

## CYTOLOGICAL STUDIES OF SOME SPECIES OF THE GENUS *Clusia* L. (GUTTIFERAE)

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### ABSTRACT

Chromosome number and meiotic behaviour are reported for the first time for five Brazilian species of *Clusia* L. (Guttiferae) which belong to three different sections of the genus: *Cordylandra*, *Eucruiva* and *Phloianthera*. All five species have  $n = 30$  chromosomes and thus do not display any polyploidy or secondary base numbers which are such striking features among species of other genera of this family.

These *Clusia* species have mainly normal meiosis with metaphase I showing predominantly bivalent formation and with more than 80% viable pollen. Some cases of univalents, secondary associations and cytomixis were observed, but no occurrence of multivalent formation such as trivalents or quadrivalents, was recorded for any pollen mother cell. These cytological data for *Clusia* are discussed within the context of available chromosome information for the family.

### INTRODUCTION

The genus *Clusia* L. comprises approximately 250 species distributed throughout tropical forests in regions of some elevation, with few representatives outside of America (Hutchinson, 1969). According to Mariz (1974) and Mariz and Weinberg (1982, 1984), 45 *Clusia* L. species are recognized in Brazil. The genus is the largest in the family in America (Maguire, 1976), with a number of species equivalent to that of the genus *Garcinia* L., which occurs in the tropical regions of the Old World. This group of plants (family Guttiferae), which is included in Guttiferales by some and in Theales by others, is quite heterogeneous and artificial in taxonomic position, as demonstrated by the discrepant assignment of genera to different taxons by inves-

tigators such as Engler (1888); Takhtajan (1969). Cronquist (1968, 1981) and Hutchinson (1973). Even considering Clusiaceae (*sensu* Hutchinson) to be independent of Hypericaceae, they continue to be heterogeneous, and the family should be divided into smaller groups containing more natural units (Maguire, 1976).

Few studies are available on the chromosome number of Guttiferae genera (*sensu* Engler). The genera *Hypericum* L. and *Garcinia* L. are, pre extensively studied terms of their in cytology. According to Robson and Adans (1968), a still debatable and doubtful series of fundamental numbers may occur in the family Guttiferae, and even for the genus *Hypericum*, in which the number may be 12, 10, 9, 8 and 7, with 12, 10 and 9 possibly being the fundamental numbers of a group of *Hypericum* sections including shrub-like species, and 8 and 7 being found in sections of more specialized herbaceous plants. These investigators proposed that evolution may have occurred through a reduction in fundamental chromosome number from 12 to 7. In the few known genera, wide variation in chromosome number is observed among species, showing the occurrence of both polyploidy and aneuploidy in the evolutionary process. The extreme values in the family are reached in *Cratoxylum formosum* (Jack) Benth.,  $2n = 14$ , and in *Garcinia mangostana* L.,  $2n = 96$  (Bolkhovskikh et al., 1969).

There are practically no reports on the chromosome numbers of members of the Clusiaceae tribe of Engler, which consists of the following genera: *Clusia* L., *Rengifia* Poepp. et Endl., *Oedematopus* Planch. et Triana, *Havetiopsis* Planch. et Triana, *Renggeria* Meisn., *Tovomita* Aubl., and *Tovomitopsis* Planch. et Triana, all of them represented in Brazil. The genus *Alanblackia* Oliv. has been added to this tribe and the chromosome number of the species *A. floribunda* from West Africa was found to be  $n = 28$  (Robson and Adans, 1968).

Another interesting feature reported by some investigators is the reproductive system of this family. Different types of apomixis occur in some genera, such as adventitious embryony in *Clusia rosea* and *C. minor* (Maguire, 1976), parthenogenetic apospory in *Hypericum perforatum* (Grant, 1971), and adventitious embryony in *Garcinia mangostana* (Grant, 1971), phenomena commonly observed in plants of high ploidy.

Thus, the objective of the present investigation was to determine for the first time the chromosome number of *Clusia* and of representatives of the tribe Clusiaceae (*sensu* Engler) in Brazil, as a contribution to the clarification of the phylogenetic relationships of a group of controversial taxonomy.

## MATERIAL AND METHODS

The five species of *Clusia* investigated in the present paper are grown in the Floriculture and Ornamental Plant Section and a dried specimen of each species was added to the Herbarium of the Instituto Agronômico (IAC) of Campinas. The species

have been grouped into sections according to the method of Mariz (1974) (Table I), which uses an adaptation of the Engler key, with modifications proposed by Maguire.

Table I - Species of the genus *Clusia* L. that have been studied cytologically.

Species	Origin (Engler, 1888; Mariz, 1974)	Site of collection	Herbarium number
Section <i>Cordylandra</i> Pl. et Tr.			
<i>C. fluminensis</i> Pl. et Tr.	Coastal woods in the State of Rio de Janeiro	Wooded hills in Ubatuba, SP	IAC 25018
<i>C. organensis</i> Pl. et Tr.	States of Minas Gerais, Rio de Janeiro and Bahia	Serra Pedra Azul, Minas Gerais	IAC 25020
Section <i>Eucriuva</i> Engl.			
<i>C. criuva</i> Camb.	Woods in the States of Rio de Janeiro, Minas Gerais, São Paulo and Federal District	Unknown	IAC 25021
Section <i>Phloianthera</i> Pl. et Tr.			
<i>G. lanceolata</i> Camb.	States of Rio de Janeiro and São Paulo	Wooded hills in Ubatuba, SP	IAC 25019
<i>C. hilariana</i> Schlecht.	States of Espírito Santo, Bahia and Pernambuco	Unknown	IAC 25024

Flower buds of male plants were collected and fixed in 3:1 Carnoy (a mixture of absolute ethyl alcohol and glacial acetic acid). The fixative was renewed three times within a period of 48 hours. The material was then cooled and stored frozen at  $-20^{\circ}\text{C}$  in the same fixative.

At the time of slide preparation, the anthers were squashed in 1.2% acetic carmine and semipermanent slides were mounted (Medina and Conagin, 1964) that can be stored in the refrigerator for approximately 15 days. Pollen mother cells that appeared to be most adequate for study were observed at different stages of meiosis, interpreted, drawn and microphotographed. Approximately 50 slides were studied from each collection.

Pollen grain viability was investigated in 5 flower buds prepared with 1.2% acetic carmine, each preparation representing several anthers of one flower in which 100 grains were counted in random fields. We always utilized mature pollen collected at anthesis from protected flowers. Analysis included the viability of microspore tetrads.

## RESULTS

The diploid number was 60 for the five *Clusia* species investigated, even in *C. criuva* in which the number varied from 56 to 62 in different pollen mother cells, but was more frequently  $2n = 60$  ( $30_{II}$ ) (Table II). The chromosomes of the various species appeared to be similar in size at corresponding phases of meiosis, but showed individual differences within the same genome.

Table II - Chromosome behavior and tetrad viability of microspores and pollen grains in *Clusia* L. species.

Species	Chromosome number	Cytomixis	Types of chromosome pairing Diakinesis and metaphase I	Formation of secondary associations	Separation at anaphase I	Viability	
						Tetrad %	Pollen %
<i>C. criuva</i>	60	Yes	$30_{II}$	Yes	30 - 30	71	80
	61		$30_{II}1_1$		30 - 31		
	62		$30_{II}2_1$		29 - 31		
	60		$29_{II}2_1$				
	60		$28_{II}4_1$				
	59		$28_{II}3_1$				
	58		$28_{II}2_1$				
	56		$27_{II}2_1$				
<i>C. fluminensis</i>	60	No	$30_{II}$	Yes	30 - 30	89	93
	60		$25_{II}10_1$				
	60		$23_{II}14_1$				
<i>C. hilariana</i>	60	Yes	$30_{II}$	No	30 - 30	96	97
	60		$29_{II}1_1$		30 - 29-1		
	60		$28_{II}4_1$		29 - 29-2 27 - 28-5		
<i>C. lanceolata</i>	60	No	$30_{II}$	No	30 - 30	98	91
	60		$28_{II}4_1$				
<i>C. organensis</i>	60	No	$30_{II}$	Yes	30 - 30 29 - 31	92	98

Some irregularities were observed at meiosis, such as lack of pairing or early separation between homologues and unequal chromosome separation within some cells. Fewer irregularities were detected in *C. organensis*. Monovalents, secondary associations and frequent cytomixis were also observed (Table II). However, we observed no B chromosomes, formation of chromatin bridges at anaphase or formation of polyvalents, possible signs of structural rearrangements. In general, a single chromosome pair was associated with the nucleolus.

Both in the species in which cytomixis was observed and those in which the phenomenon did not occur, the frequency of normal microspore tetrads was high (above 89%), except for *C. criuva* (71%). This high frequency of normal tetrads was similarly confirmed in pollen, which showed viability ranging from 80% in *C. criuva* to 98% in *C. organensis*. Tetrads and pollen grains were identically stained and showed no variation in size or shape among species.

a) *C. criuva* presented  $n = 30$  chromosomes in most cells analyzed. We observed cells with different chromosome numbers at diakinesis ( $30_{II} + 2_I$ ,  $28_{II} + 2_I$  and  $27_{II} + 2_I$ ), as well as at metaphase I ( $30_{II} + 2_I$ ,  $30_{II} + 1_I$  and  $28_{II} + 3_I$ ). Lag-gards were observed at anaphase I and cytomixis was observed at anaphase I and II, as well as bivalents in secondary association. The formation of normal tetrads was 71% and pollen viability 80% (Table II).

b) *C. fluminensis* presented  $n = 30$  chromosomes.  $30_{II}$  were always observed at diakinesis, but sporadic cases of cells with a large number of monovalents at metaphase I (Figure 1A) and secondary association were observed. However, the separations were regular at anaphase I and II, with the formation of 89% normal tetrads (Figure 1B) and of 93% viable pollen (Table II).

c) *C. hilariana* presented  $n = 30$  chromosomes, but some irregularities were observed. Some chromosomes became laggards at anaphase I and II (Figure 1G) and cytomixis was observed at all phases of meiosis (Figure 1H, I). However, formation of normal tetrads was 96% and pollen viability was 97% (Table II).

d) *C. lanceolata* presented  $n = 30$  chromosomes, forming  $30_{II}$  at diakinesis and metaphase I. In some metaphase I cells there were monovalents (Figure 1F), but separation was regular at anaphase I and II. Formation of normal tetrads was 98% and pollen viability 91% (Table II).

e) *C. organensis* presented  $n = 30$  chromosomes and normal meiotic behavior, forming  $30_{II}$  at diakinesis (Figure 1C) and metaphase I. Secondary associations were observed between some bivalents at metaphase I (Figure 1D), with regular separations at anaphase I and II. Formation of normal tetrads was 92% and pollen viability was 98% (Figure 1E and Table II).

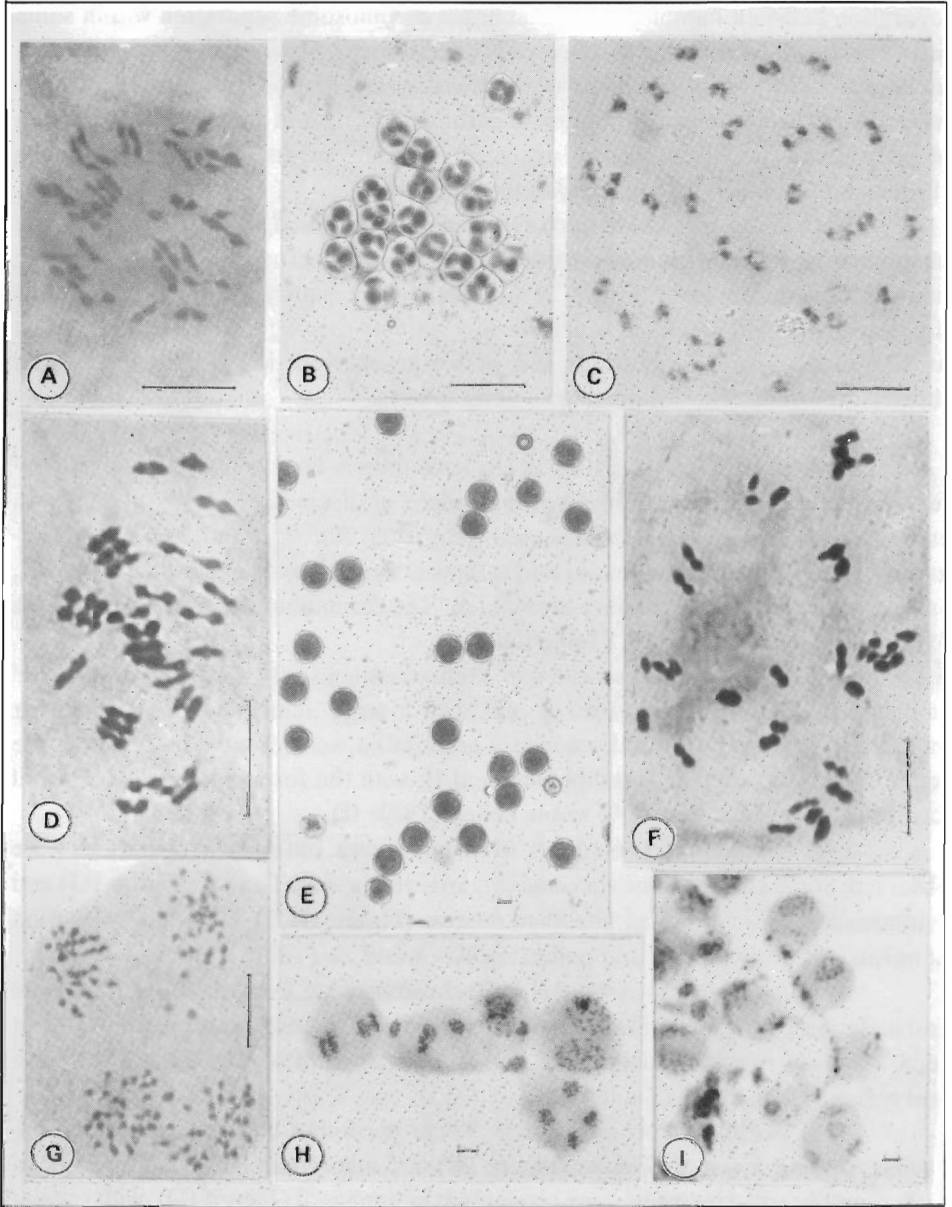


Figure 1 - Photomicrographs obtained with a light microscope showing aspects of the microsporogenesis of *Clusia* L.. A and B, *C. fluminensis*, metaphase I with  $25_{II} + 10_{I}$  and normal microspore tetrads. C, D and E, *C. organensis*, diakinesis with  $30_{II}$ , metaphase I with  $30_{II}$  showing secondary association and normal pollen grains. F, *C. lanceolata*,  $M_{1}$  with  $28_{II} + 4_{I}$ . G, H and J, *C. hilariana*,  $A_{II}$  with laggards and fields of pollen mother cells with cytomixis. The bar in each figure is  $10 \mu\text{m}$ .

## DISCUSSION

*Chromosome number in Guttiferae*

The groups comprising the Guttiferae of Engler (1888) or even those comprising the Theales of Cronquist (1981) are heterogeneous, with considerably discrepant morphological traits present in groups more or less related. There are no biotaxonomic studies that might permit a better clarification of the real position of the different taxons and of the more natural relations among families, genera and species. Indeed, data on geographic distribution and morphology studies have not proved to be sufficient thus far for the delimitation of taxons. Few genera of the family Guttiferae have been studied in terms of chromosome number and behavior, or even in terms of any other aspect of reproduction. There are no reports on the chromosome numbers of members of the subfamily Clusiaceae (Engler, 1888; Barroso *et al.*, 1978) whose genera are almost all represented in Brazil, even for those genera with a larger number of species, as is the case for *Clusia* L. (Bolkhovskikh *et al.*, 1969).

Because of this lack of information on chromosome number and morphology in the Clusiaceae, it is difficult to even make a proposal about the evolutive trends in the group by associating such data with those of external plant specialization and geographic distribution. With respect to the members of the family Guttiferae for which chromosome counts are available there is very wide variation in chromosome number among genera and even among species such as the well-studied *Hypericum* and *Garcinia*, for which different haploid numbers have been detected for each species (Nielsen, 1924; Hoar and Haertl, 1932; Robson and Adans, 1968; Bolkhovskikh *et al.*, 1969). In *Hypericum*,  $x$  ranges from 7 to 12 and 16 has been suggested for *Garcinia*, in which chromosome numbers of 8 and 9 have also been suggested to exist (Robson and Adans, 1968). Despite the limited data available, however, the existence of aneuploid and euploid variations in chromosome number as a factor in speciation is very clear.

*Chromosome of Clusia L.*

In *Clusia*,  $n = 30$  seems to be constant for the genus, even though very few species have been analyzed. In *C. criuva*, some pollen mother cells presented a chromosome number differing from 60. These cells may derive from a persistence of numerical variation from the somatic tissue to the sporogenous tissue, or may have originated from errors in cell division in premeiotic sectors.

In polyploids this aspect may be of importance in the formation of natural aneuploids in which the gametes formed have a better chance of being viable and capable of fertilization. These numerical variations may become stable in the population through polyploidy or through gene balance if they show adaptive superiority, or

may become lost through selection. The secondary associations observed in some of the species studied here and the high chromosome number ( $n = 30$ ) confirm the polyploid origin of the genus. However, on the basis of the constant chromosome number observed in the five *Clusia* species studied here which belong to three different sections, speciation may differ from the type observed in *Hypericum* and *Garcinia*, whose species show extremely variable numbers.

Some investigators believe that the cytomixis phenomenon may be due to technical artifacts, as proposed by Takats (1959) who defended the idea that the canals linking the pollen mother cells may be an effect of the fixative. The evidence of the presence of cytomixis observed in only two of the five species studied, *C. criuva* and *C. hilariana*, suggests that the phenomenon is spontaneous and originates from an unbalanced genetic system in the plant. This idea is based on the fact that the five species coexist in the same cultivation environment and that the cytological preparations were similarly processed, with the same time of collection, the same fixative and the same method of squashing of pollen mother cells in carmine solution between a slide and coverslip. This genetic unbalance is more or less confirmed because cytomixis is more commonly encountered among polyploid plants, hybrids and apomictic plants and in plants subjected to fluctuations in temperature (George and Geethamma, 1983; Basaivaiah and Murthy, 1987), thus also occurring in many diploid species (Omara, 1976). George and Geethamma (1983) detected a direct relationship between cytomixis frequency and pollen sterility in *Jasminum* species. These authors proposed that the phenomenon may be the consequence of the action of a peculiar gene or a defect in gene function.

The occurrence of cytomixis seems to be a phenomenon varying in behavior and intensity from species to species, but little is known as yet about this phenomenon, and especially about the role of cytomixis in evolution. A controversial suggestion is that cytomixis may give origin to polyploids and B chromosomes (Sarvella, 1958; Sallés, 1970; Omara, 1976).

It should be pointed out that the *Clusia* species studied here were well defined both in terms of morphology of the normal meiotic behavior and high viability of pollen grains. A few monovalents were observed that could be interpreted to be smaller bivalents which at times form a smaller number of chiasmata owing to the fact that the frequency of these monovalents is low. However, the chromosome number ( $n = 30$ ) suggested an origin due to polyploidy in the genus. In *C. criuva*, in which a chromosome number differing from 60 was detected in some pollen mother cells, one may assume that cytomixis occurs already in premeiotic cells, even though the phenomenon was observed during the meiotic process. However, the uniform size and high viability of the pollen grains and the constancy of the chromosome number in the genus appear to indicate that selection occurs at different stages of gametogenesis, whereby only gametes with 30 chromosomes would be functional at the time of flower anthesis.

If we consider the heterogeneous Guttiferae group with respect to morphological and cytological aspects, and the smaller Clusiace group, for which there is no cytological information, it is not possible to determine whether *Clusia* may be part of a polyploid complex or of a hybrid complex (Grant, 1971). The number  $x = 30$ , which is constant for the genus, indicates that this is a probable polyploid whose original  $x$  is not predictable within the series mentioned by Robson and Adans (1968) and Bolkhovskikh *et al.* (1969).

If the origin was by hybridization, it is natural to think about a process of allopolyploidy, since only bivalents were observed. Grant (1971) believes that polyploidization occurs after hybridization between related species whose genomes may be partially different. However, the origin may have been by autopolyploidization as confirmed by the secondary associations observed, by irregularities in mitotic division or by chromosome disjunction in gamete formation. In the latter case, the lack of polyvalent formation may be explained by genome differentiation in an ancestral autopolyploid or by the action of a gene or genes regulating the lack of polyvalent formation (Grant, 1971).

However, the cytological data for the family, with an extremely variable  $n$  and obligate crossed reproduction (dioecism), plus the existence of apomixis mentioned in the literature (Maguire, 1976) and possible vegetative propagation do suggest an originally hybrid complex that later became polyploid. Woody plants with an  $x$  of more than 12 may normally be considered to be of polyploid origin. The existence of plants in the family Guttiferae that are highly diversified in temperate and tropical regions and mostly polyploid shows that polyploidy must have played an important role in the evolution of the family. Evolutionists believe that the polyploid series may have originated from plants with lower numbers and that these ancestors may already be extinct (Stebbins, 1971).

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### RESUMO

A contagem do número de cromossomos e o comportamento meiótico são relatados, pela primeira vez, para cinco espécies de *Clusia* L. do Brasil pertencentes à três seções diferentes: *Cordylandra*, *Eucruiva* e *Phloianthera*. O primeiro registro para as cinco espécies do gênero *Clusia* mostrou  $n = 30$  cromossomos em todas elas e não revelou variações de ploidia. O número básico de cromossomos nas Guttiferae é variável e os gêneros têm mostrado números diferentes para cada espécie, com números básicos secundários e ciclos de poliploidia. Assim sendo, é impraticável sugerir as tendências evolutivas dentro de Clusiace porque não há dados citológicos para qualquer espécie dos outros gêneros do grupo.

As espécies estudadas exibem meiose um pouco irregular, mostram a formação predominante de bivalentes em metafase I e mais de 80,0% dos grãos de pólen são viáveis. Porém, alguns casos de monovalentes, associações secundárias e citomixia foram observados. Contudo, multivalentes como trivalentes ou tetravalentes não foram vistos em qualquer célula mãe de pólen. Um possível mecanismo de origem do gênero é discutido.

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