Chromosome Studies in Bignonieae (Bignoniaceae): The First Record of Polyploidy in *Anemopaegma*

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Summary *Anemopaegma* (Bignonieae, Bignoniaceae) includes 45 species distributed from Mexico to Northeastern Argentina. The genus is monophyletic and well supported by morphological and molecular characters. However, the delimitation of species within *Anemopaegma* is complex. In this study, we determined chromosome numbers and interphase nuclei structure for 5 shrubby species of *Anemopaegma*, 3 of which belong to the *Anemopaegma arvense* species complex. While most shrubby species analyzed were polyploids ($2n=4x=80$), polyploidy was not encountered among the climbing members of the genus. Furthermore, interphase nuclei was shown to vary among species. In particular, *A. album* was the only species that presented semi-reticulate interphase nuclei, while *A. glaucum* and *A. scabriusculum* presented areticulate nuclei. *Anemopaegma acutifolium* and *A. arvense*, on the other hand, presented an interphase nuclei structure that was intermediate between the areticulate and semi-reticulate types. Overall, our findings suggest that ploidy level may have had an important role in the diversification of the Neotropical genus *Anemopaegma*.

Key words *Anemopaegma*, Chromosome number, Cytotaxonomy, Interphase nuclei, Evolution, Polyploidy.

Bignoniaceae comprises 81 genera and 840 species (Lohmann and Ulloa 2010) of shrubs, trees and climbers, and represents one of the most diverse plant families in South America (Gentry 1974). Species of Bignoniaceae are mainly distributed through tropical rain forests and dry areas (Fischer *et al.* 2004), representing the second most diverse family of wood plants in seasonal dry forests (Gentry 1988). The family has been traditionally divided into 8 tribes (Gentry 1980): Bignonieae, Crescentieae, Tourrettieae, Eccremocarpeae, Schlegelieae, Tecomeae, Coleeae and Oroxyleae. However, recent phylogenetic studies have indicated that Tecomeae is highly paraphyletic, while Schlegelieae is best treated as a separate family (Spangler and Olmstead 1999, Olmstead *et al.* 2009). The most recent classification for the group (Olmstead *et al.* 2009) recognizes 9 main clades: Bignonieae, Catalpeae, Oroxyleae, Tabebuia Alliance Clade, Paleotropical Clade, *Delostoma*, Tecomeae, Tourrettieae, and Jacarandaeae.

Bignonieae, with 360 species and 21 genera, mainly includes tendrillate climbers of Neotropical distribution (Gentry 1976), and represents the largest clade in the family (Lohmann 2006, Lohmann in press). Within Bignonieae, *Anemopaegma*, with 45 species of shrubs and lianas, is the third largest genus (Lohmann in press). *Anemopaegma* is distributed through dry and wet

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forests, savannas and caatingas from Mexico to Brazil and Argentina, and is characterized by cylindrical stems, foliaceous prophylls of the axillary buds, racemose inflorescences, yellow flowers, glandular calyx and stipitate ovary and fruits (Lohmann in press). Despite the clear circumscription of the genus, the delimitation of the species of *Anemopaegma* is known to be complicated, perhaps due to hybridization (Gentry 1973). In particular, natural hybridization has been documented in representatives of the “*Anemopaegma arvense* species complex” (Firetti-Leggieri 2009). This species complex includes 3 species (*A. acutifolium*, *A. arvense* and *A. glaucum*), and several taxonomic varieties (De Candolle 1845, Bureau and Schumman 1896) that are taxonomically complex.

Chromosome data have been useful for the establishment of species limits in several taxonomic groups (Stace 1991, Briggs and Walters 1997). In particular, similar chromosome numbers suggest a close relationship among taxa while different chromosome numbers imply more distant relationships due to the higher levels of reproductive isolation in those taxa. The type of interphasic nuclei, the proportion of condensed and diffused euchromatin, and the general nuclear structure are also known to represent important taxonomic characters at different taxonomic levels (Guerra 1985). Despite the great potential of chromosome information for taxonomy, chromosome studies in the Bignoniaceae are still scarce and fragmentary (Goldblatt and Gentry 1979). Out of the 860 species of Bignoniaceae (Fischer *et al.* 2004), only 113 (13.4%) species had their chromosome numbers counted (Goldblatt and Gentry 1979, Gentry 1980, Goldblatt 1989, Alcorcés de Guerra 2002, Chen *et al.* 2004).

Here, we studied chromosome numbers in the “*Anemopaegma arvense* species complex,” and in 2 phylogenetically distant species of *Anemopaegma* in order to evaluate the potential of chromosome numbers as taxonomic characters in this genus.

Materials and methods

Sampling

Five species of *Anemopaegma* were sampled: *A. acutifolium*, *A. album*, *A. arvense*, *A. glaucum* and *A. scabriusculum*. Most of these species belong to the “*Anemopaegma arvense* species complex” except for *A. album* and *A. scabriusculum*. In addition, 2 populations corresponding to 2 different morphological forms of *A. acutifolium*, *A. arvense* and *A. glaucum* were sampled. These populations were here named as follows: *A. acutifolium* “scandent morph,” *A. acutifolium* “shrubby morph,” *A. arvense* “linear leaflet morph,” *A. arvense* “lanceolated leaflet morph,” *A. glaucum* “pubescent morph,” and *A. glaucum* “glabrous morph.” The exact taxa sampled and locations of the sampled populations are presented in Table 1.

Materials were collected in natural populations of the Brazilian Cerrado and Campo Rupestre vegetation types. During fieldwork, ripe fruits were collected and seeds selected for germination

Table 1. Species of *Anemopaegma* sampled, followed by collection location and voucher number. Vegetation types: CERR=Cerrado; CR=Campo rupestre

Species	Location	Collection number
<i>A. acutifolium</i> “shrubby morph”	Minas Gerais, near Uberlândia; CERR	F. Firetti <i>et al.</i> 43
<i>A. acutifolium</i> “scandent morph”	Distrito Federal, Brasília; CERR	F. Firetti and C.P. Firetti 193
<i>A. album</i>	Bahia, Mucugê, CR	F. Firetti <i>et al.</i> 111
<i>A. arvense</i> “linear leaflet morph”	Distrito Federal, Brasília; CERR	F. Firetti and C.P. Firetti 185
<i>A. arvense</i> “lanceolated leaflet morph”	Distrito Federal, Brasília; CERR	F. Firetti and C.P. Firetti 53
<i>A. glaucum</i> “pubescent morph”	São Paulo, Assis; CERR	F. Firetti <i>et al.</i> 27
<i>A. glaucum</i> “glabrous morph”	Distrito Federal, Brasília; CERR	F. Firetti and C. P. Firetti 178
<i>A. scabriusculum</i>	Bahia, Mucugê; CR	F. Firetti <i>et al.</i> 100

studies. Vouchers of all sampled taxa were deposited in the herbarium UEC (Universidade Estadual de Campinas, UNICAMP).

Chromosome counts and interphase nuclei structure

To obtain mitotic metaphases, seeds with embryos from all studied individuals were germinated in wet paper in Petri dishes kept between 28–30°C. Root tips were pre-treated with 2 mM 8-hydroxyquinoline for 24 h, at 8°C and fixed in Farmer solution (3 ethanol : 1 acetic acid) until the preparation of slides. Roots were hydrolyzed in HCl 5N for 20 min, and squashed in a drop of 45% acetic acid solution. Slides were stained with Giemsa 2% solution (Guerra 1983), and subsequently examined under light microscopy. Mitotic cells with a good chromosome condensation and spreading were recorded with a photomicroscope. Chromosome numbers were determined through the analysis of at least 10 metaphases per individual. Interphase nuclei were classified following Guerra (1985).

Results

The species of “*Anemopaegma arvense* complex” and *A. scabriusculum* presented $2n=80$, while *A. album* presented $2n=40$. In the “*Anemopaegma arvense* complex”, there was constancy in the chromosome number of the different populations (morphotypes) of their 3 species (Table 2).

Anemopaegma glaucum and *A. scabriusculum* presented areticate nuclei, while *A. album* presented a semi-reticulate interphase nuclei. *Anemopaegma acutifolium* and *A. arvense* presented interphase nuclei with a structure that was intermediate between the areticate and semi-reticulate types (Fig. 1, Table 2). The intermediate structure of interphasic nuclei is characterized by a greater condensation of the chromatin content than the areticate type, and a lower condensation than the semi-reticulate type.

Discussion

Chromosome counts

Prior to this study, only 2 species of *Anemopaegma* had their chromosome numbers studied: *A. chamberlaynii* (Venkatasubban 1944 *apud* Darlington and Wylie 1961) and *A. orbiculatum* (Goldblatt and Gentry 1979), both of which presented $2n=40$. This study contributed with chromosome counts for 5 additional species of *Anemopaegma*, increasing total chromosome sampling in *Anemopaegma* to 16% (7 of 45 species).

Table 2. Chromosome numbers and interphase nuclei types in *Anemopaegma*. Interphase nuclei: ARET: Areticulate; S-RET: Semi-reticulate; and, INTERM: Intermediate structure between areticate and semi-reticulate. Habit: S: Shrub, L: Liana

Species	$2n$	Interphase nuclei	Habit	Reference
<i>A. acutifolium</i> “shrubby morph”	80	INTERM	S	Present study
<i>A. acutifolium</i> “scandent morph”	80	INTERM	S	Present study
<i>A. album</i>	40	S-RET	S	Present study
<i>A. arvense</i> “linear leaflet morph”	80	INTERM	S	Present study
<i>A. arvense</i> “lanceolated leaflet morph”	80	INTERM	S	Present study
<i>A. chamberlaynii</i>	40	Unknown	L	Venkatasubban 1944
<i>A. glaucum</i> “pubescent morph”	80	ARET	S	Present study
<i>A. glaucum</i> “glabrous morph”	80	ARET	S	Present study
<i>A. orbiculatum</i>	40	Unknown	L	Goldblatt and Gentry 1979
<i>A. scabriusculum</i>	80	ARET	S	Present study

Fig. 1. Chromosomes and interphase nuclei in *Anemopaegma*. A. *A. album*, $2n=40$. B. *A. acutifolium* "shrubby morph.", $2n=80$. C. Interphase nuclei of *A. scabriusculum* illustrating the areticate nuclei. D. Interphase nuclei of *A. album* illustrating the semi-reticulate nuclei. Bar: 10 μm .

The basic chromosome number for all species of *Anemopaegma* studied was $x=20$, corroborating previous findings by Goldblatt and Gentry (1979) and Raven (1975) for Bignoniaceae. Even though $x=20$ represents the most frequent chromosome number in Bignoniaceae, different chromosome numbers were documented in some clades as Oroxyloae, Tecomeae, Jacarandae, *Delostoma*, and in the Tabebuia Alliance. In Oroxyloae, basic chromosome numbers varied between $x=14$ in *Oroxylum* and $x=15$ in *Millingtonia* (Goldblatt 1976). In Tecomeae, chromosome numbers different from $x=20$ were recorded in *Incarvillea* ($x=11$, Chen *et al.* 2004), *Tecoma* ($x=17$, Nakajima 1936 and $x=18$, Goldblatt and Gentry 1979), *Tecomaria* ($x=17$, Venkatasubban 1944 *apud* Darlington and Wylie 1961 and $x=18$, Goldblatt and Gentry 1979), *Campsis* ($x=19$, Venkatasubban 1944 *apud* Darlington and Wylie 1961), and *Pandorea* ($x=19$, Nakajima 1936). In Jacarandae, chromosome numbers different from $x=20$ were recorded in *Jacaranda* ($x=18$, Goldblatt and Gentry 1979, Piazzano 1998), while the chromosome number $x=21$ were recorded in *Delostoma* (Goldblatt and Gentry 1979). In the Tabebuia Alliance Clade, chromosome numbers different from $x=20$ were recorded in *Handroanthus* ($x=19$, Alcorcés de Guerra 2002).

Interphase nuclei structure

Apart from the uniformity of chromosome number within the *Anemopaegma arvense* species complex ($2n=80$), the interphase nuclei structure varied among species from this complex and was useful for species characterization. In particular, *Anemopaegma glaucum* presented an areticate interphase nuclei, while *A. acutifolium* and *A. arvense* presented an interphase nuclei structure that was intermediate between the areticate and semi-reticulate types. *Anemopaegma scabriusculum* showed the same type of interphase nuclei as *A. glaucum* (areticate interphase nuclei), while *Anemopaegma album* presented a semi-reticulate interphase nuclei (Fig. 1, Table 2). These findings suggest that interphase nuclei structure is useful character for taxonomic delimitation in the plant

family Bignoniaceae.

Ploidy level

Most species studied, *Anemopaegma acutifolium*, *A. arvense*, *A. glaucum* and *A. scabriusculum* presented $2n=80$, except for *A. album* which presented $2n=40$. These results indicate that 4 out of the 5 species studied (*A. acutifolium*, *A. arvense*, *A. glaucum*, and *A. scabriusculum*) were tetraploids ($2n=4x=80$), representing the first record of polyploidy in *Anemopaegma*. Even though polyploidy had already been reported for the Bignoniaceae (Piazzano 1998), polyploidy is thought to represent a rare phenomenon in the family (Goldblatt and Gentry 1979). In Bignoniaceae specifically, tetraploidy was documented in *Dolichandra unguis-cati* ($2n=80$, Jullier 1989, Piazzano 1998), while triploidy was reported for *Pyrostegia venusta* ($2n=60$, Joshi and Hardas 1956). In the Tabebuia Alliance Clade, tetraploidy was documented in *Handroanthus chrysotrichus* ($2n=80$, Piazzano 1998). In this clade, variations in ploidy levels were documented in different populations of *Handroanthus chrysotrichus*, with diploid and tetraploid cytotypes (Ortolani 2007).

Anemopaegma album and the 2 other species of *Anemopaegma* previously studied, *A. chamberlayni* (Venkatasubban 1944 *apud* Darlington and Wylie 1961) and *A. orbiculatum* (Goldblatt and Gentry 1979) were shown to be diploid (Table 2). The fact that 4 out of 5 species shrubby species studied are tetraploids, indicates a predominance of polyploidy on shrubby species. Varying chromosome numbers in taxa with different habits was also recorded in Sapindaceae (Lombello and Forni-Martins 1998) and Malpighiaceae (Lombello and Forni-Martins 2003). In Sapindaceae, lianoid taxa were shown to represent more derived lineages and to have evolved from ancestors with a shrubby habit; in this case, a reduction on chromosome numbers followed by an increase in chromatin length was hypothesized on the lianoid taxa. For Malpighiaceae, the lianoid species (subfamily Malpighioideae) were shown to present basic chromosome numbers $x=5$ or $x=10$, while shrubby representatives (Byrsonimoideae) presented $x=6$, the plesiomorphic condition in the family.

Polyploidy promotes hybrid fertility (Grant 1971, Rieseberg 1997, Ramsey and Schemske 1998, Otto and Whitton 2000, Stace 2000, Soltis and Soltis 2000) and is known to present an important role for the hybrid establishment (Stebbins 1971, Rieseberg 1997). Specifically in *Anemopaegma*, hybridization had already been hypothesized based on observations of individuals with intermediate morphologies (Gentry 1973). In the case of the polyploid species from the *Anemopaegma arvense* species complex, it is possible that successive hybridization events may have occurred between parental diploids with $2n=40$. Hand pollination inter-specific events among sympatric species indicate that species from this complex are indeed inter-fertile (Firetti-Leggieri 2009). In natural conditions, gene flow among species of this complex is possible due to their shared floral phenology, pollination strategy, and distribution (Firetti-Leggieri 2009). In sympatric populations of *A. arvense* and *A. glaucum*, hybrid zones containing individuals with intermediate morphology have been observed (Firetti-Leggieri 2009), corroborating the hypothesis of a polyploid or hybrid origin of species in this complex.

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