CSIRO PUBLISHING

www.publish.csiro.au/journals/ajb *Australian Journal of Botany*, 2006, **54**, 409–415

Chromosome studies in species of *Eugenia*, *Myrciaria* and *Plinia* (Myrtaceae) from south-eastern Brazil

Itayguara Ribeiro da Costa[A,C] *and Eliana Regina Forni-Martins*[B]

[A]Programa de Pós-graduação em Biologia Vegetal, Instituto de Biologia (IB), Universidade Estadual de Campinas (UNICAMP), Caixa Postal 6109, Campinas, 13083-970 São Paulo, Brazil.

[B]Departamento de Botânica, Instituto de Biologia (IB), Universidade Estadual de Campinas (UNICAMP), Caixa Postal 6109, Campinas, 13083-970 São Paulo, Brazil.

[C]Corresponding author. Email: itayguara@yahoo.com

Abstract. The chromosome numbers of Brazilian species of Myrtaceae were reassessed in the context of chromosomal evolution in fleshy-fruited Myrteae. The chromosome numbers of 14 species of *Eugenia*, three of *Myrciaria* and two of *Plinia* were determined, 14 of which had not been published before. In *Eugenia*, a diploid state ($2n = 22$) was found in nine species, polyploid ($2n = 33$ or $2n = 44$) in three species, and both diploid and polyploid cytotypes in another three species. The percentage of *Eugenia* species with a known chromosome number increased from 19 to 31 species, 22.6% of which were polyploid (3 triploid, 1 tetraploid and 3 hexaploid) and a further 16.1% either dysploid from the triploid level or had both diploid and polyploid races, giving a total of 38.7% in which polyploidy is recorded. In *Myrciaria* (3 species) and *Plinia* (2 species), the chromosome number was $2n = 22$, with no polyploidy known in these genera. The results reinforce the previous indications that polyploidy is of great importance in the evolution of fleshy-fruited Myrteae.

Introduction

The family Myrtaceae is composed of ~3600 species, grouped in 150 genera, with a widespread distribution in tropical and subtropical regions, as well as in temperate regions of Australia (Cronquist 1981). This family was traditionally divided into two subfamilies (Berg 1857; Candolle 1828), Myrtoideae (one tribe Myrteae, divided into the following three subtribes: Eugeniinae, Myrciinae and Myrtinae), with a pantropical distribution, and Leptospermoideae (two tribes), which is essentially Australasian (McVaugh 1968). Alternatively, Wilson *et al.* (2005) suggested expanding the limits of the family and recognising two subfamilies, Myrtoideae (with 15 tribes) and Psiloxyloideae (two tribes). All of the Neotropical species of Myrtaceae occur in the fleshy-fruited tribe Myrteae (*sensu* Wilson *et al.* 2005) and have baccoid fruits and opposite leaves. The Myrteae (*sensu* Wilson *et al.* 2005) comprises some genera of Eugeniinae, Myrciinae and Myrtinae (*sensu* Berg 1857 and Candolle 1828). In this work, we adopted the intrafamilial classification of Wilson *et al.* (2005).

McVaugh (1956) considered the American Myrtaceae a complex group that needed considerable taxonomic studies. Barroso (1991) also indicated the need for regional surveys and biosystematic studies in order to provide a more precise definition of the taxa.

Chromosome studies in Neotropical Myrtaceae (fleshy-fruited Myrteae) are still scarce. There are few chromosome counts for species of *Campomanesia* (Forni-Martins and Martins 2000), *Eugenia* (Andrade and Forni-Martins 1998; Coleman 1982; Forni-Martins and Martins 2000), *Myrceugenia* (Landrum 1981; Sanders *et al.* 1983); *Myrcia* (Forni-Martins and Martins 2000) and *Psidium* (Atchinson 1947; Forni-Martins and Martins 2000). There are no chromosome data for other genera of this group, such as *Blepharocalyx*, *Calycorectes*, *Calyptranthes*, *Gomidesia*, *Hexachlamys*, *Marlierea*, *Neomitranthes*, *Siphoneugena* and *Plinia*. Most chromosome studies

Candolle 1828) is a paraphyletic group (Lucas *et al.* 2005). major groups and characterising most genera. According

10.1071/BT04199 0067-1924/06/040409

to Rye (1979), dysploid species are much more common in the dry-fruited Myrtaceae, whereas polyploid species (and their dysploid derivates) are more frequent in the fleshy-fruited taxa. Andrade and Forni-Martins (1998) analysed some Brazilian species of Myrteae and indicated the importance of polyploidy in the evolution of the family. Polyploid series were also cited in fleshy-fruited Syzygieae, culminating in $2n = 110$ in *Syzygium samarangense* (Blume) Merr. & L.M.Perry (Roy and Jha 1962).

In this work, we investigated the chromosome numbers of some Brazilian species of Myrteae (genera *Eugenia*, *Myrciaria* and *Plinia*) and reassessed the importance of polyploidy in the evolution of this group.

Material and methods

Nineteen species in three genera (*Eugenia*, *Myrciaria* and *Plinia*) of fleshy-fruited tribeMyrteae were collected in different habitats (*cerrado strictu sensu*, '*campos rupestres*', tropical rainforest, among others) in south-easternBrazil. The species or populationswere selected according to availability of materialfor chromosomic studies (floralbuds or mature fruits with seeds). We analysed a variable number of plants (1–5) for each species or population studied. In four species of *Eugenia*, two or three populations were analysed. The species were identified by comparison with specimens in herbaria and literature reports confirmed by a specialist (Marcos Sobral—UFMG). Voucher specimens were deposited in the UEC Herbarium (Universidade Estadual de Campinas) (Table 1).

For meiotic studies, floral buds were fixed in Farmer solution (ethanol : acetic acid, 3 : 1, v/v) for 24 h, and stored in 70% alcohol at a freezer. The cytological preparations were obtained by squashing the anthers in aceto-carmine 1.2% (Medina and Conagin 1964).

To obtain mitotic metaphases, seeds were germinated at temperatures of 28–30°C. The root tip meristems were pre-treated with 2 mM 8-hydroxyquinoline for 24 h, at 8°C. The roots were fixed in Farmer solution and stored in 70% alcohol and frozen until slide preparation and staining by the Giemsa technique (Guerra 1983).

The slides were examined by light microscopy and meiotic and mitotic cellswith a good chromosomiccondensation and spreading were photographed with a photomicroscope.

The pollenstainability in three *Eugenia* species (*E. aurata*, *E. uvalha* and *E. moosenii*) was assessed by a slightly modified technique of Medina and Conagin (1964), using acetocarmine at 1.2%. About 1200 pollen grains were counted for each species.

Results

The chromosome numbers for 19 species of Neotropical Myrtaceae (14 *Eugenia*, three *Myrciaria* and two *Plinia* species) are listed in Table 1. Most of the counts obtained were somatic numbers. In four species the gametic number was recorded as $n = 11$ (Fig. 1*A*). Only in *E. bracteata* and *E. uniflora* was it possible to determine both the gametic ($n = 11$) and somatic ($2n = 22$) numbers. Polyploidy was observed in the following six *Eugenia* species: *E. dysenterica* (Fig. 1*C*), *E. khlotschiana* and *E. pyriformis* all individuals were triploid ($2n = 33$), and the other three (*E. hyemalis*, cytotypes ($2n = 33$ or $2n = 44$) in separated populations (Table 1, Fig. 1*B*, *D*). In *Myrciaria* and *Plinia*, all analysed species had $2n = 22$ (Table 1, Fig. 1*E*, *F*).

Meiotic counts in selected diploid species revealed no abnormalities in chromosome pairing and disjunction. The pollen grains in three of these showed a high degree of staining, with a mean value of 93% (92.3% in *E. aurata*, 91.4% in *E. uvalha* and 95.4% in *E. moosenii*).

Discussion

The chromosome numbers obtained for *Eugenia*, *Myrciaria* and *Plinia* species, with a predominance of $n = 11/2n = 22$, agreed with those reported in most of the literature (Table 1). Prior to the present study, the chromosome number was known in only 19 of the estimated 350 species of *Eugenia* (Landrum and Kawasaki 1997). With the 12 new records reported here (Table 1), this number is now 31 species.

The records obtained for all of the *Myrciaria* species were new and agreed with that of *M. dubia*, the only previous record for the genus (Uchiyama and Koyama 1993). The results for *Plinia cauliflora* and *P. glomerata* are the first records for the genus *Plinia*. For *Myrcianthes*, the only two species previously documented in the literature, *M. cisplatensis* (Bernadello *et al.* 1990) and *M. fragans* (Landrum 1981) also had $2n = 22$. There are no chromosome data for any of the other American genera of the subtribe Eugeniinae (*sensu* Berg, 1857, and Candolle, 1828) as *Calycorectes*, *Hexachlamys*, *Neomitranthes* and *Siphoneugena*.

Diploidy ($2n = 22$) has a high frequency in *Eugenia*, with 19 species of the 31 sampled known only from diploid counts, and a further four species having both diploid and polyploid races. Including the latter four species and a single dysploid species based on the triploid level (*E. bimarginata*, $2n = 32$, Forni-Martins and Martins 2000), polyploidy is known in 38.7% of the species examined. These include seven polyploid species, with three triploid ($2n = 3x = 33$), three hexaploid ($2n = 6x = 66$) and just one tetraploid ($2n = 4x = 44$) species.

In *Eugenia*, the intra-specific differentiation in the degree of ploidy appears to be common. The occurrence of polyploid cytotypes in *E. hyemalis* (*Pop1*, $2n = 22$; *Pop2*, $2n = 44$) and *E. pitanga* (*Pop1*, $2n = 44$; *Pop2*, $2n = 22$), in addition to *E. punicifolia* (*Pop1* and *Pop2*, $2n = 22$; *Pop3*, $2n = 33$), is very interesting because it involves three of the four species for which more than one population was analysed. Only for *E. brasiliensis* did the two populations sampled have the same chromosome number. This suggests that if more populations could be sampled for other taxa, some of these would also prove to have both diploid and polyploid cytotypes. Indeed, Singhal *et al.* (1980, 1985) reported diploid and triploid populations of *E. uniflora*, for which only the diploid level

Table 1. Cromosome numbers in *Eugenia*, *Myrciaria* and *Plinia* species

n and *2n*, gametic and somatic chromosome number, respectively. * and **, first descriptions for species and genus, respectively. State: MG, Minas Gerais; SP, São Paulo. Habitat: AR, rocky outcrop; CE, cerrado *strictu sensu*; CL, cultivated; CR, 'campo rupestre'; FA, tropical rainforest; FS, semideciduous forest. Number of analysed individuals (species/genus) is given in parentheses

Genus/species	Habitat/state, municipality/collector	*n*	*2n*	Reference
Eugenia				
E. aurata O.Berg (2)	—	11	—	Forni-Martins and Martins (2000)
	CE/SP, Mogi Guaçu/I. R. Costa, 429	11	—	This work
E. baruensis (Jacq.) Jacq.	—	—	22	Moussel (1965)
E. bimarginata DC.	—	—	32	Forni-Martins and Martins (2000)
E. bracteata Vell. (3)*	CL/SP, Campinas/I. R. Costa, 434	11	22	This work
E. brasiliensis L. (*Pop1*) (3)*	CE/SP, Itirapina/I. R. Costa, 484	—	22	This work
E. brasiliensis L. (*Pop2*) (2)*	CE/SP, Assis/I. R. Costa, 505	—	22	This work
E. costata O.Berg.	—	—	22	Moussel (1965)
E. dysenterica DC. (3)*	CR/MG, Sa. do Cipó/I. R. Costa, 455	—	33	This work
E. formosa Wall.	—	11	—	Sarkar *et al*. (1982)
E. frondosa DC.	—	33	—	Mehra (1972), (1976)
E. guabiju O.Berg	—	—	24	Moussel (1965)
E. hyemalis DC. (*Pop1*) (4)*	AF/SP, Atibaia/I. R. Costa, 426	—	22	This work
E. hyemalis DC. (*Pop2*) (2)*	CR/MG, Sa. do Cipó/I. R. Costa, 455	—	44	This work
E. khasiana Duthie	—	11	—	Mehra and Khosla (1969, 1972)
E. khlotzschiana O.Berg*	CE/SP, Itirapina/F. R. Martins, s/n	—	33	This work
E. kurzii Duthie	—	33	—	Mehra and Khosla (1969, 1972), Mehra (1976)
E. lilloana Legrand	—	11	—	Coleman (1982)
E. mangifolia Wall.	—	11	—	Mehra and Khosla (1969, 1972), Mehra (1976)
E. michelli Lam.	—	—	22	Bhaduri *et al.* (1949)
E. micrantha (Kunth) DC.	—	—	44	Gill (1974)
E. moosenii O.Berg (2)*	FA/SP, Sete Barras/I. R. Costa, 512	11	—	This work
E. operculata Roxb.	—	11	—	Mehra and Khosla (1972), Mehra (1976)
E. pardensis O.Berg	—	—	22	Delay (1947)
E. pitanga (O.Berg) Kiaersk. (*Pop1*) (3)*	CE/SP, Mogi Guaçu/I. R. Costa, 429	—	44	This work
E. pitanga (O.Berg) Kiaersk. (*Pop2*) (2)*	CE/SP, Assis/I. R. Costa, 505	—	22	This work
E. pluriflora DC.	—	33	—	Andrade and Forni-Martins (1998)
E. punicifolia (Kunth) DC. (*Pop1*) (5)*	CR/MG, Sa.do Cipó/I. R. Costa, 454	—	22	This work
E. punicifolia (Kunth) DC. (*Pop2*) (4)*	CE/SP, Itirapina/I. R. Costa, 492	—	22	This work
E. punicifolia (Kunth) DC. (*Pop3*) (2)*	CR/MG, Sa.do Cabral/K. F. Rodrigues, s/n	—	33	This work
E. pyriformes L.*	—	—	33	This work
E. ramosissima Wall.	—	11	—	Mehra and Khosla (1969, 1972), Mehra (1976)
E. revoluta Griseb.	—	—	22	Gill (1974)
E. sandwicensis A.Gray	—	11	—	Carr (1978)
E. uniflora L.	—	11	22, 33	Singhal *et al.* (1980, 1984, 1985)
E. uniflora L. (5)	CL/SP, Campinas/I. R. Costa, 420	11	22	This work
E. uvalha L. (2)*	CE/SP, Itirapina/I. R. Costa, 491	—	22	This work
Eugenia sp. 1 (2)*	FA/SP, Cananéia/C. Urbanetz, 243	—	22	This work
Eugenia sp. 2 (2)*	FS/SP, Itatiba/R. Macedo, s/n	—	22	This work
Myrciaria				
M. delicatula (DC.) O.Berg (5)*	AF/SP, Atibaia/I. R. Costa, 425	—	22	This work
M. dubia (Kunth) McVaugh	—	—	22	Uchiyama and Koyama (1993)
M. tenella (DC.) O.Berg (4)*	CE/SP, Mogi Guaçu/I. R. Costa, 494	—	22	This work
Myrciaria sp. (2)*	AF/SP, Atibaia/I. R. Costa, 488	—	22	This work
Plinia				
P. cauliflora L. (3)**	CL/SP, Campinas/I. R. Costa, 421	—	22	This work
P. glomerata DC. (2)**	CL/SP, Campinas/I. R. Costa, 462	—	22	This work