Acta Botánica Mexicana (1992), 17:45-51

NEW CHROMOSOME COUNTS IN LYTHRACEAE - SYSTEMATIC AND EVOLUTIONARY IMPLICATIONS

SHIRLEY A. GRAHAM

Department of Biological Sciences, Kent State University,
Kent, Ohio, 44242, U.S.A.

ABSTRACT

Chromosome numbers of twenty four taxa in seven genera of the Lythraceae are reported: 16 species and 1 variety of *Cuphea*; 2 species of *Rotala*; and 1 species each of *Diplusodon, Ginoria, Lafoensia, Pleurophora*, and *Woodfordia*. Plant materials originated in Mexico, Guatemala, Ecuador, Brazil, Dominican Republic, Ethiopia, and China. Nineteen counts are first reports; the others represent populations from geographic areas distant from populations previously counted. Systematic and evolutionary implications of the counts are discussed.

RESUMEN

Se dan a conocer los números cromosómicos de 24 taxa de siete géneros de la familia Lythraceae: 16 especies y una variedad de *Cuphea*; dos especies de *Rotala* y una de cada uno de los siguientes géneros: *Diplusodon, Ginoria, Lafoensia, Pleurophora* y *Woodfordia*. El material vegetal proviene de México, Guatemala, Ecuador, Brasil, República Dominicana, Etiopía y China. Diecinueve de los recuentos son los primeros efectuados para el taxon correspondiente. Los demás representan poblaciones de áreas geográficas distantes de las estudiadas previamente. Se discuten las implicaciones sistemáticas y evolutivas de los recuentos.

The Lythraceae are a family of world-wide distribution, growing primarily in subtropical and tropical climates and comprising ca. 31 genera and 600 species. An overall understanding of the family and the phylogenetic relationships among the genera are currently being addressed through revisionary studies (e.g. S. Graham, 1988, 1989a; Lourteig, 1986a, 1986b, 1989), palynological (A. Graham et al., 1985, 1987, 1990), anatomical (Baas & Zweypfenning, 1979; Baas, 1986), and cytological and genetic studies (Knapp & Tagliani, 1989; Ray et al., 1989). Chromosome numbers counted in the family through 1985 have been summarized (Tobe et al., 1986). Chromosome numbers reported for *Cuphea*, the largest lythraceous genus with ca. 300 species, recently also have been summarized (S. Graham, 1989b). The base number of the family is $\underline{x} = 8$ which has been established for 13 genera. A base number of $\underline{x} = 5$ has been reported for two genera (Tobe et al., 1986). This paper presents chromosome numbers for 24 taxa in seven genera of the Lythraceae including: 16 species and 1 variety of *Cuphea*; 2 species of *Rotala*; and 1 species each of *Diplusodon, Ginoria, Lafoensia, Pleurophora*, and *Woodfordia*. Plant materials originated in Mexico, Guatemala, Ecuador, Brazil, Dominican Republic, Ethiopia, and China. Nineteen counts are first reports for the taxa, whereas the remainder represent

additional reports for populations geographically distant from populations previously counted.

MATERIALS AND METHODS

Flower buds were collected from wild plants in the field or from plants grown in the greenhouse from seeds obtained from wild plants. Field-collected buds were fixed in 3 parts 95% ethyl alcohol: 1 part glacial acetic acid. Greenhouse-collected buds were fixed in 4 parts chloroform: 3 parts 95% ethyl alcohol: 1 part glacial acetic acid for a minimum of 24 hours. Root tips were pre-treated in a cold, saturated solution of paradichlorobenzene for 2-3 hours, then fixed in 3:1 alcohol:glacial acetic acid. Buds and root tips were hydrolyzed in 1 N HCl for 15 min. at 60° C. Single anther preparations were squashed in acetocarmine then briefly warmed on glass slides to clear the cytoplasm for observation. Root tips were stained in Schiff's reagent (Lillie, 1951) and squashed in acetocarmine.

RESULTS AND DISCUSSION

Results are summarized in Table 1 and relevance of the numbers reported is discussed below by genus and in the case of *Cuphea*, by infrageneric classification.

Cuphea - The greatest number of new reports has been made in this speciose genus where 17 taxa have been counted. The species reported are classified in seven of the 14 sections of the genus.

In sect. *Cuphea, C. utriculosa* with $\underline{n} = 11$, has exactly half the number of the similar and presumably most closely related species *C. salicifolia* with $\underline{n} = 22$ (S. Graham, 1989b). The species also share the same unusual habitat, growing attached to rocks in fast-flowing shallow streams. *Cuphea utriculosa* ranges from Jalisco to Panama while the tetraploid *C. salicifolia* is more restricted, occurring from San Luis Potosí to Chiapas. Other members of the section have gametic numbers of 8, 10, 16, 20, 22, and 24.

In sect. *Brachyandra* Koehne, the chromosome number of *C. urens*, an endemic species of the Dominican Republic with $\underline{n} = 8$, conforms to the pattern of the section. Of the eight species counted, six have $\underline{n} = 8$ and two are tetraploids with $\underline{n} = 16$. The section appears to be a natural one defined by the floral synapomorphy of deeply inserted stamens and the base chromosome number of 8. *Cuphea carthagenensis*, $\underline{n} = 8$, is also a member of this section. It is the most widely distributed species of the genus and extremely variable vegetatively but uniform in floral morphology. The chromosome numbers of populations counted from North Carolina, USA; Veracruz and Chiapas, México; and Paraná, Brazil are all $\underline{n} = 8$.

Section *Euandra* Koehne is represented by *C. pertenuis*. This diminutive species was collected a number of times by George Hinton in the 1930's but rarely has been collected since. It is highly unusual in the genus because of its fragile habit, flowers just 5 mm long with practically no nectariferous disc, and exceptionally long-exserted stamens. Foster (1945) was unable to confidently place it near any other species of the genus. It remains an enigma, but is here classified in sect. *Euandra* because the small flowers with exserted stamens lead it to be keyed there. Section *Euandra* is primarily South American in

Table 1. New chromosome number reports in the Lythraceae. All materials are vouchered in KE-Graham herbarium with additional duplicate collections of Graham at MICH and duplicate collections of others as indicated.

Taxon	Count	Voucher
Cuphea		
C. axilliflora (Koehne) Koehne	n= ca. 84-86	Guatemala. Alta Verapaz:*Graham 1019*
C. carthagenensis (Jacq.) Macbr.	n= 8	Mexico. Chiapas: *Graham 1084.*
C. cyanea DC.	n= 15	Mexico. Veracruz: *Graham 1008;* Guerrero: *Graham 1060*; Oaxaca: *Graham 1070*
C. megalophylla S. F. Blake	n= 16	Mexico. Oaxaca: *Graham 1074*
C. micropetala var. *micropetala* Kunth	n= 16	Mexico. Mexico: *Graham 1051*
C. *micropetala* var. *hirtella* Koehne	n= 16	Mexico. Michoacan: *Graham 1048*
C. nudicostata Hemsl.	n= 30	Mexico. Oaxaca: *Graham 1068*
C. painteri Rose in Koehne	n= 12	Mexico. Jalisco: *Graham 1034*
C. pertenuis R. C. Foster	n= 12	Mexico. Guerrero: *Graham 1057*
C. pinetorum Hemsl.	2n= 22	Mexico. Chiapas: *Graham 1085*
C. pulcherrima R. C. Foster	n= 8	Mexico. Mexico: *Graham 1052*
C. rasilis S. Graham	n= 16	Mexico. Nayarit: *Graham 1027*
C. salvadorensis (Standley) Standley	n= 16	Mexico. Chiapas: *Graham 1076*
C. schumannii Koehne	n= 16	Mexico. Oaxaca: *Graham 1090*
C. urens Koehne	n= 8	Dominican Republic: *Zanoni 41811, 41517*
C. utriculosa Koehne	n= 11	Mexico. Chiapas: *Graham 1086*
C. watsoniana Koehne	n= 16	Mexico. Jalisco: *Graham 1040*
Diplusodon hexander DC.	n= 15	Brazil. Minas Gerais: *Graham 979*
Ginoria nudiflora (Hemsl.) Koehne	2n= ca. 56	Mexico. Veracruz: *Gutierrez B. 3099*
Lafoensia acuminata (Ruiz & Pavon) DC.	2 n=16	Ecuador. *Neill 8930* (MO)
Pleurophora anomala (St. Hil.) Koehne	n= 7	Brazil. Bahia: *Graham 941* (NY)
Rotala		
R. indica (Willd.) Koehne	2 n= 32	China. *Guizhou Bot. Exped. 2354* (GH)
R. ramosior (L.) Koehne	n= 8	Mexico. Chiapas: *Davidse 30163* (MO); Jalisco: *Graham 1039*
Woodfordia uniflora (A. Rich.) Koehne	2n= 16	Ethiopia. Keffa region: *Tadesse 6063*

distribution and displays an array of haploid chromosome numbers of 7, 8, 9, 14, 16, 18, 24, and 36. The chromosome number of *C. pertenuis* is the only n = 12 reported for the section. The unique morphology and different chromosome number suggests the species should be placed in a new section or a new subsection of *Euandra.*

First chromosome counts are reported for two species of sect. *Leptocalyx* Koehne. The gametic count of 16 in *Cuphea megalophylla* is the same as that of the morphologically similar *C. appendiculata* (n = 16) in this polyphyletic section, and further separates the two species from three others in the section with reported numbers of n = 12 (S. Graham, 1989a). *Cuphea axilliflora*, n = ca. 82-86, has previously been treated as a variety of *C. appendiculata* because the two taxa cannot be consistently separated on the basis of floral morphology (S. Graham, 1989a). *Cuphea appendiculata* grows erect and has variable, multi-flowered, mostly terminal inflorescences. Observations on the growth habit of *C. axilliflora* in the field and greenhouse, first made after the revision of sect. *Leptocalyx* was

completed, reveal it to be a large shrub with 2-2.5 m trailing stems and elongated internodes that consistently bear only one flower at a node. The vegetative differences between *C. appendiculata* and *C. axilliflora*, together with the difference in chromosome numbers reported here, now lead me to regard *C. axilliflora* as a distinct species.

The large section *Melvilla* Koehne, with 46 species, is poorly known chromosomally. Many species of the section are narrow endemics that have rarely been collected. Three species of this description, *C. rasilis* (from Nayarit, Mexico), *C. schumannii* (from southern Veracruz and adjacent northern Oaxaca), and *C. salvadorensis* (disjunctly in Chiapas, Mexico, Guatemala, and El Salvador), have $\underline{n}$ = 16. In addition, the western Mexican *C. watsoniana* and *C. micropetala* var. *micropetala* and var. *hirtella* are reported with $\underline{n}$ = 16. Two species previously counted, *C. jorullensis* and *C. quaternata*, also have a gametic number of 16. The preponderance of tetraploid species now reported suggests the North American species of this section have evolved from a tetraploid ancestor with $\underline{n}$ = 16. However, a possible second evolutionary line based on 15 is represented by three species counted earlier *(C. ignea*; *C. caeciliae*; and *C. flavovirens*, this last species with $\underline{n}$ = 14 probably derived by additional aneuploid loss) (S. Graham, 1989b). Chromosome number differences, supported by morphological characters, lead to the hypothesis that the North American species of sect. *Melvilla* are paraphyletic, based on polyploid lines of 16 and 15. No chromosome numbers are known for any South American species of the section. Prior published counts for *Cuphea micropetala* of $\underline{n}$= 13, 27 are based on cultivated material lacking original source data (S. Graham, 1989b). Counts of both varieties of *C. micropetala* ($\underline{n}$ = 16) reported in this paper are based on flower buds collected in the field in Mexico. Attempts were made to obtain accurate counts of *C. heterophylla* in sect. *Melvilla* but failed. Two collections of the species from the state of Mexico (*Graham 994, 998*) had more than 50 bivalents and 1-3 univalents, but the sticky, clumped figures (trivalents?) allow only an estimate of chromosome number.

Sections *Diploptychia* Koehne and *Melvilla* are together considered to include the most highly evolved species of the genus. This is based on their advanced floral morphology, seed oil chemistry (S. Graham, 1989c), partial distribution into Andean South America from Mexico and Central America (S. Graham, 1989b), and the high chromosome numbers reported for some species. In section *Diploptychia, Cuphea cyanea* has been previously recorded with $\underline{n}$ = 30 from Oaxaca. In this study, three populations of the species from southern Oaxaca, Veracruz, and Guerrero all had $\underline{n}$ = 15. Either two ploidy levels are present, or the $\underline{n}$ = 30 number was incorrectly counted. Two ploidy levels have been recorded in other species of the genus (i.e. *C. jorullensis*, *C. confertiflora*, *C. ericoides*). Plants of *C. cyanea* from Guerrero are semi-shrubs reaching 1-2 meters whereas those from Oaxaca and Veracruz tend to be small herbaceous perennials of less than 1 m. Both forms have the same chromosome number so that the difference in habit is not attributable to additional polyploid events. *Cuphea cyanea* and *C. nitidula* ($\underline{n}$ = 30) are closely related in the section. *Cuphea nudicostata*, $\underline{n}$ = 30, occurring from western Oaxaca to Honduras, is related to *C. spectabilis*, $\underline{n}$ = 15, from the mountains of Guerrero. These species possibly were derived from an ancestral tetraploid with $\underline{n}$ = 16. Chromosome numbers above 13 are considered of polyploid origin in the genus (S. Graham, 1989b).

Also in sect. *Diploptychia* are *C. painteri* and *C. pinetorum*, diploids with $\underline{n}$ = 12 and 11, respectively. Both species are fully fertile outcrossing members of the section. Both

closely resemble the partially to completely sterile, apomictic *C. hookeriana* (n = ca. 40), a common component of disturbed roadside and forest margin vegetation from Sonora, Mexico, to northern Nicaragua. Experimental studies are needed to establish relationships among these taxa. The section *Diploptychia*, like sect. *Melvilla*, appears to be polyphyletic based on chromosome number and morphological data.

In sect. *Ornithocuphea* (Koehne) Bullock, *Cuphea pulcherrima* (n = 8) is closely related to *Cuphea avigera* (n = 10). Only a few minimally different morphological characters separate the species. Revisionary studies are in progress which will address the species limits in this section. The chromosome number difference adds an important character for evaluating species' relationships and taxonomy.

Numbers presented in this study bring reports of chromosome numbers in *Cuphea* from 78 to 91 species, or ca. one-third of the genus.

Diplusodon - The genus is endemic to eastern Brazil. Chromosome numbers for three species have been reported, all n = 15 (S. Graham, 1985). This report adds a fourth species with the same number. An additional species, *D. candollei* (Minas Gerais: Gouveia, *Graham 985*) is provisionally recorded as n = 15, but there were insufficient pollen mother cells to confirm the count. Pollen mother cells in *Diplusodon* are unusual, perhaps unique, in the family due to their large size which is at least double that of any other genus. (PMC's in squashes were 100 μm or more in diameter, but actual size was not measured; uniqueness lies in the relative difference in PMC cell size between *Diplusodon* and other genera.) The contrastingly small chromosomes of the metaphase stage can be difficult to distinguish from dark-staining inclusions that fill the cell. *Diplusodon* might be regarded as having a base number of 5. However, the gametic number of 15 most likely arose from an ancient polyploid with a haploid set of 16 through loss of a chromosome. In contrast, the derivation of x = 5 in *Lythrum* and *Peplis*, the only other lythraceous genera with x = 5, is thought to be by aneuploid reduction from x = 8 (Tobe et al., 1986). Unlike *Diplusodon*, chromosome numbers in *Lythrum* vary, with n = 5, 10, 15 and 30.

Ginoria - Roottip counts of ca. 2n = 56 for the Mexican *Ginoria nudiflora* compare favorably with 2n = 56 for the Cuban endemic *Ginoria glabra* (Tobe et al., 1986). The remaining species of the genus are narrow endemics of the Caribbean, primarily of Cuba, and are as yet unknown chromosomally. Ploidy levels above the tetraploid level are uncommon in the family. Only *Crenea* (2n = 64) has a higher level (probably octoploid) than *Ginoria*.

Lafoensia - *L. acuminata* (2n = 16) from Ecuador retains the base number of the family. The same number has been recorded for *Lafoensia vandelliana* Cham. & Schldl., a Brazilian endemic (Sharma, 1970). A third species *L. pacari*, is reported as n = ca. 10 (Coleman & Smith, 1969). Chromosome numbers of the two remaining members of the genus are uncounted.

Pleurophora - This genus is most closely related to *Cuphea* based on a number of shared derived characters. They are the only truly zygomorphic genera of the family as defined by the reduction of the ventral (abaxial) ovary locule and concomitant strong dorsal-ventral floral morphology. Basic stamen number in both genera is 11, and both share unusual internal spiral hairs in the outer epidermal cells of the seed coat. Whereas the base number of *Cuphea* is eight, in *Pleurophora* it appears to be x = 7, as determined from n = 7 occurring in the two most generalized and widespread species, *P. anomala*,

reported here, and the earlier counted *P. saccocarpa* (Tobe et al., 1986). The base number in *Pleurophora* is believed derived from $\underline{x}$ = 8 in the common ancestor of *Pleurophora* and *Cuphea.*

Rotala - The counts of *R. ramosior* from two states in Mexico ($2\underline{n}$ = 16) confirm the previous report of its diploid status in Mexico ($\underline{n}$ = 8 from Guerrero; S. Graham, 1987). The species is widespread in the Americas and the Caribbean between ca. 50° N lat. and 30° S lat. (Cook, 1979). Only tetraploid populations have been recorded in the northern part of its range in the United States (Lewis et al., 1962; Graham, 1969). The southern and northern populations have been recognized as distinct species based on differences in the length of the bracteoles and appendages. Plants from Mexico and Central America tend to have longer bracteoles and appendages (*R. catholica* or *R. dentifera*) than those in the United States (*R. ramosior*). Cook (1979) saw continuous variation in these characters, with a clinal reduction in length to the north and south of Central Mexico. The limits of the tetraploid race need to be determined and the possibility of correlation of ploidy level to morphological differences investigated.

Woodfordia - The genus is ditypic. With this count of $2\underline{n}$ = 16 from *W. uniflora* from Ethiopia, both species are recorded as diploids. They retain the primitive base number of the family.

The new counts presented above add two genera to our knowledge of generic base numbers in the family Lythraceae (Tobe et al., 1986). The two additions, in *Diplusodon* and *Pleurophora*, are both aneuploid derivatives from an ancestral base of 8, or in the case of *Diplusodon*, from an ancient tetraploid of 16. Fourteen of 28 genera in the family are now known to retain the primitive base number of the family. Three genera have $\underline{x}$ = 5. Base numbers remain to be determined for seven genera. Chromosome numbers remain to be counted for the monotypic genera *Koehneria* and *Lourtella*, and the ditypic *Haitia*. In *Cuphea*, chromosome numbers are providing useful information for estimating relationships among the species of this genus. *Cuphea* continues to be the most diverse lythraceous genus chromosomally. The presence of diploids, dysploids, and neopolyploids in the most advanced genus in the family represents yet another example of the correlation of morphological, ecological, and karyological parameters now being recognized in woody tropical families (Ehrendorfer, 1989).

ACKNOWLEDGEMENTS

The study was supported by NSF grant BSR-8806523. The author gratefully acknowledges that support and the aid of Alan Graham, D. Boufford et al., David Neill, Thomas Zanoni, C. Gutiérrez B., J. Pérez, I. Calzada and M. Tadesse in obtaining material used in this study.

LITERATURE CITED

Baas, P. 1986. Wood anatomy of Lythraceae - additional genera (*Capuronia*, *Galpinia, Haitia, Orias*, and *Pleurophora*). Ann. Missouri Bot. Gard. 73: 810-819.

Baas, P. & R. C. V. J. Zweypfenning. 1979. Wood anatomy of the Lythraceae. Acta. Bot. Neerl. 28: 117-155.

Coleman, J. R. & L. B. Smith. 1969. Chromosome numbers in Brazilian angiosperms. Rhodora 71: 548-551.

Cook, C. D. K. 1979. A revision of the genus *Rotala* (Lythraceae). Boissiera 29: 1-156.

Ehrendorfer, F. 1989. Progrès des connaissances sur la différenciation caryologique et sur l'évolution des plantes ligneuses tropicales. Bull. Soc. Bot. Fr. 136: 197-206.

Foster, R. C. 1945. Miscellaneous diagnoses and transfers. Contr. Gray Herb. 155: 64-70.

Graham, A., J. Nowicke, J. Skvarla, S. Graham, V. Patel, & S. Lee. 1985. Palynology and systematics of the Lythraceae. I. Introduction and genera *Adenaria* through *Ginoria*. Amer. J. Bot. 72: 1012-1031.

Graham, A. 1987. Palynology and systematics of the Lythraceae. II. Genera *Haitia* through *Peplis*. Amer. J. Bot. 74: 829-850.

Graham, A., S. Graham, J. Nowicke, V. Patel, & S. Lee. 1990. Palynology and systematics of the Lythraceae. III. Genera *Physocalymma* through *Woodfordia*, addenda, and conclusions. Amer. J. Bot. 77: 159-177.

Graham, S. A. 1969. IOPB chromosome number reports. XXIV. Taxon 18: 684.

Graham, S. A. 1985. Chromosome number reports LXXXVIII. Taxon 34: 547.

Graham, S. A. 1987. Chromosome number reports XCIV. Taxon 36: 283.

Graham, S. A. 1988. Revision of *Cuphea* section *Heterodon* (Lythraceae). Syst. Bot. Monogr. 20: 1-168.

Graham, S. A. 1989a. Revision of *Cuphea* section *Leptocalyx* (Lythraceae). Syst. Bot. 14: 43-76.

Graham, S. A. 1989b. Chromosome numbers in *Cuphea* (Lythraceae): new counts and a summary. Amer. J. Bot. 76: 1530-1540.

Graham, S. A. 1989c. *Cuphea*: A new plant source of medium-chain fatty acids. CRC Crit. Rev. Food Sci. Nutri. 28: 139-173.

Knapp, S. J. & L. A. Tagliani. 1989. Genetics of allozyme variation in *Cuphea lanceolata* Ait. Genome 32: 57-63.

Lewis, W. H., H. L. Stripling, & R. G. Ross. 1962. Chromosome numbers for some angiosperms of the southern United States and Mexico. Rhodora 64: 147-161.

Lillie, R. D. 1951. Simplification of the manufacture of Schiff reagent for use in histochemical procedures. Stain Tech. 26: 163-165.

Lourteig, A. 1986a. Revisión del genero *Lafoensia* Vandelli (Litraceas). Mem. Soc. Ci. Nat. La Salle 45: 115-157.

Lourteig, A. 1986b. Revisión del genero *Crenea* Aublet (Litraceas). Caldasia 15: 121-142.

Lourteig, A. 1989. Lythraceae austroamericanae. Addenda et Corrigenda III. *Diplusodon*. Bradea 5: 205-242.

Ray, D. T., A. C. Gathman, & A. E. Thompson. 1989. Cytogenetic analysis of interspecific hybrids in *Cuphea*. J. Heredity 80: 329-332.

Sharma, A. K. 1970. Annual report, 1967-1968. Res. Bull. Univ. Calcutta (Cytogenetics Lab) 2: 1-50.

Tobe, H., P. Raven, & S. Graham. 1986. Chromosome counts for some Lythraceae sens. str. (Myrtales), and the base number of the family. Taxon 35: 13-20.