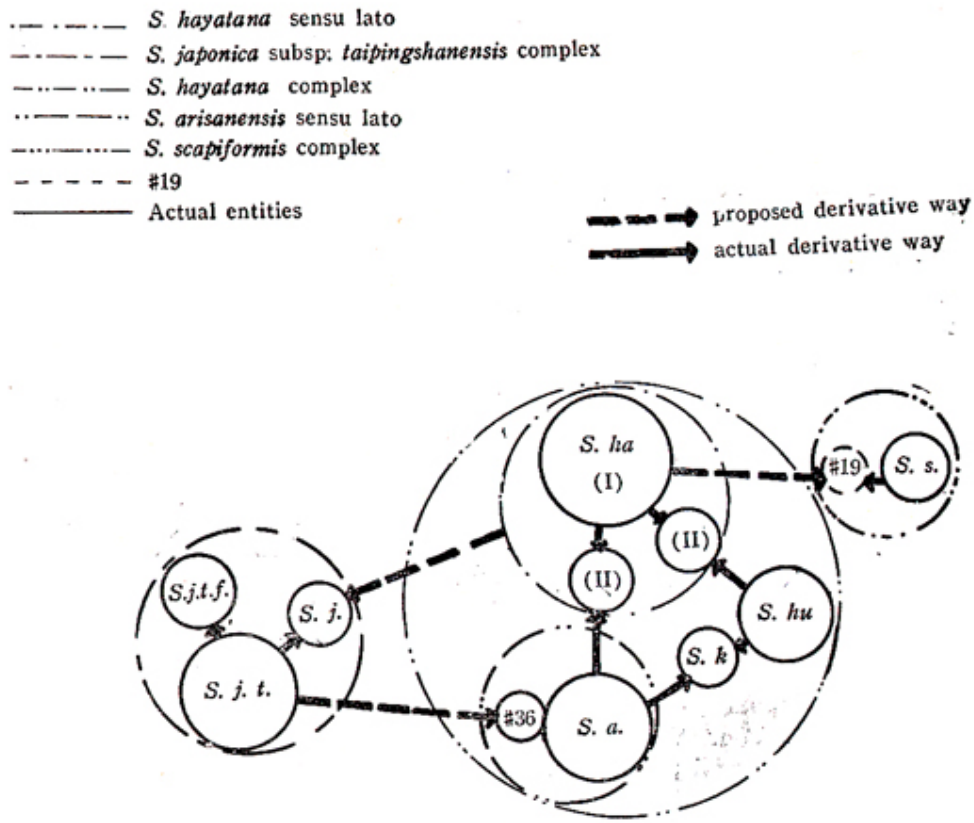

Fig. 12. Concept of reticulate relationships in Formosan *Salvia*. Where *S. j.*=*S. japonica*; *S. j. t.*=*S. japonica* subsp. *taipingshanensis*; *S. j. t. f.*=*S. japonica* subsp. *taipingshanensis* var. *filicifolia*; *S. a.*=*S. arisanensis*; *S. ha*=*S. hayatana*; *S. hu*=*S. hualiensis*; *S. s.*=*S. scapiformis*; *S. k*=*S. keitaoensis*.

Species, or subspecies, of *Salvia* characteristically differ from each other in many ways. Species difference does not depend on any single character but is a composite of many characters. Most of the entities have their own species specific morphological characteristics. But taxa whose morphological characters are intermediate, such as *S. hayatana* (II) and *S. keiatoensis*, are accompained with the qualitative and quantitative chemical tests which are intermediate, and with pollen which is much alike. All of the informations studied agree with the suggestions that *S. hayatana* (II) is derived from *S. hayatana* (I)×*arisanensis* or from *S. hayatana* (I)×*hualiensis* and *S. keitaoensis* is derived from *S. hualiensis*×*arisanensis*. The reason for the wide variation of morphological characteristics in *S. hayatana* (II), introgressing from *hayatana* (I) to *hualiensis* or *arisanensis*, is that introgressive hybridization has caused the spread of other entities into the gene complex of *S. hayatana*.

The chemical and morphological evidences suggest that *S. scapiformis*, *S. arisanensis*, *S. hayatana* (I), *S. hualiensis* and subspecies *taipingshanensis* of *S. japonica* are definitively ancestral rather than derivative and favor the recognition of variety *filicifolia* as being a morphologically instead of chemically differentiated

derivative of subspecies *taipingshanensis*. Such taxa as *S. japonica*, *S. hayatana* (II) and *S. keitaoensis* are undoubtly secondary derivatives.

From chromosome studies, it is observed that all entities are diploid with $n=8$, except for some individuals of *S. japonica* and *S. arisanensis* which are tetraploids. Chromatographic evidence suggests that the tetraploid of *S. arisanensis* is auto-polyploid and that *S. japonica* probably came from allopolyploidy stock which might have originated from the hybridization of subspecies *taipingshanensis* with a *hayatana-like* ancestor.

Based on our chemical, morphological, chromosome, anatomical and palynological studies, *S. hayatana* is relatively close to *S. arisanensis* and *S. hualiensis*. *S. arisanensis* is more or less related to subspecies *taipingshanensis* of *S. japonica*, and is genetically closer to *S. hualiensis* than to subsp. *taipingshanensis* of *S. japonica*. The reticulate phylogentic interrelationships of these species to each other is shown in Fig. 12.

Speciation based on pollen characters is seen in some of the tetraploid species, which show an increase in aperature number from 6 through 7 to 8-colpate and this is probably their speciation pathway.

All evidences show that the speciation of Formosan *Salvia* has been accomplished by two mechanisms: first, by hybridization to increase the ability of adaptation to various ecological environments; and second, by chromosome doubling to stabilize the genetic substances which were caused by hybridization or genetic drift.

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