Cytotaxonomy of the Family Bromeliaceae

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Introduction

The family Bromeliaceae is represented in India both by wild and cultivated species. A large number of the species is valued from the horticultural standpoint, for their flowers, leaves and in case of *Ananas sativus* (Pineapple) for the fruit.

Taxonomists have classified Bromeliaceae into four tribes though the relationship between them is obscure. The phylogenetic position of the different genera is still debated (Pittendrigh 1948). In spite of the economic importance of the family as well as the taxonomic importance of several genera, very little work has been done on the cytology of different taxa. The work that has been done upto date (Billings 1904, Collins and Kerns 1931, 1935, Collins 1933, Lindschau 1933, Tschischow 1956, Diers 1961, Gadella and Kliphuis 1964, Favarger and Huynh 1965) has been confined to chromosome counts only and no information is available regarding its morphology. The lack of data in this family is principally due to the very small size of the chromosomes, which taken in conjunction with heavy cytoplasmic content provided extreme difficulty in fixation. It was therefore thought necessary to work out a method suitable for the study of chromosome morphology in Bromeliaceae. A detailed study of chromosome is essential to solve the problem of taxonomic dispute of this family and also to provide an understanding for a new project in improvement. In the text, fifteen different species belonging to seven genera under the family Bromeliaceae have been incorporated.

Materials and methods

For the present investigation, fifteen species and varieties under seven genera were collected and observed.

They are all ornamental plants, cultivated solely for their variegated foliage and their colourful flowers. Although a few species are propagated by seeds, the main method of propagation is through cuttings during the rainy season.

The plants were procured from the local nurseries and grown in earthenware pots in the University garden. Unfortunately due to the scarcity of flowering in the plants, meiosis could not be studied in most of them.

Studies of the somatic chromosomes, were mainly made from temporary squash preparations. Different pretreating chemicals were tried. Pretreatment in saturated solution of paradichlorobenzene in dist. water again saturated with aesculin, for two hours and fifteen minutes at 8°C–10°C, was found most suitable and as such it was used as a common pretreating agent in all cases. The root tips were then fixed in acidulated alcohol (1 part conc. HCl: 4 parts of rectified spirit) for 40 minutes and kept in 45% acetic acid for an hour and stained in 2% aceto-orcein and (N) HCl mixture (9 : 1). They were kept over night in the stain at 45°C and finally squashed in 45% acetic acid. Meiosis was studied mainly from aceto-carmine smears. The preparations were made permanent by inverting the slides in butyl alcohol grades and finally mounting in euparal.

Figures were drawn with ×20 eye-piece and 1.3 apochromatic objective with a condenser of 1.3 N.A. at a table magnification of approximately× 2,900. In the figures and idiograms, the chromosomes with secondary constrictions have been drawn in outline.

Observations

The present study of the family Bromeliaceae reveals a natural assemblage. The chromosomes are generally very small in size. Besides, the different species and varieties differ from each other by their definite chromosome characteristics and these may be considered as the criteria for their identification. The chromosome complements studied here show similarities in their morphology and size and for this a general karyotype is described for the family. The general types which can be identified are :

Type A : Medium sized chromosome with two constrictions, primary and secondary, one nearly median and other nearly sub-terminal at the shorter end.

Type B : Medium sized chromosome with two constrictions located at the opposite ends of the long middle arm, on nearly sub-median and the other nearly sub-terminal in position.

Type C : Medium sized chromosome with a nearly median primary constriction and a satellite at the distal end of one arm, attached by a SAT-thread.

Type D : Medium sized to very short chromosome with nearly sub-median to median primary constriction.

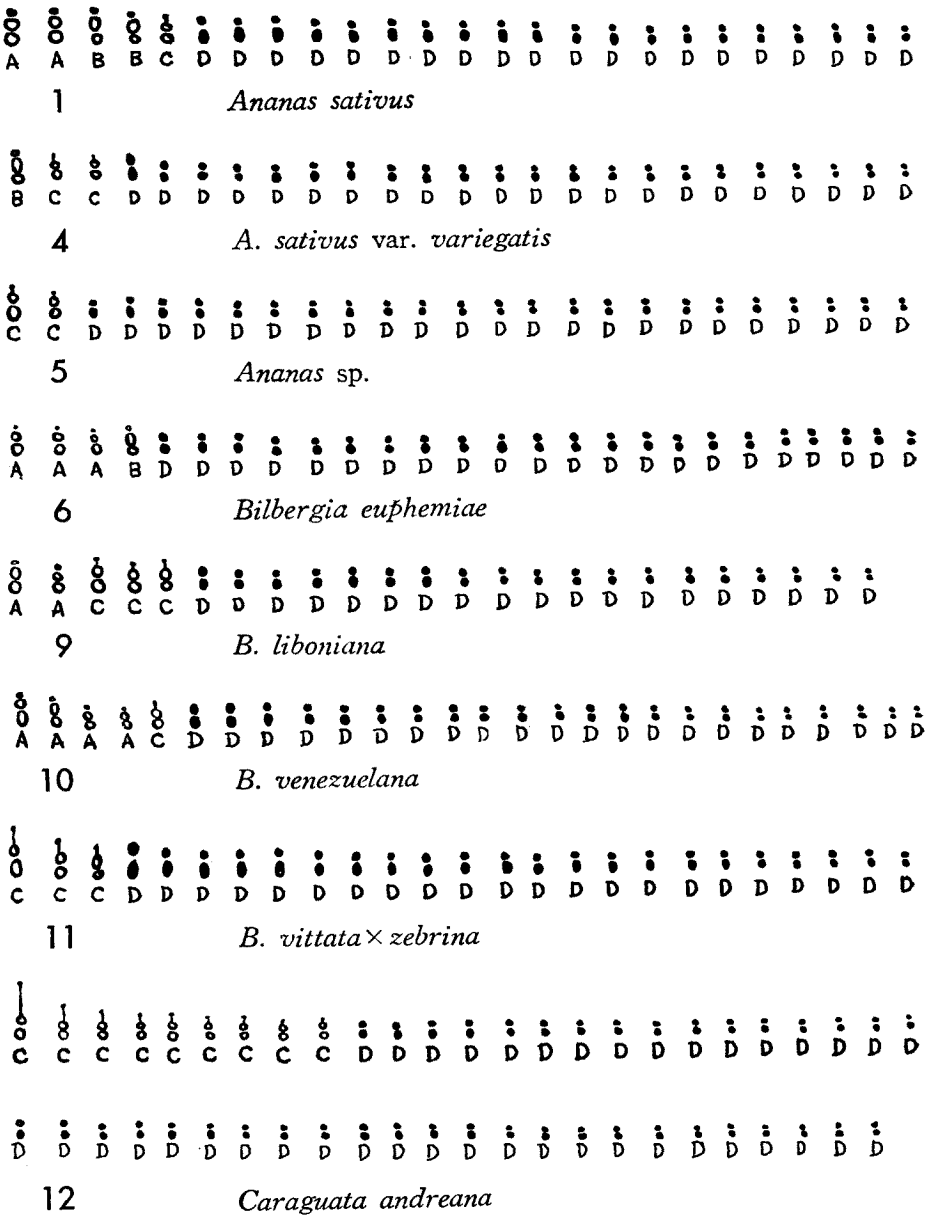
Genus—*Ananas* Tourn. ex Linn.

Two species and one variety of this genus have been studied. All of them show a constant chromosome number, viz. 2n=50. Chromosomes are very short. Secondary constrictions are found to be present in two to five pairs of chromosomes. Gradation in size is noticed.

1. *Ananas sativus* Schultz $2n = 50 = A_4 + B_4 + C_2 + D_{40} = 1.7\mu - 0.89\mu$ (Fig. 1).

Somatic nuclei showing varying chromosome numbers 2n=28 and 34 (Figs. 2, 3) have also been observed.

2. *A. sativus* Schultz, var. *variegatis* 2n=50=$B_2+C_4+D_{44}$=1.5μ−0.68μ (Fig. 4).



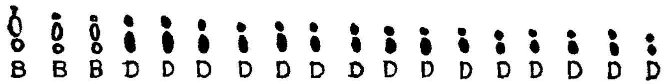
Figs. 1, 4, 5, 6, 9, 10 and 12. Showing the idiograms of *Ananas sativus* (2n=50), *A. sativus* var. *variegatis* (2n=50), *Ananas* sp. (2n=48), *Bilbergia euphemiae* (2n=52), *B. liboniana* (2n=48), *B. venezuelana* (2n=54), *B. vittata×zebrina* (2n=50), *Caraguata andreana* (2=98) respectively.

3. *Ananas* species (horticultural) $2n=50=C_4+D_{46}=1.7\mu-0.51\mu$ (Fig. 5).

Genus—*Bilbergia* Thunb.

The four species of this genus explored differ in their somatic chromosome number. Chromosomes are medium sized to very short. Secondary constrictions are located on three to five pairs. The different species show similarity in their general chromosome morphology.

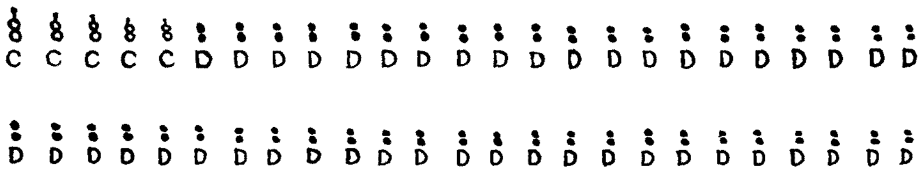
4. *Bilbergia euphemiae* E. Morr. $2n=52=A_6+B_2+D_{44}=1.3\mu-0.68\mu$



16 *Cryptanthus bivittatus*

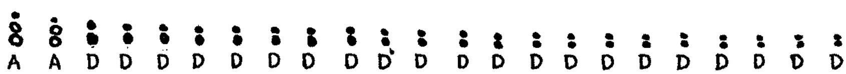
17 *C. bromelioides*

18 *C. praetextus*

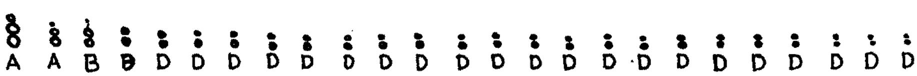


19 *Dyckia argentea*

20 *D. sulphurea*



21 *Neoregelia spectabilis*



22 *Pitcairnia punicea*

Figs. 16, 17, 18, 19, 20, 21 and 22. Showing the idiograms of *Cryptanthus bivittatus* (2n=36), *C. bromeloides* (2n=34), *C. praetextus* (2n=34), *Dyckia argentea* (2n=100), *D. sulphurea* (2n=50), *Neoregelia spectabilis* (2n=46) and *Pitcairnia punicea* (2n=50) respectively.

(Fig. 6). Variant nuclei with 2n = 42, 56 (Figs. 1, 8) have been observed.

5. *Bilbergia liboniana* D. Forst. 2n = 48 = $A_4 + C_6 + D_{38} = 1.5\mu - 0.5\mu$ (Fig. 9).
6. *B. venezuelana* Mez. 2n = 54 = $A_8 + C_2 + D_{44} = 1.7\mu - 0.5\mu$ (Fig. 10).
7. *B. vittata* Brongn × *B. zebrina* Lindl. 2n = 50 = $C_6 + D_{44} = 2.4\mu - 0.89\mu$ (Fig- 11).

Genus—*Caraguata* Lindl.

Only one species under this genus has been worked out. Chromosomes are comparatively long to very small in size.

8. *Caraguata andreana* E. Morr. 2n = 98 = $C_{18} + D_{80} = 2.7\mu - 0.51\mu$ (Fig. 12).

Somatic nuclei showing variations in chromosome number with 2n = 82, 86 and 94 (Figs. 13, 14 and 15) have also been observed.

Genus *Cryptanthus* Otto et Dietr.

Three species under this genus have been studied. Two of them contain 2n = 34 chromosomes and one 2n = 36 chromosomes. Chromosomes are medium sized to small. Secondary constrictions are located on six to eight of them.

9 *C. bivittatus* Regel. 2n = 36 = $B_6 + D_{30} = 2.2\mu - 1.0\mu$ (Fig. 16).
10. *C. bromeloides* Otto and Dietr 2n = 34 = $A_8 + D_{26} = 2\text{-}4\mu - 1.0\mu$ (Fig. 17).
11. *C. praetextus* Morr. ex Baker 2n = 34 = $B_8 + C_2 + D_{24} = 2.4\mu - 1.0\mu$ (Fig. 18).

Genus *Dyckia* Shuft.

Two species under this genus have been investigated one with 2n = 50 and the other with 2n = 100 chromosomes. Chromosomes are in general short.

12. *Dyckia argentea* Nichols. 2n = 100 = $C_{10} + D_{90} = 1.5\mu - 0.98\mu$ (Fig. 19).
13. *D. sulphurea* C. Koch. 2n = 50 = $A_4 + B_4 + C_2 + D_{40} = 1.8\mu - 0.89\mu$ (Fig. 20).

Genus—*Neoregelia* L. B. Smith

Only one species has been studied.

14. *N. spectabilis* L. B. Smith 2n = 46 = $A_4 + D_{42} = 1.5\mu - 0.51\mu$ (Fig. 21).

Genus—*Pitcairnia* L. Herit.

Only one species under this genus has been studied.

15. *P. punicea* Lindl. ex Hassk 2n = 50 = $A_4 + C_2 + D_{44} = 1.3\mu - 0.51\mu$ (Fig. 22).

In meiosis twenty five bivalents have been noted in metaphase I (Fig. 23). No irregularities were seen.

Ananas sativus 2n=50

A. sativus var. *variegatis* 2n=50

Ananas sp. 2n=50

Bilbergia euphemiae 2n=52

B. liboniana 2n=48

B. venezuelana 2n=54

B. vittata×*zebrina* 2n=50

Caraguata andreana 2n=98

Cryptanthus bivittatus 2n=36

C. bromelioides 2n=34

C. praetextus 2n=34

Dyckia argentea 2n=100

D. sulphurea 2n=50

Neoregelia spectabilis 2n=46

Pitcairnia punicea 2n=50

Discussion

Different tribes of Bromeliaceae have shown the existence of varying basic numbers in the constituent genera—the implications of which will be discussed later. But karyotype analysis of all the taxa of this family has revealed facts of fundamental interest. The idiograms, in general, show outstanding similarity among different species and genera. In the gross morphology of the chromosomes, as well as the chromatin content,—the uniformity is conspicuous and is indeed remarkable in a large family like Bromeliaceae where the number of genera is quite large, distributed under several tribes. Except in *Cryptanthus*, where a few chromosomes are slightly larger than the rest, all other taxa are characterised by medium to short chromosomes with slight gradation in size. Asymmetry in relation to chromosome size is mostly lacking in most of the genera. The primary constrictions, too vary from median to submedian in position which, taken in conjunction with the size, present a uniform appearance in the idiogram. The total amount of chromatin matter varies between $47\mu - 21\mu$ (vide histogram) and the increase and decrease are principally correlated with the corresponding increase or decrease in chromosome number. The number of secondary constrictions varies between four to eighteen and the morphology of the chromosomes bearing them differs widely.

All these facts, taken together, distinctly suggest the homogeneity of the family Bromeliaceae (*vide* Smith 1934).

Taking for granted the homogeneity of the family, the critical details of the karyotype showed differences confirming the status of different taxa from the cytological stand point. In *Caraguata andreana* specially, the exaggerated appearance of the satellite is remarkable. Moreover, the number and combination of secondary constrictions vary from species to species implying the importance of structural alteration in the evolution of species (Dobzhansky 1947, Sharma and Sharma 1959).

Karyotype analysis and taxonomic status of different tribes

The first tribe Navieae of Hutchinson (1959) is monotypic with one genus *Navia*. No cytological investigation has so far been carried out in this genus and as such it is not possible to ascertain the validity of its status as the first genus of this family.

Of the second tribe Pitcairnieae, five genera have so far been studied namely, *Puya*, *Dyckia*, *Lindmannia*, *Hechtia* and *Pitcairnia*—including the present investigations on *Dyckia* and *Pitcairnia*. In the species of *Dyckia*,

Figs. 2, 3, 7, 8, 13, 14, 15. Variation nuclei of *Ananas sativus* (2n=38, 34), *Bilbergia euphemiae* (2n=42, 56) and *Caraguata andreana* (2n=82, 86 and 94 chromosomes) respectively. Fig. 23. Pollen mother cell division of *Pitcairnia punicea* showing 25 bivalents. Histogram showing the total amount of chromatin matter is length of haploid complements of the different species of Bromeliaceae.

clear 2n=50 and 100 chromosomes have been reported in the present investigation, whereas in *Pitcairnia* 2n=50 have been observed. In such individuals, meiosis shows 25 bivalents. Multiples of 25 chromosomes are common in other species of these genera as well (Lindschau 1933, Matsuura and Suto 1935). Similarly, in *Puya* (Lindschau 1933, Diers 1961, Favarger and Huynh 1965), *Lindmania* (Lindschau 1933) and *Hectia* (Lindschau 1933) too, the number represents a multiple of 25 chromosomes. Only in one species of *Puya*, that is *P. chilensis* Md. n=24 (Tschischow 1956) has been observed which may be a later derivation. In *Lindmannia penduliflora*, the 2n chromosome number is approximately 120 (Lindschau 1933) which being an approximate number cannot be seriously taken into account. On the whole, the tribe Pitcairnieae has a uniform chromosome number which undoubtedly proves its homogeneity. In chromosome morphology, two species of *Dyckia* and one species of *Pitcairnia* show remarkable similarity, though the karyotypes differ in the number of satellited chromosomes. Therefore both from chromosome number and chromosome morphology—the tribe Pitcairnieae may be considered to represent a uniform grouping.

Of the tribe Tillandsieae, *Caraguata* and *Tillandsia* are the two genera which so far have been studied. They differ in their chromosome number (vide idiogram). All the species so far studied in *Tillandsia* indicate a basic set of 16 or 8 (2n=32, 64 and 96) whereas the species of *Caraguata* represent a series of n=14 (Lindschau 1933). In *C. zahnii*, Lindschau (1933) recorded 2n=56 chromosomes, whereas in *C. andreana*, 2n=98 chromosomes have been observed and counted in the present investigation. Evidently, the basic set may be 7 or 14. In absence of any data on the other genera in this family, it is difficult to ascertain the prevalent chromosome series in this group. However, the chromosome morphology of the species of *Caraguata* does not differ markedly from that of the other genera of this family. In fact a chromosome pair with extremely exaggerated secondary constriction region is common in both C-type chromosomes of *Bilbergia vittata*×*zebrina* and C-type chromosomes in *Caraguata andreana*. Future researches are necessary to find out as to which of the two basic sets (7, 14, or 8, 16) represents the ancestral set for these genera. Chromosome morphology however shows a striking similarity between the taxa, and one can be easily derived from the other.

In *C. andreana*, in addition to normal karyotype, a high frequency of nuclei in the somatic tissue has been noted to contain 82, 86 and 94 chromosomes. Such altered nuclei may help in the formation of new genotypes by vegetative propagation through their participation in the formation of daughter shoots (Sharma 1956).

A number of genera in the tribe Bromelieae has been subjected to cytological study. Species of *Ananas*, because of the edible value, are extensively cultivated and all of those cultivated species show a haploid set of

25 chromosomes. In *A. comosus*, Collins and Kerns (1935) observed diploid, triploid and tetraploid individuals. Evidently intraspecific polyploidy has been an important factor in their evolution though otherwise the species principally differ in respect to their karyotype, indicating importance of structural changes of chromosomes in the evolution of species. Regarding chromosome number, all species of *Ananas* represent a rather uniform series. In *Acanthostachys*, *Aechmea*, *Cannistrum* and *Pseudananas*, similar series of 25 chromosomes has been recorded by Lindschau (1933). On the other hand different numbers have been noted especially in *Cryptanthus*, *Bilbergia*, *Neoregelia*, *Bromelia* and *Nidularium* (Collins and Kerns 1931, Lindschau 1933, Matsuura and Suto 1935). In *Cryptanthus*, previous report shows a distinct series of $n=9$ and 18 chromosomes ($2n=36$ and 54). Intraspecific polyploidy was recorded by Lindschau (1933) in *C. bivittatus*. In *C. acaulis*, aneuploid number has been found ($2n=34$ and 36, Matsuura and Suto 1935). On the other hand, of the 3 species studied in the present investigation *C. bivittatus* shows $2n=36$ whereas $2n=34$ chromosomes have been found in *C. praetextus* and *C. bromeloides*. In view of the facts that $n=18$ is widespread in this genus and that in *C. acaulis*, 34 chromosomes have been found in certain individuals, $n=17$ appears to be a later derivation. The derived nature of *C. praetextus* ($2n=34$) is also borne out by 3 pairs of chromosome in its karyotype having constrictions at two ends of chromosome (type B in *C. praetextus*).

The genus *Bilbergia* was characterized by the presence of multiples of 9 or 18 chromosomes in its 3 species as previously noted (Lindschau 1933, Matsuura and Suto 1935). In the 4 species studied by the present author $2n=48$, 50, 52 and 54 chromosomes have been recorded showing aneuploidy in this genus. The karyotypes however, indicate remarkable homogeneity not only in the size of chromosomes but also in the nature of constrictions. The species differ only in relation to minute change specially in chromosomes with secondary constrictions. In this genus therefore polyploidy, aneuploidy and structural changes of chromosomes have been effective factors in evolution. In all probability $n=9$ and 18 represent the basic sets for this genus.

Summing up the situation from the karyotype data, meiotic study and chromosome number in Bromeliaceae, there seems little doubt that the entire family represents a homogeneous assemblage. This is specially borne out by their karyotypes which show remarkable uniformity in spite of minor differences accounting for the status of a taxon. The chromosome series though found to be varied yet show relation between one another, specially evidenced by the intraspecific variations. Certain chromosome series are however, conspicuous in particular tribes.

The four conspicuous series that have been found in different tribes are 8, 9, 25 and 7 restricted to a few species. It is not unlikely that 8 represents the original basic set from which other numbers might have been derived by

either loss or addition of a chromosome. The number 25 is, in all probability, the result of extensive hybridization between species with 16 and 9 chromosomes respectively. In Tillandsieae the present chromosome series is 8 or 16. In Bromelieae, it is probably 9 and 25 whereas in Pitcairnieae—it is 25. In view of the fact that the karyotype data shows a homogeneity and the chromosome numbers show preponderance of different numbers in different tribes, a rearrangement of the different tribes may be suggested. Tillandsieae with the probable basic set of 8 should represent a primitive level whereas Pitcairnieae with the deep-seated series of 25 is the present climax in evolution. The intermediate stages are possibly represented by the genera included in Bromelieae, showing variable numbers, some of which are common to Tillandsieae whereas others find parallel in Pitcairnieae. In absence of any data on Navieae, it is difficult to ascertain its systematic status. For the present it should be retained in its present position as given by Hutchinson.

The present investigation therefore puts forward an idea that in this family polyploidy, aneuploidy, extensive hybridization and structural alteration of chromosomes have been principally responsible for evolution. The cytological data presented above, taken in conjunction with the previous records, confirm the homogeneity of the family Bromeliaceae but suggest a rearrangement of the tribes on phylogenetic grounds.

Summary

Detailed karyotype studies have been carried out on 15 different species and varieties belonging to 7 genera of Bromeliaceae, viz. *Ananas*, *Bilbergia*, *Caraguata*, *Cryptanthus*, *Dyckia*, *Neoregelia* and *Pitcairnia*.

Although gross homogeneity in the karyotype among the members of the 7 genera is noticed, yet a critical analysis shows that each species and variety is characterised by the distinctive karyotype of its own, particularly in nature of secondary constriction—so structural alteration of chromosomes has been the prime factor in evolution within the family.

Out of the 4 tribes within this family, in Tillandsieae, the prevalent chromosome series is 8 or 16; in Bromelieae it is 9, 18 and 25 and in Pitcairnieae it is 25. It has been suggested that 8 represents the basic set for this family from which other numbers might have been derived. On the basis of past report and present investigations a rearrangement of different tribes is suggested—Tillandsieae with its basic set of 8 should represent primitive whereas Pitcairnieae with a series of 25 forms the present climax of evolution. The intermediate stages are possibly represented by Bromelieae some of which are common to Tillandsieae and others with Pitcairnieae.

Addenda

Since the paper went to press, an extensive and critical work on Bromeliaceae has been published by C. J. Marchant in Kew Bulletin, 21:

161-168 (1967-1968). It has been suggested that terrestrial Pitcairnioideae with uniform chromosome size represents a primitive level (Smith 1934) from which varying degrees of bimodality in some Bromelioid genera and the universally bimodal complement typifying the advanced and epithytic Tillandsioideae have been evolved. The primitive basic number has been suggested to be 25. This suggestion has been based as a large number of species studied and reinvestigated by him.

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