

# CYTOTAXONOMIC STUDIES IN THE EUPHORBIACEAE, SUBTRIBE PHYLLANTHINAE<sup>1</sup>

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## ABSTRACT

WEBSTER, GRADY L. (Purdue U., Lafayette, Ind.), and J. R. ELLIS. Cytotaxonomic studies in the Euphorbiaceae, subtribe Phyllanthinae. Amer. Jour. Bot. 49:(1): 14-18. Illus. 1962.—Chromosome numbers are reported for 18 species of mostly West Indian Euphorbiaceae, subtribe Phyllanthinae, 13 of these for the first time (including the first published count for the genus *Margaritaria*). For 4 species, a number different from previous determinations has been recorded. The base chromosome number in *Breynia*, *Fluggea*, *Margaritaria*, and most species of *Phyllanthus* appears to be 13. However, in *Phyllanthus* subg. *Isocladius* haploid numbers of 8 and 18 were observed. One species, *Phyllanthus pulcher*, is a sterile hexaploid ( $n = 39$ ) of presumably hybrid origin. The cytological data do not support Perry's suggestion that annual taxa are primitive in the Euphorbiaceae.

THE 7,000 species of the family Euphorbiaceae present such a vegetative and floral diversity that systematic treatment of the group has always been controversial. Not only have there been many schools of thought in delimiting taxa within the family, but its recognition as a natural group has been seriously questioned on the basis that it is polyphyletic in origin. Hutchinson (1959) suggests derivation of the family from at least 4 different orders (Bixales, Tiliales, Malvales, and Celastrales). Erdtman (1952), on the basis of palynological studies, has suggested possible relationships with the Buxaceae and Thymelaeaceae. Hurusawa (1954) proposes a division of the Euphorbiaceae into 4 families.

In contrast to such families as the Gramineae, Leguminosae, and Compositae, the Euphorbiaceae have been relatively little studied cytologically. Of the 7,000 species, fewer than 250 have been reported on, and the bulk of the counts are in the single genus *Euphorbia*. The subfamily Phyllanthoideae has been especially under-represented, as heretofore counts have been published on less than 2% of the 1500 species. In the subtribe Phyllanthinae, which includes about 1000 species (700 of these in *Phyllanthus*), counts have been published for only 8 species, and only 1 of these may be

regarded as unequivocally confirmed. The few workers who have studied taxa of the subtribe include Perry (1943), Raghavan (1957), Raghavan and Arora (1958), Janaki Ammal and Raghavan (1958), and Thombre (1959).

In the present contribution we wish to report the results of cytological observations on 18 species belonging to 4 genera of subtribe Phyllanthinae, most of which are either native to or cultivated in the West Indies. In view of the paucity of data available up until now, we believe that these counts are more significant than a similar number of miscellaneous reports in more extensively studied taxa. However, the tentative and exploratory nature of our conclusions must be recognized, since even with the addition of the records in this paper, chromosome numbers are known for only about 3% of the species of *Phyllanthus*.

**MATERIALS AND PROCEDURES**—Studies were made of plants growing in the field in the West Indies during the summer of 1959, and were later supplemented by observations on plants grown in the greenhouses at Purdue. Observations of mitotic chromosomes were made from root-tip squashes. Root-tips were pre-treated for 2-3 hr in a saturated solution of paradichlorobenzene, fixed overnight in acetic alcohol, hydrolyzed in 1 N HCl at 60 C for 18 min, stained in Feulgen for 2 hr and squashed in aceto-carmine. For meiotic observations, buds were collected in the field and fixed directly in acetic alcohol in which the acetic acid component had been saturated with ferric acetate; the anthers were then dissected from the male flowers and squashed in aceto-carmine. Voucher herbarium specimens of the plants studied have been deposited in the Kriebel Herbarium, Purdue University (PUL).

**RESULTS AND DISCUSSION**—In Table 1 are presented counts made by us for 18 species belonging to 4 genera of the subtribe Phyllanthinae, of which 17 represent either different determinations com-

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pared with previous reports or counts recorded for the first time. For convenience of comparison, published observations by other workers are included in the table, but are set in italic type. The circumscription of the Phyllanthinae and the arrangement of subgeneric taxa within *Phyllanthus* follows the treatment of Webster (1956–58), which differs in a number of respects from the classification of Pax and Hoffmann (1931).

It is interesting that in the 3 other genera studied (besides *Phyllanthus*)  $x = 13$ . Perry's record of  $2n = 56$  in *Breynia nivos*a is questionable, inasmuch as we have found  $2n = 52$  in meiotic preparations of *B. disticha* (the correct name for the same plant). Likewise, his report of  $2n = 16$  in *Fluggea obovata* is probably erroneous, for in our plant *F. virosa* the diploid number is clearly 26. Since *Fluggea obovata* and *F. virosa* are synonymous names for the same plant, this large discrepancy in reported chromosome number suggests that perhaps Perry's determination was for some completely different plant.

*Margaritaria* has not been reported on cytologically before, possibly because the commonest species *M. nobilis* is disguised in many books as a species of *Phyllanthus*. Cytologically, it agrees closely with *Fluggea* in having a diploid complement of 26 chromosomes (Fig. 1). *Margaritaria* and *Fluggea* are, therefore, diploid genera, so far as we know, whereas *Breynia* is tetraploid. This relationship is not unexpected, since the former genera have been considered primitive on the basis of floral and vegetative morphology, whereas *Breynia* is obviously more specialized.

The 16 species of *Phyllanthus* reported on here, together with 1 species (*P. emblica*) studied by Indian workers, represent 13 sections belonging to 8 subgenera. Although these species constitute less than 3% of those in the genus, they are sufficient for a preliminary survey of cytological relationships at the supraspecific level.

One of the most striking results of our studies is the demonstration that the base number in most sections of *Phyllanthus* is 13, as in the 3 genera

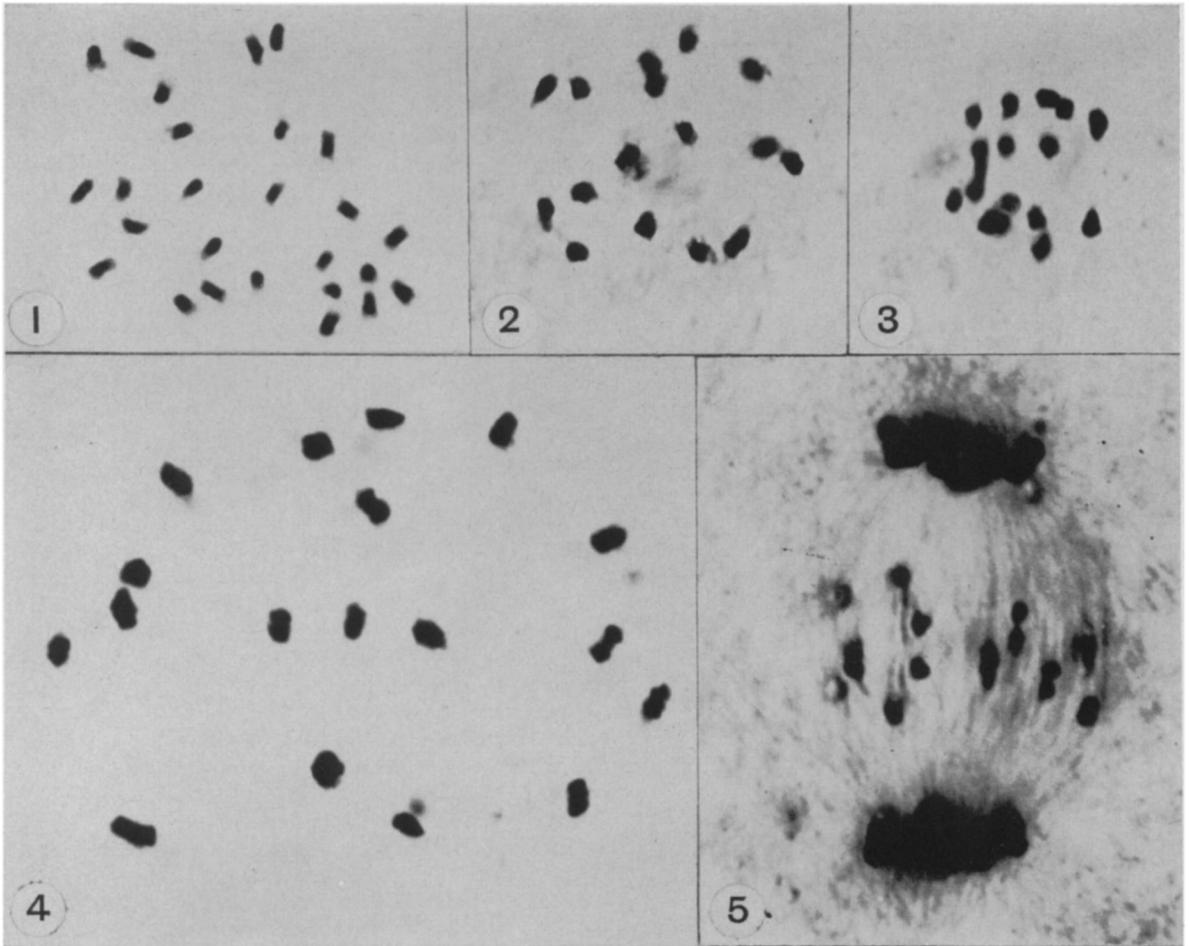


Fig. 1-5. Chromosomes of *Margaritaria* and *Phyllanthus*.—Fig. 1. *Margaritaria nobilis*, mitotic,  $2n = 26$ ,  $\times 2800$ .—Fig. 2-3. *Phyllanthus polygonoides*, diakinesis, anaphase I,  $2n = 16$ ,  $\times 1900$ .—Fig. 4. *Phyllanthus caroliniensis*, diakinesis,  $2n = 36$ ,  $\times 1900$ .—Fig. 5. *Phyllanthus pulcher*, anaphase I,  $\times 2300$ .

mentioned above, but that in 2 sections it is discordant. In *P. polygonoides* of sect. *Isocladus* (not West Indian but included here because of its interest)  $n = 8$  (Fig. 2, 3), while in *P. caroliniensis* of sect. *Loxopodium*,  $n = 18$  (Fig. 4). Perry's report of  $2n = 28$  in the latter species appears very dubious, even though he may have studied a different subspecies. It is noteworthy that sections *Isocladus* and *Loxopodium* both belong to subgenus

*Isocladus*, which consists of species with unspecialized branching. On the other hand, all taxa with  $n = 13$  have phyllanthoid branching (except for sect. *Botryanthus*). This would appear to suggest that the taxa in subgenus *Isocladus* may be phylogenetically isolated from the other groups within *Phyllanthus*. However, Arora (according to Janaki Ammal and Raghavan, 1958) has reported a chromosome count of  $2n = 52$  for *P. maderaspatensis*,

TABLE 1. *Chromosome numbers in the Phyllanthinae*

Taxon	Chromosome number		Reference or voucher collection
	gam.	spor.	
<i>Breynia disticha</i> Forst.	26		Jamaica; Webster et al. 8069
<i>Fluggea 'obovata'</i>		56	Perry, 1943 <sup>a</sup>
<i>Fluggea virosa</i> (Willd.) Baill.	13	16	Perry, 1943 <sup>b</sup>
<i>Margaritaria nobilis</i> L. f.		26	Jamaica; Proctor 8131
<i>Phyllanthus</i>			Guadeloupe; Webster et al. 9157
Subgenus ISOCLADUS			
Section ISOCLADUS			
<i>P. polygonoides</i> Nutt. ex Spr.		16	Texas; Johnston 3547
<i>P. maderaspatensis</i> L.		52	Arora (cited by Janaki Ammal and Raghavan, 1958)
Section LOXOPODIUM			
<i>P. caroliniensis</i> Walt.			
ssp. <i>caroliniensis</i>		28	Perry, 1943 <sup>c</sup>
ssp. <i>guianensis</i> (Kl.) Webster		36	Martinique; Webster et al. 9200
Subgenus & section KIRGANELIA			
<i>P. reticulatus</i> Poir.	13		Webster and Wilson 5237
Subgenus & section CICCIA			
<i>P. acidus</i> (L.) Skeels		26	Jamaica, cult.
	14		Thombre, 1959
Subgenus PHYLLANTHUS			
Section EMBLICA			
<i>P. emblica</i> L.		28	Perry, 1943
		98	Raghavan, 1957
		98-104	Janaki Ammal and Raghavan, 1958
Section URINARIA			
<i>P. urinaria</i> L.	26		Jamaica; Webster et al. 8177
		52	Raghavan and Arora, 1958
Section PHYLLANTHUS			
<i>P. amarus</i> Schum. & Thonn.	26		Jamaica; Webster et al. 8025A
<i>P. 'niruri'</i>		26	Raghavan, 1957
<i>P. pentaphyllus</i> Wright ex Griseb.		52	Florida; Webster et al. 9983
Subgenus & section CONAMI			
<i>P. acuminatus</i> Vahl	26		Puerto Rico; Webster et al. 8851
<i>P. subglomeratus</i> Poir.	26		Guadeloupe; Webster et al. 8985
Subgenus & section ERIOCOCCUS			
<i>P. pulcher</i> Wall. ex Muell. Arg.	c.39		Trinidad; Webster et al. 9969
Subgenus & section BOTRYANTHUS			
<i>P. nutans</i> Sw. ssp. <i>nutans</i>	26		Jamaica; Webster et al. 8019
Subgenus XYLOPHYLLA			
Section ASTERANDRA			
<i>P. juglandifolius</i> Willd.			
ssp. <i>juglandifolius</i>		c.156	Puerto Rico; Webster et al. 8594B
Section EPISTYLUM			
<i>P. axillaris</i> Sw.	26		Jamaica; Webster et al. 8472
Section HEMIPHYLLANTHUS			
<i>P. mimosoides</i> Sw.	26		Guadeloupe; Webster et al. 8987
<i>P. ovatus</i> Poir.	26		Martinique; Webster et al. 9172

<sup>a</sup> Determined by Perry as *Breynia nivosa*; this is a synonym of *B. disticha*.

<sup>b</sup> Determined by Perry as *Fluggea obovata*; if the determination is correct, the plant is the same as *F. virosa*.

<sup>c</sup> Perry's plant came from a nursery in Virginia, so presumably it is the subspecies native there.

a species which certainly belongs in sect. *Isocladus* along with *P. polygonoides*. We think that this report should be reinvestigated, as 1 pair of chromosomes in *P. polygonoides* has very large satellites (not apparent in Fig. 2, 3 due to higher degrees of constriction in meiotic material), which during prophase and early metaphase could easily be mistaken for small chromosomes. If in *P. maderaspatensis*  $x = 8$  also, it may well be a hexaploid in which  $2n = 48$ .

Whatever the diploid number of *P. maderaspatensis* may prove to be, it is clear that subgenus *Isocladus* does not fit into the cytological pattern established for the *Phyllanthinae*. However, until additional taxa in subgenus *Isocladus* can be studied, it would be unwise to draw any phylogenetic conclusions or to make any formal taxonomic adjustments. It is quite possible, for instance, that still other base numbers may be found within this rather diverse group.

Turning to the other subgenera of *Phyllanthus*, in which  $x = 13$ , we find a more understandable correlation between chromosome number and taxonomic status. Here most of the species studied are polyploid, and since the diploid species belong to the smaller sections, it is clear that by far the greater part of the species in the genus will prove to be polyploid when studied. Only in subgenera *Kirganelia* and *Cicca* did we find diploids, with  $2n = 26$ . Since these 2 subgenera are the only diploid taxa with phyllanthoid branching, it follows that they occupy a strategic position in the phylogenetic sense. In this connection, it is interesting that Janaki Ammal and Raghavan (1958) found very high chromosome numbers,  $2n = 98-104$ , in *P. emblica*, which has often been placed in the *Cicca* group because of its drupaceous fruit. If  $x = 13$  in *P. emblica*, as seems highly probable, then the species should be an octoploid with the primary number of  $2n = 104$ . Perry's (1943) account of  $2n = 28$  in this species is again open to

suspicion, since in the other 2 species (*Breynia disticha* and *Phyllanthus caroliniensis*) in which he reported  $x = 7$ , his counts could not be confirmed. At any rate, further cytological investigation of this variable and economically important species appears warranted.

Considering the great diversity of the indigenous West Indian species belonging to subgenera *Conami*, *Botryanthus*, and *Xylophylla*, it was interesting to find that all species investigated are tetraploids ( $2n = 52$ ), with 1 exception. In *P. juglandifolius* of sect. *Asterandra*, the somatic number proves to be ca. 156, so that the plant must be a dodecaploid; this is the highest chromosome number yet recorded in the subfamily *Phyllanthoideae*. Of course, since only 7 of the 44 West Indian members of the 3 subgenera have been investigated, it is quite possible that other species may prove to have chromosome numbers at intermediate ploidy levels. The high chromosome number of *P. juglandifolius* does not seem to be correlated with any great degree of morphological advancement, since the plant is actually rather unspecialized within subgenus *Xylophylla*. More significant is the fact that the other 6 species, belonging to 4 sections in 3 subgenera, have a common number of  $2n = 52$  despite their many superficial differences. This adds support to the conservative philosophy of retaining all these taxa within *Phyllanthus* rather than segregating *Conami* and *Asterandra* as distinct genera (as treated by Britton and Wilson, 1924).

The native herbaceous West Indian species of *Phyllanthus* with phyllanthoid branching, as well as their Old World relatives, have been placed in subgenus *Phyllanthus*. Our studies show that most of the species investigated are also tetraploids, with  $2n = 52$  (Fig. 8). However, Raghavan (1957) has reported  $2n = 26$  for *Phyllanthus 'niruri'* collected in India. Since true *P. niruri* is an American species not known to be immigrant in India, the identity of Raghavan's plant is doubtful; it may not even

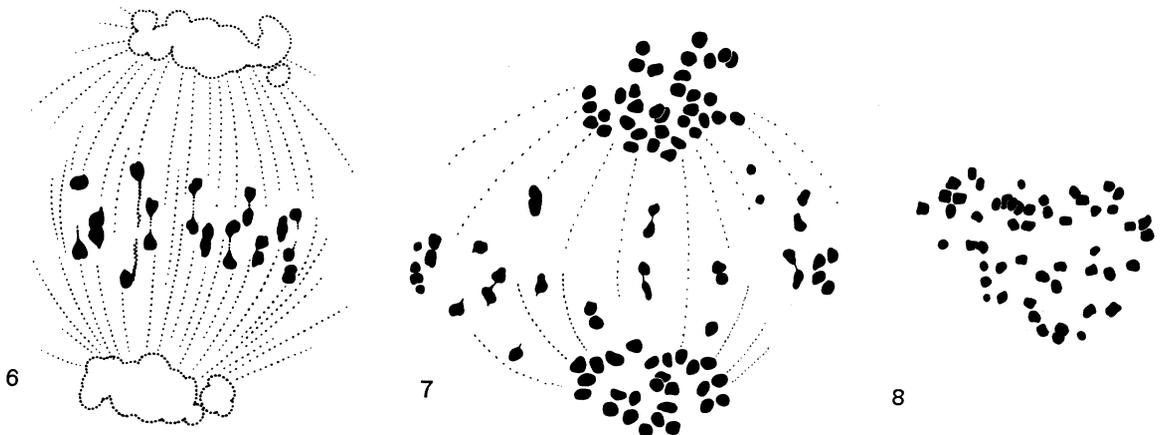


Fig. 6-8. Chromosomes of *Phyllanthus*.—Fig. 6. *Phyllanthus pulcher*, anaphase I, showing 12 dividing univalents,  $\times 2200$ .—Fig. 7. *Phyllanthus pulcher*, anaphase I, showing 16 dividing univalents and  $2n = 78$ ,  $\times 2200$ .—Fig. 8. *Phyllanthus pentaphyllus*, mitotic,  $2n = 52$ ,  $\times 2000$ .

belong to subgenus *Phyllanthus*. For the time being, therefore, there is no unequivocal record of an herbaceous diploid species except for the ones with unspecialized branching in subgenus *Isocladus*.

A quite exceptional case is provided by *P. pulcher* (subgenus *Eriococcus*), a hexaploid plant with  $2n = 78$ . The species as observed by us in Trinidad flowers freely but never produces seed. Cytological observations (Fig. 5-7) showed very irregular meiotic divisions, due to a variable number (10-16) of chromosomes which failed to pair. It appears, therefore, to be a sterile hybrid between 2 distinct species of sect. *Eriococcus*, but its parentage is still unknown, nor is it known whether other species of the section are also hexaploid. We commend this interesting group to the attention of cytotaxonomists in eastern Asia.

CONCLUSIONS—It is now clear that in most taxa of the subtribe Phyllanthinae the original base number is 13, and that the taxa which would be regarded as most primitive on the basis of gross morphology are diploids. However, the majority of both herbaceous and woody species of *Phyllanthus* are at the tetraploid level or higher. Janaki Ammal and Raghavan (1958) suggest that the herbaceous species of *Phyllanthus*, with  $x = 13$ , have a different base number from the woody ones, with  $x = 7$ . Presumably their suggestion is influenced by Perry's (1943) report of  $2n = 28$  in *P. emblica* (and perhaps also by his record of  $2n = 56$  in *Breynia disticha*) which would suggest  $x = 7$ . Thombre's (1959) report of  $n = 14$  in *Phyllanthus acidus* may reflect the same influence. However, we believe that Perry's observations are incorrect and that  $x = 13$  in the woody as well as the herbaceous species.

The demonstration of  $x = 13$  in most of the Phyllanthinae is especially interesting in view of the fact that Mangenot and Mangenot (1958) have reported  $n = 13$  in *Antidesma*, *Bridelia*, *Hymenocardia*, *Protomegabaria*, *Spondianthus*, and *Uapaca*. Since these genera represent subtribes Antidesminae and Uapacinae of the Phyllanthaceae, as well as tribe Brideliaceae, it is apparent that 13 is a very widespread base number in the Phyllanthoideae. However, it is not necessarily the original number for the subfamily, for Reese (1957) has reported  $n = 12$  in *Andrachne*, which is more primitive than most of the genera mentioned above, and Mangenot and Mangenot (1957) have found  $n = 20$  in *Drypetes*, which is a primitive taxon in many respects. Sampling of genera in the still unstudied subtribes will be necessary before a clear picture of karyotype evolution in the Phyllanthoideae can be constructed.

Finally, it may be noted that the data here collected do not support Perry's thesis that perennial taxa have evolved from annual ones in the Euphorbiaceae. The most primitive genera in the Phyllanthinae, *Fluggea* and *Margaritaria*, are either trees or shrubs and are diploid. With the exception of subgenus *Isocladus*, the more primitive groups within *Phyllanthus* are woody and diploid, while the herbaceous species so far investigated are tetraploid. From other lines of evidence it is clear that these herbaceous species are not ancestral to the tetraploid woody species. In the evolution of the Phyllanthinae there has been a tendency toward higher chromosome numbers and a trend from the woody to the herbaceous condition, but these trends are not strictly correlated in all groups.

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