CHROMOSOME STUDIES IN VERBENA WITH SPECIAL REFERENCE

TO THE COMMERCIAL VARIETIES

By

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29. Manton, I. 1937. The problem of Biscutella laevigata. Ann. Bot. New Series 1: 439-462. No. 3.

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of probable prototypes, namely: V. chamaedryfolia Juss. (V. peruviana (L.) Britton), V. phlogiflora Cham., V. incisa Hook., and V. teucrioides Gill. et Hook. (V. platensis Spreng.). Though a few more species are mentioned as being used by hybridizers, their influence, if any, seems to have been slight and transient.

That the garden varieties of the genus Verbena have phenotypical characters common to certain wild species is evident in discussions of the garden varieties by early workers. Hegi (21) lists several species which supposedly entered into the development of the garden forms. He mentions the four prototypes suggested by Bailey and also V. erinoides Lam. Hegi (21) further states that the garden hybrids having names such as Defiance and Scarlet Verbena are probably hybrids between V. phlogiflora Cham. and V. chamaedryfolia Juss. (V. peruviana (L.) Britton) and the blue and red eyed types are probably hybrids between V. teucrioides Gill. et Hook. (V. platensis) and V. chamaedryfolia Juss. (V. peruviana (L.) Britton)

More evidence for the probable influence of V. chamaedryfolia Juss. (V. peruviana (L.) Britton) and V. phlogiflora Cham. as parental species is found in Briquet's discussion of the Verbenaceae in Engler-Prantl (10). The two species are mentioned as being early garden favorites which, through natural and induced hybridization, have lost the original species characteristics.

Cytological literature dealing specifically with the genus Verbena has been meager. When the present work was begun in the fall of 1935, cytological literature pertaining to the Verbenaceae was limited to a field and laboratory study of three species of Verbena by Kanda (23) and a morphological survey of several representatives of the Verbenaceae by Schwencke (38). There was no work dealing with the chromosome numbers or morphology of the chromosomes until Dermen (14) published a cytological and hybridization study on two sections of Verbena. Noack (33) followed a year later with a similar study. The results of these papers as far as the chromosome numbers are concerned are presented in Table I.

It is the purpose of this investigation to survey the chromosome number of many varieties of the genus Verbena and to note any similarities of these, in so far as chromosome number and morphology are concerned, to the wild species.

MATERIALS AND METHODS

Seeds were assembled from several sources in North America, South America, Europe, Asia and Africa. Plants were grown both in the field and under glass. Root tips and buds for chromosome study were obtained from plants grown in flower pots. Somatic studies were made on root tips fixed in a modified Navaschin's fixative, embedded after the usual ethyl alcohol-xylol-paraffin and butyl alcohol-paraffin methods and cut at 10-13 microns. Sections were stained according to the iron alum-haematoxylin technique. The aceto-carmine smear method was used to obtain haploid numbers in pollen mother cells. Some haploid numbers were determined from sectioned materials.

Chromosome counts were made with the aid of a Zeiss apochromatic oil immersion objective with a numerical aperture of 1.3 and a 15x compensating ocular. Photomicrographs were taken with the same apparatus. Magnifications of the photomicrographs are indicated on the individual plates.

In an attempt to induce polyploidy, a specially designed heat chamber patterned after the apparatus of Brink (9) was employed. The heat chamber consisted of a double-walled bell jar containing distilled water between the walls. A low-lag heating unit was placed in the distilled water and the temperature was controlled within one degree plus or minus over periods as long as one hour by means of a rheostat. The plant was placed inside the jar and the bottom of the jar was sealed with asbestos board. Cotton was placed around the stem at the point of insertion at the bottom of the apparatus. Plants were also heat treated in the specially designed heat chamber at the U.S.D.A. Horticultural Station at Beltsville, Maryland through the kind cooperation of Dr. S. A. Emsweller and Dr. D. V. Lumsden.

V. rigida Spreng., *V. canadensis* (L.) Britton, *V. racemosa* Eggert and *V. erinoides* Lam. along with 15 varieties of *V. hybrida* Hort. were treated from one to three days between the hours of 10 in the morning to 2 in the afternoon. Time of exposure varied from 15 minutes to one hour. After exposure to heat the plants were placed at normal greenhouse temperature and later self-pollinated by hand when the anthers had developed.

Moist heat was applied to 56 plants representing 13 different varieties of V. hybrida Hort. at the Beltsville Station. The heat chamber at Beltsville permits treatment of several plants at one time and the entire plant is exposed by this method. The temperature within the heat chamber was brought to 48 degrees and the plants were then placed in the chamber and treated for 20 minutes. The temperature inside the chamber was controlled to within 0.5 degrees C. After treatment the plants were allowed to remain at normal greenhouse temperature and subsequently self-pollinated.

Efforts were also made to induce polyploidy by the use of the drug colchicine. Aqueous solutions varying from 0.5 - 1.5 per cent were used for soaking cuttings of White's Blue and White's Pink varieties. These were placed in colchicine solutions and allowed to remain for intervals of 6 hours and 20 hours. After treatment cuttings were planted in 4-inch pots. Roots were then collected and sectioned. Much the same technic has been recently reported by Walker (43).

Application of colchicine in lanolin paste was made on the growing points of several species and varieties. Concentration of colchicine in this case was about 0.5 per cent.

Hybridization experiments were carried out between species within the section Glandularia and between species of the section Glandularia and V. hybrida Hort.

Cytological results were compared with the taxonomic grouping of the Verbenaceae by Schauer (37), Briquet (10) and Perry (34). Taxonomical characters of the whole genus have been most ably worked out by Schauer (37) and his classification has been used as a basis for the taxonomical considerations in this work, though Perry's monograph has proved to be helpful in the naming and classification of North American species.

Herbarium specimens were made of all species and most of the varieties studied. These specimens were sent to Dr. H. N. Moldenke and Dr. L. M. Perry for identification. Herbarium specimens in the New York Botanical Garden Herbarium and the National Herbarium in Washington, D. C. were also consulted for accurate nomenclature of the species studied cytologically.

Table 1. Chromosome numbers previously reported for the genus Verbena

Section I. Verbenaca Schauer	2n	n
Leptostachyae Schauer		
V. bracteata Cav.	14(N)	-
V. Halei Small	14(D)	-
V. hastata L.	14(D)(N)	6(K) 7(N)
V. prostrata R. Br. (V. lasiostachys Link)	14(N)	7(D)(N)
V. neomexicana Small	-	7(D)
V. officinalis L.	14(N)	6(S)6(Sf)7(N)
V. angustifolia Michx. (V. simplex Lehm.)	14(D)(N)	4(K)
V. stricta Vent.	14(D)(N)	6(K)
V. urticifolia L.	14(D)(N)	7(N)6(S)
Pachystachyae Schauer		
V. hispida R. et P.	14(D)(N)	7(N)
V. bonariensis L.	28(D)(N)	-
V. litoralis H. B. K.	28(D)(N)	-
V. venosa Gill. et Hook. (V. rigida Spreng.)	42(D)(N)	21(N)
V. corymbosa R. et P.	56(N)	-
V. ovata Cham.	72(N)	-
Nobiles Schauer		
V. teucrioides Gill. et Hook. (V. platensis Spreng.)	10(D)(N)	5(N)
Section II. Glandularia Schauer		
V. erinoides Lam.	10(D)(N)	5(N)
V. grandiflora Ortega	10(D)	-
V. Aubletia Jacq. (V. canadensis (L.) Britton)	30(D)(N)	15(N)
V. ambrosifolis forma eglandosa Perry	30(D)	-
V. racemosa Eggert	30(D)	-
*V. hybrida Hort.	10(N)	5(N)

*Classification by Noack
Key to investigators: (K) - Kanda, (S) - Schwencke, (Sf) - Schnarf (quoted by Noack), (D) - Dermen.

Table 2. Chromosome numbers in V. hybrida Hort.

Name	Source	2n No.
Mammoth Fancy Helen Willmott	Michell (Phila.)	10
" " Purple Mantle	"	10
Newport Salmon	"	10
Etna	"	10
Royale	"	10
Erinoides	"	10
Erinoides elegans	"	10
Heliotrope Blue	"	42**
Hybrida gigantea White	Burpee (Phila.)	10
" " Apple Blossom	"	10
" " Etna	"	10
Drummondi	Mette (Germany)	30*
venosa argentea alba	"	42**
venosa lilacina	"	42**
auriculaeflora	"	10
candidissima	"	10
coccinea	"	10
coerula	"	10
Defiance	"	10
compacta alba	"	10
" carnea	"	10
" coerula	"	10
" Defiance	"	10
" rosea	"	10
Mammoth atrococcinea	"	10
" Cardinal	"	15***
" Fairy Queen	"	10
" Lucifer	"	10
" rosea	"	10
Defiance	Thompson & Morgan (Eng.)	10
Miss Willmott	"	10
Purple Garnet	"	10
compacta Cameo Pink	"	10
" Snowball	"	10
" coccinea	"	10
Mammoth Rose Queen	"	10
" Scarlet Queen	"	10
" Snow Queen	"	10
venosa	"	42**
" lilacina	"	42**
White's Blue	Campus Greenhouse	10
" Pink	"	15***

Note. * V. racemosa Eggert var., ** V. rigida Spreng. var., *** Triploid Vars.

Table 3. Chromosome numbers in Verbena species

Species	2n	n	Distribution
Section I. Verbenaca Schauer			
Leptostachyae Schauer			
V. hastata L.	14	7	North America
V. lasiostachys Link.	14		
V. officinalis L.	14		
V. simplex Lehm.	14	7	N. Eur., Asia and N.Amer.
V. stricta Vent.	14		
V. urticifolia L.	14	7	N. and Central America
Pachystachyae Schauer			
V. hispida R. et P.	14		Tropical and subtropical South America
V. bonariensis L.	28	14	
*V. brasiliensis Vell.	28	14	
V. litoralis H. B. et K.	28		
V. rigida Spreng.	42		
Nobiles Schauer (L.) Britton			
*V. peruviana	10	5	Temperate South America
V. hybrida Hort.	10	5	
Section II. Glandularia Schauer			
V. erinoides Lam.	10	5	Temperate South America
*V. tenuisecta Briq.	10	5	
*V. bipinnatifida Nutt.	30	15	Temperate North America
V. canadensis (L.) Britton	30	15	
V. racemosa Eggert	30		

* Species hitherto unreported

Table 4. Chromosome numbers in other genera of the Verbenaceae

	2n
Vitex agnus-castus L.	35
Vitex incisa Lam.	35
Lippia lanceolata Michx.	28
Lippia nodiflora (L.) Michx.	35
Lantana camara Cham.	42
Clerodendron myricoides (Hochst.) Gurke	175±

RESULTS

Chromosome numbers. The somatic numbers of 18 species of the genus Verbena, 35 representatives of V. hybrida Hort. and 9 species of 5 other genera of the Verbenaceae were determined. The results of these determinations are found in Tables II, III and IV, respectively. The species under consideration fell into two cytological classes, one group with a basic chromosome number of n-7 and a second group with a basic number of n-5.

The genus Verbena is divided into two sections, Verbenaca and Glandularia. The section Verbenaca is represented in this study by three subsections, Leptostachyae, Pachystachyae and Nobiles. The subsections Leptostachyae and Pachystachyae fall within the first cytological group, n-7. The Nobiles, however, is to be classified within the second class, n-5, along with the entire second section, the Glandularia.

The Leptostachyae subsection is entirely diploid. All species have the chromosome number 2n-14. The Pachystachyae subsection exhibits a polyploid series and contains only one diploid species, V. hispida. The species V. bonariensis and V. litoralis are tetraploid, 2n-28, and V. rigida is hexaploid, 2n-42. Two representatives of the subsection Nobiles indicate a condition of diploidy for the subsection. V. teucrioides Gill. et Hook. (V. platensis Spreng.) has been reported as a diploid by Dermen (14) and Noack (33) while this study includes another diploid species V. peruviana (L.) Britton, 2n-10. Diploidy for this subsection is further indicated by the fact that phenotypical characters of the diploid hybrid garden forms are present in the wild species of the group.

The section Glandularia, which includes the large-flowered Verbenas of our western plains and also a few species native to temperate South America, has two somatic numbers, 2n-10 and 2n-30. The species V. erinoides Lam. and V. tenuisecta Briq. have the chromosome number 2n-10 and are both native to temperate South America. The North American species all have the chromosome number 2n-30. Thus the section Glandularia is divided into two groups.

Varieties of the garden Verbenas are generally diploid, 2n-10 and n-5, with the exception of a large-flowered pink variety which has a somatic number of 2n-15 and a variety named Cardinal obtained from Mette in Germany which has given rise to both diploid and triploid seedlings.

Chromosome numbers in other genera of the Verbenaceae seem to indicate a basic number of 7. (Table IV) The species Vitex agnus-castus L. and Vitex incisa Lam. along with Lippia nodiflora (L.) Michx. gave somatic numbers of 2n-35. Lippia lanceolata Michx. was tetraploid with 2n-28 chromosomes. Lantana camara Cham. was hexaploid with 2n-42 chromosomes. The highest chromosome count in the genus was obtained from Clerodendron myricoides (Hochst.) Gurke, 2n-175-.

Chromosome size. Chromosomes in the genus Verbena vary in size from 1 to 4 microns in length. The smallest chromosomes are found in the Leptostachyae and measure from approximately 1.0 micron to about 1.5 microns. The Pachystachyae measure about the same for the smaller chromosomes but the larger chromosomes sometimes reach a length of 1.9 microns. Only one species of the subsection Nobiles was available for study. This species had chromosomes which were a little larger than those of the Pachystachyae, being from 1.8 to 2.2 microns in length. The longest chromosomes were found in the variety White's Blue. These measured 4 microns. Other varieties of V. hybrida Hort. varied in length from 2 to 3.8 microns. Thus, in the section Verbenaca, the size of chromosomes seems to increase with each succeeding subsection.

The section Glandularia has larger chromosomes as a whole than has the section Verbenaca. Chromosomes are all at least 2 microns long and reach a maximum length of about 3.5 microns. The chromosomes of the Glandularia are not as thick in proportion to their length as those of the section Verbenaca.

Artificial induction of polyploidy. Heat treatments to flower buds, whether by the application of dry or moist heat, gave negative results at the temperatures used. Some treated flower heads developed and set seed after treatment but the seed failed to germinate. For the most part treatment proved lethal to the young meristematic parts of the plants. The flower heads withered and drooped soon after treatment. *V. canadensis* (L.) Britton and most commercial varieties appeared to survive the treatments best.

Most of the efforts to induce polyploidy by the use of the drug colchicine were unsuccessful. Many of the cuttings soaked in the colchicine solutions died a few days after being placed in pots. In one case, White's Blue variety cuttings soaked in 0.5 per cent colchicine solution for 20 hours, 2 out of 20 cuttings survived treatment and rooted. However, root tip counts revealed no change in chromosome number. At the time of writing, the cuttings had neither flowered nor formed flower buds. Therefore, an investigation of meiotic chromosomes such as was carried out by Walker (43) was not possible.

Application of 0.5 per cent colchicine in lanolin paste by means of a pipette to the growing points, also gave negative results. The growing points turned brown, drooped and finally died.

Hybridization. Hybridization was attempted in crosses between species of the section Glandularia and commercial varieties and interspecific crosses within the section Glandularia. Although seeds were set in some cases the resulting progeny proved to have maternal characteristics indicating self-pollination had taken place.

Crosses between the 2n-10 chromosome species and the 2n-30 chromosome species gave negative results. Crosses between the 2n-30 chromosome species produced progeny which showed variation in depth of flower color. However, other phenotypical characters were so much alike that it is doubtful that actual hybrids were produced. Moreover, chromosome plates gave no indication of hybridity. Meiotic studies were not made of the hybrids. Since variation in flower color seems to be a characteristic, common even to species of the Verbenaceae, it is untenable that a change in depth of color would indicate interspecific hybridity.

Satellites. The type species of the genus *V. officinalis* L., exhibits two satellites (Fig. 7). No satellites were observed in any other species. However, satellites were often observed in commercial varieties. One satellite per chromosome plate was quite common, two per plate were rare and never more than two were observed in any plate.

DISCUSSION

Chromosome determinations in this study have confirmed counts made by Dermen (14) and Noack (33). In addition to these counts, somatic and haploid chromosome numbers of four more species of the genus Verbena have been determined. These species are as follows: V. brasiliensis Vell. (n-14, 2n-28), V. peruviana (L.) Britton (n-5, 2n-10), V. tenuisecta Briq. (n-5, 2n-10) and V. bipinnatifida Nutt. (n-15, 2n-30). The species V. grandiflora Ortega, reported by Dermen (14) as having a chromosome number of 2n-10 and belonging to the section Glandularia, is considered by Perry (34) to be a species of doubtful classification and is not included in her monograph as a member of the genus Verbena.

Results of the present study are not in agreement with the counts reported by Kanda (23), Schwencke (38) and Schnarf (33). Kanda (23) has reported V. angustifolia Michx. to have 4 haploid chromosomes, V. hastata L. as n-6, V. stricta Vent. as n-6 and an intermediate form as n-6. Schwencke (38) found 6 haploid chromosomes in V. officinalis L. and V. urticifolia L. Schnarf is quoted by Noack (33) as giving the haploid number of V. officinalis L. as n-6.

Special emphasis is placed on the commercial hybrids known collectively as V. hybrida Hort. To date no intensive survey of this group has been made cytologically, though Noack (33) has classified V. hybrida Hort. under the section Glandularia. However, this classification is to be questioned. According to Bailey (4), four species of the subsection Nobiles of the section Verbenaca are generally recognized as the prototypes of the garden varieties. This view is supported by Hegi (21) and Briquet (10) and is based on studies of taxonomical characters common to the wild species and the garden forms. The most salient character which separates the two sections of Verbena is the presence or absence of appendages on the upper anthers. The fact that none of the garden hybrids has this character would seem to eliminate the possibility of their belonging to the section Glandularia. Though a few members of the Glandularia are mentioned as being used by hybridizers their influence, if any, has been slight and transient.

The similarity of chromosome number in the species of the subsection Nobiles and V. hybrida Hort. is further indication of relation between the two groups. Unfortunately, only two species of the subsection Nobiles have been studied. V. teucrioides Gill. et Hook. (V. platensis Spreng.) has been reported by Dermen (14) and Noack (33) as having a 2n-10 chromosome number while the present study includes V. peruviana (L.) Britton as 2n-10. All commercial varieties have a chromosome number of 2n-10 with the exception of two which exhibited triploidy.

The relation of the chromosome number of other groups of the genus to the taxonomic classification has been emphasized by Dermen (14). He has shown that the species studied fall into two cytological classes, one with a basic number of n-5 chromosomes, the other with n-7. The cytological classification was found to correspond to the taxonomical classification of the North American species by Perry (34) in that the section Verbenaca with its two subsections Leptostachyae and Pachystachyae, has the basic number of n-7 while all the species with n-5 and n-15 chromosomes belong to the section Glandularia. However, Perry's monograph is concerned only with the North American species and the South American subsection Nobiles is not included in her study. The species of the Nobiles are all listed as being native to South America by Briquet in Engler-Prantl (10), Hegi (21) and Schauer (37). If the genus as a whole is to be considered, the classification suggested by Dermen (14) must be modified. The section Verbenaca should be listed as containing two subsections, the Leptostachyae and Pachystachyae, with the basic number n-7 and one subsection, Nobiles, with the basic number n-5. Further investigation of the remaining subsections may disclose the necessity of even more modification.

During the course of examining somatic chromosome plates it was noted that there appeared to be an abnormally high percentage of naturally occurring polyploid cells. Current literature reveals that this phenomenon is not uncommon. Darlington (13) is of the opinion that somatic doubling in tissues may be due to a special racial propensity. Increase in chromosome number by natural means has been reported by Breslawetz (7 and 8), Langlet (24), Meurman (30), Wulff (45), Lorz (27) and many others. Lorz (27) has discussed the causes of increase in chromosome number in somatic tissue. He explains doubling of chromosome number as being caused by asynchronous chromosomal and nuclear division. The division and separation of the chromosomes occurring at a faster rate that the division of the nucleus as a whole, ultimately results in two chromosomal cleavages occurring within one nuclear cycle.

Doubling of chromosome number was observed in the root tips of several species and varieties of Verbena. Diploid and tetraploid cells were found in the same root tip of the species V. peruviana (L.) Britton (Figs. 20, 21, 22). The variety Purple Mantle showed somatic doubling (Figs. 9 and 10).

Other variations in chromosome number were also noted. Indefinite increase in chromosome number was observed in V. bipinnatifida Nutt. Root tips of V. litoralis H. B. K., a normally 2n-28 species, were observed to have the following numbers: 2n-14, 2n-21 (Fig. 13), 2n-28 (Fig. 14) and 2n-42. Dermen (14) has shown that this species also has irregular meiotic divisions. The species V. canadensis (L.) Britton, normally 2n-30, had root tips with 2n-20 chromosomes (Figs. 11 and 12). This number is of special interest in that Dermen (14) has reported hybrids with 2n-20 chromosomes between V. racemosa (2n-30) and V. erinoides (2n-10). It is possible that the 2n-20 plant found in with V. canadensis may have been a hybrid between V. canadensis and V. erinoides, although no marked variation was observed among the plants observed cytologically. However, variation in V. canadensis is quite extensive and determination of hybridity by phenotypical characters is not dependable.

Probably the most interesting variation in chromosome number is that observed in commercial hybrids. White's Pink, a variety which has been carried along in the greenhouses at College Park for a period of about 10 years, has a somatic number of 2n-15. This variety has desirable commercial attributes which may or may not be due to the increase of chromosome number. The flowers are larger and appear earlier than the diploid varieties, vegetative growth is much more vigorous and the plants appear to be comparatively disease-resistant. Plants are sterile, though pollen appears to be fair under the microscope. Sterility in Verbena hybrids does not detract from their commercial importance, however, because of the ease of propagating by vegetative cuttings. Seeds of another variety, Cardinal (Mette) have given rise to both diploid and triploid seedlings. The occurrence of triploidy in these two varieties led to the above-mentioned efforts to induce polyploidy by artificial means. The negative results obtained from the experiments do not necessarily indicate that it is impossible to obtain polyploids by these methods. There is the possibility that correct correlation between chromosome divisions, correct temperature and length of exposure was not obtained in the case of the heat treatments and that the correct concentration of the drug along with the correct time and length of exposure was not obtained in the colchicine experiments. Furthermore, it is now the concensus of opinion that colchicine treatment is more effective if the drug is applied to germinating seeds rather than to the more mature tissues.

That polyploidy may be induced in nature by unfavorable environmental factors is shown by Hagerup (17). His study of 29 different plants of the southern part of the Sahara Desert reveals that some of the plants are polyploid and, in comparison with the parent species, have acquired new genetical and morphological characters which enable them to grow in different habitats. In another study Hagerup (18) found that the form of Vaccinium ulinosum L. with the greatest number of chromosomes was the most resistant to unfavorable conditions. Similar studies by Muntzing (32), Heilborn (22), Anderson and Woodson (1), Anderson and Sax (2), Anderson (3), Wulff (46), Tischler (42), Manton (28 and 29), Strelkova (41) and Sokolskaya (40) indicate that a correlation exists between polyploidy and geographic distribution.

The agreement between geographic distribution and polyploidy in the genus Verbena has been noted by Noack (33). He has observed: (1) That the members of the subsection Leptostachyae all have 2n-14 chromosomes and that all are native to temperate North America with the exception of V. officinalis L. which is native to middle and northern Europe and Asia. (2) That the members of the subsection Pachystachyae are native to tropical and subtropical South America and that they exhibit polyploidy ranging from 2n-14 to 2n-72. (3) That the polyploidy extant in the Pachystachyae may be explained by the extreme conditions in the climate of their habitat.

In addition to these facts pointed out by Noack (33), it is to be noted that the section Glandularia may be divided into two geographo-taxonomic groups on the basis of chromosome numbers. (1) A 2n-10 group containing the species V. erinoides Lam. and V. tenuisecta Briq. which are native to temperate South America and (2) A 2n-30 group including the species

V. bipinnatifida Nutt., V. canadensis (L.) Britton and V. racemosa Eggert. The species V. ambrosifolis forma eglandosa Perry, reported by Dermen (14) may also be included in the second group.

Hybridization in the genus Verbena is reported as early as November 23, 1751 in Linnaeus' Amoenitates Academicae. Roberts (36) has included both a translation of the description and illustrations of the hybrid between Veronica maritima x Verbena officinalis which was reported by Johannes Haartman. Another hybrid, Verbena hastata x Verbena spuria (V. officinalis) is mentioned in the same report and is stated to have originated naturally in the Botanical Garden in 1748, perishing two years later. The hybrid appeared in the same bed with the two species named above.

Moldenke (31) has found several naturally occurring hybrids. Among these are: V. Blanchardi, a name proposed for the natural hybrid between V. simplex Lehm, and V. hastata L., V. Deamii, a natural hybrid between V. bracteata Lag. & Rodr. and V. stricta Vent., V. Engelmanni, a hybrid between V. hastata L. and V. urticifolia, V. illicita, a hybrid between V. stricta Vent. and V. urticifolia L., V. moechina, a hybrid between V. simplex Lehm. and V. stricta Vent., V. Perriana, a hybrid between V. bracteata Lag. & Rodr. and V. urticifolia L. and V. Rydbergii, a hybrid between V. hastata L. and V. stricta Vent. These hybrids are results of natural crosses between members of the subsection Leptostachyae which has a 2n-14 chromosome number.

That hybridization occurs between certain groups in the genus Verbena has been established by Dermen (14). He reports the following successful crosses in his n-7 group which included the Leptostachyae and Pachystachyae of the section Verbenaca:

V. hispida x V. prostrata (V. lasiostachys Link.)
" x V. hastata
" x V. officinalis
" x V. simplex
" x V. urticifolia
" x V. litoralis
" x V. bonariensis

V. officinalis x V. prostrata
V. Halei x "
" x V. neomexicana
" x V. officinalis
V. hastata x V. urticifolia
V. urticifolia x V. prostrata
V. urticifolia x V. neomexicana

From the above crosses it is indicated that the Leptostachyae and Pachystachyae are interfertile only in the case of V. hispida. This species is interfertile with five species of the Leptostachyae. The cross between V. hispida (n-7) and V. litoralis (n-14) was successful only when V. hispida was used as a female parent. The resulting hybrid plants were pentaploid with 35 chromosomes. Triploid plants (n-21) were obtained from the cross V. hispida (n-7 x V. bonariensis (n-14). The n-21 chromosome form found among V. litoralis plants (Fig. 13) in the present study may have been a similar hybrid, but here again, no marked variation was observed in the plants used for cytological examination.

For the n-5 and n-15 group, Dermen has reported the following successful crosses:

V. hybrida x V. erinoides
Crosses between V. canadensis, V. canadensis var. atroviolacea, V. canadensis var. compacta and V. racemosa
V. racemosa x V. erinoides
V. racemosa x V. erinoides alba

Hybrids between V. racemosa x V. erinoides and V. erinoides alba were tetraploid with 2n-20 chromosomes.

Hybridization experiments carried out during the present investigation substantiate Dermen (14) in so far as crosses involving n-5 and n-15 species crossed with n-7 species are concerned. No seeds were obtained in attempted crosses between any species of the Leptostachyae x any species of the Glandularia, any species of the Pachystachyae x any species of Glandularia or in any crosses involving the Leptostachyae or Pachystachyae with any commercial hybrid.

The following crosses yielded seeds which failed to germinate: V. canadensis x V. erinoides and the reciprocal, V. canadensis x var. Helen Willmott and the reciprocal, V. canadensis x var. Royale and the reciprocal and V. canadensis x var. Erinoides elegans and the reciprocal. It is to be noted that each of these crosses involves V. canadensis, an n-15 species with various representatives of the n-5 chromosome group. The species V. racemosa with which Dermen (14) reports success in crosses with V. erinoides and V. erinoides alba was not used in hybridization experiments.

Seeds and seedlings were obtained from the following crosses: var. Purple Mantle x Royale and Purple Mantle x V. erinoides. Root tips from the seedling hybrids of the former gave no indication of hybridity because both plants had the chromosome number n-5. In the latter case seedling hybrids were maternal in appearance and it is presumed that the female parent was self-pollinated. No difference in chromosome morphology was observed in root tips of the seedlings.

The above crossing experiments indicate that crosses between the two sections of Verbena are not readily obtained.

Only one species was found to exhibit satellites. V. officinalis L., the type species of the genus, had satellites on two of its four somatic chromosomes. On the other hand, Noack (33) has reported satellites in fourteen species of the Leptostachyae, V. angustifolia Michx. (V. simplex Lehm.), V. bracteata Cav., V. hastata L. and V. officinalis L., while Dermen (14) has found satellites on V. angustifolia Michx. (V. simplex Lehm.), V. urticifolia L. and V. officinalis. The only species of those studied in the Leptostachyae for which no satellites have been reported is V. lasiostachys Link.

No satellites were found in the subsection Pachystachyae in the present study of by Dermen (14). Noack (33) figures a somatic plate with one satellite in the species V. hispida Ruiz et Pav., the only diploid species of the Pachystachyae.

Of the subsection Nobiles, only two species have been studied. Noack (33) reports four satellites in somatic plates of V. teucrioides Gill. et Hook. (V. platensis Spreng.) while Dermen (14) working with the same species has reported no satellites. No satellites were found in V. peruviana (L.) Britton during the present investigation. Both Dermen (14) and Noack (33) report satellites in various forms of V. hybrida Hort.

Noack (33) is the only worker to report satellites in the section Glandularia. He has found two satellites in each of two species V. erinoides Lam. and V. Aubletia Jacq. (V. canadensis (L.) Britton.)

The lack of agreement between workers concerning the presence or absence of satellites may be due to difference in fixation. Noack (33) notes this possibility as follows: "Da die fixierung auf die Erscheinungsform der Trabanten oft von groszem Einflusz ist..." Thus, the presence or absence of satellites is not to be considered of major importance until they can be seen with more regularity.

SUMMARY

1. Chromosome numbers of 18 species and 35 varieties of Verbena and 9 species of 5 other genera of the Verbenaceae are reported.

2. The genus Verbena can be divided into two cytological groups on the basis of chromosome number. (1) An n-5 group and (2) An n-7 group. The n-5 group contains the subsection Nobiles of the section Verbenaca and the entire Glandularia section. The n-7 group is composed of the subsections Leptostachyae and Pachystachyae of the section Verbenaca.

3. The subsection Leptostachyae is made up entirely of diploid species. The subsection Pachystachyae exhibits a wide range of polyploid species and the subsection Nobiles is probably diploid.

4. The section Glandularia may be subdivided into two cyto-geographic groups. (1) An n-5 group of species native to South America and (2) An n-15 group native to North America.

5. The origin of the commercial hybrids known collectively as V. hybrida Hort. is discussed.

6. Hybridization, chromosome relations and satellites, artificial induction of polyploidy and geographic distribution of the genus Verbena in its relation to polyploidy is discussed.

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Fig. 1

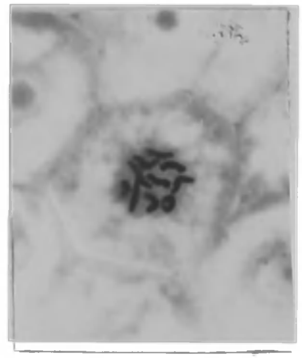
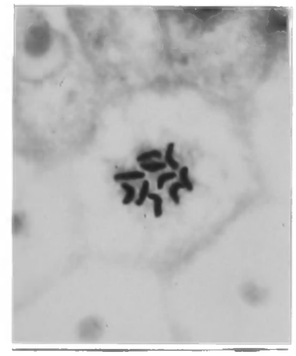


Fig. 2



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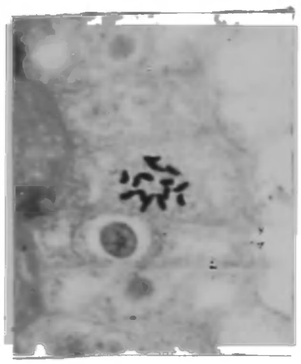
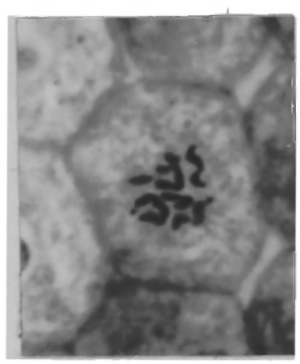


Fig. 4

Fig. 5

Fig. 6



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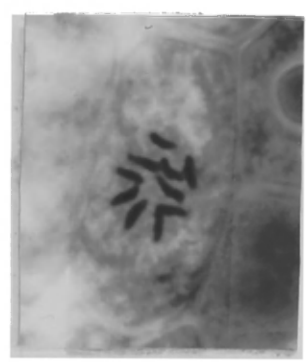

Fig. 8

Fig. 1. V. hybrida var. Royale, 10 chromosomes (x1350). Fig. 2. V. erinoides, 10 chromosomes (x1350). Fig. 3. V. hybrida var. Erinoides elegans, 10 chromosomes (x1350). Fig. 4. V. lasiostachys, 14 chromosomes (1350). Fig. 5. V. hybrida var. White's Blue, 10 chromosomes and two satellites (x1750). Fig. 6. V. hybrida var. White's Pink, 15 chromosomes (x2000). Fig. 7. V. officinalis, 14 chromosomes and two satellites (x2000). Fig. 8. V. peruviana, 10 chromosomes (x2000).

Fig. 9

Fig. 10

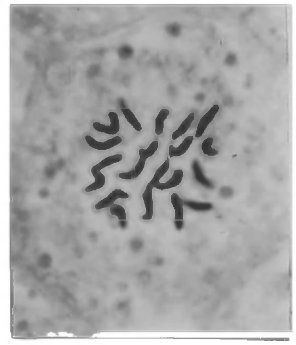

Fig. 11

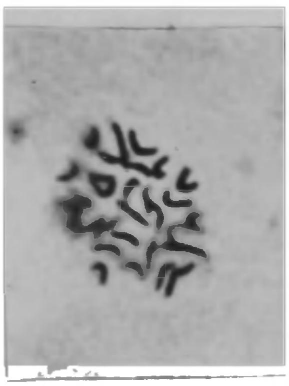

Fig. 12

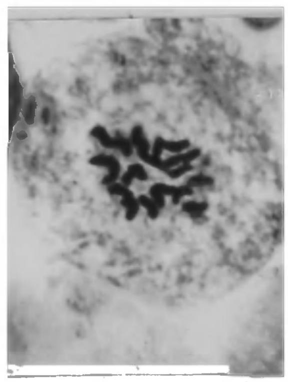

Fig. 13

Fig. 14

Fig. 15

Fig. 16

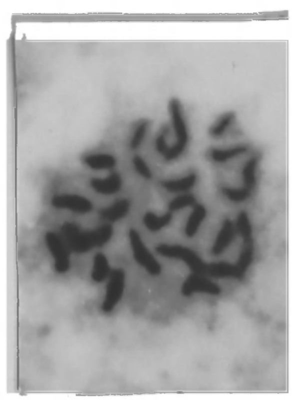

Fig. 17

Fig. 9. V. hybrida var. Purple Mantle, 10 somatic chromosomes (x 1750). Fig. 10. V. hybrida var. Purple Mantle, 20 somatic chromosomes (x 1750). Fig. 11. V. canadensis, 20 chromosomes (x1500). Fig. 12. V. canadensis, 30 chromosomes (x1500). Fig. 13. V. litoralis, 21 chromosomes (x2000). Fig. 14. V. litoralis, 28 chromosomes (x2000). Fig. 15. V. hybrida var. Etna, 10 somatic chromosomes with one satellite (x1750). Fig. 16. V. hybrida var. Erinoides elegans (x2500). Fig. 17. V. bonariensis, 28 chromosomes (x2500).

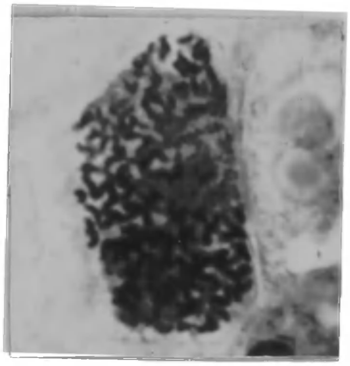

Fig. 18

Fig. 19

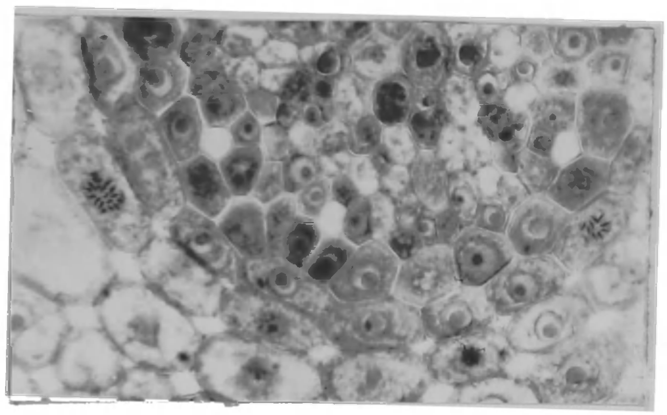

Fig. 20

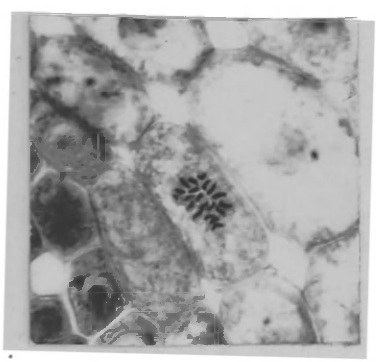

Fig. 21

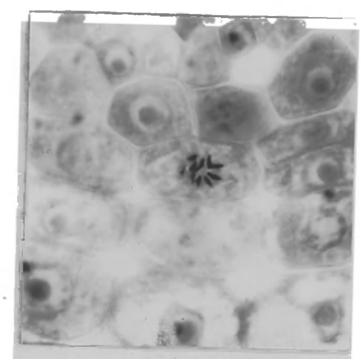

Fig. 22

Fig. 18. Clerodendron myricoides, approximately 175 chromosomes (x2500).
Fig. 19. Normal hexaploid plate, 30 chromosomes, of V. bipinnatifida (x3000).
Fig. 20. Section of a root tip of V. peruviana showing both diploid and tetraploid plates (x660).
Fig. 21. Enlargement of tetraploid plate of Fig. 20 (x750)
Fig. 22. Enlargement of diploid plate of Fig. 20 (x750)