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Karyological Studies in Argentinian Species of *Eryngium* (Apiaceae)

Rubén Darío Almada[1], Guillermo Seijo[2,*] and Julio Daviña[1]

[1] Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Rivadavia 588, 3300 Posadas, Argentina
[2] Instituto de Botánica del Nordeste (UNNE-CONICET), Casilla de Correo 209, 3400 Corrientes, Argentina

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Summary In this study, mitotic and meiotic counts are informed for 10 species that belong to 5 out of the 7 sections represented in Argentina. The chromosome numbers of *E. coronatum* ($n=8$) and *E. pristis* ($2n=16$) constitute the first counts for the species, chromosome numbers that differ from previous counts are given for some species, and counts for other taxa are confirmed. Furthermore, the karyotype analysis of *E. ebracteatum* is provided, which is the first description for the New World species. Chromosome analysis showed that *E. pristis*, *E. ekmanii*, *E. ebracteatum*, *E. elegans*, *E. coronatum* and *E. floribundum* are diploids and *E. pandanifolium* is octoploid with $x=8$, while *E. horridum* and *E. mudicaule* are diploids with $x=7$. These data demonstrate that the genus is at least dibasic and it is proposed that variation in basic chromosome number may have occurred by dysploid change. Chromosome counts showed that most of the analysed species are diploids, except in section *Panniculata*, which has a relatively high percentage of polyploid species. Therefore, dysploidy and polyploidy are both mechanism that may have been involved in chromosome number changes during the evolution of *Eryngium* species.

Key words Chromosome number, Karyotype, Dysploidy, Polyploidy.

Eryngium is one of the largest genus of Apiaceae, subfamily Saniculoideae, tribe Saniculeae, which comprises around 317 species, distributed in tropical and temperate regions of the world (Wörz 1999, Pontiroli 1965, Irgang 1974). In Argentina, the genus is represented by 30 species, concentrating the major specific diversity in the Northeast and Centre of the country (Martínez and Calviño 2000). These species are annual or perennial herbs, generally glabrous with entire or pinnatisected leaves with parallel or divergent nerves. The flowers may be white, pink or blue, arranged in capitula or dense spikes (Pontiroli 1965). Most of the species constitute important colonizers and weeds in grasslands, characters that are potenciated by their perennial habit and vegetative multiplication by vigorous rhizomes (Marzocca 1976).

From a karyological point of view, the Saniculoideae subfamily is characterized by the presence of different basic chromosome numbers being the most frequent $x=8$, present in the 73.5% of the species, and $x=7$, in 17.5% of the studied taxa (Moore 1971). This fact is also valid for *Eryngium*, in which most of the already studied species have the mentioned basic numbers. In this genus chromosome numbers were reported for several South American species, however only few Argentinian populations were included in those studies (Vianna and Irgang 1971, Constance *et al.* 1971, 1976, Hunziker *et al.* 1985, Almada *et al.* 2000). Here to for, most of the counts were performed in meiotic cells which ranged from $n=5$ to $n=48$, and in several cases, different chromosome numbers were reported for the same species. Moreover, some polyploid species were reported in the genus, and suggestions of polyploid complexes occurrence have been pointed out (Constance *et al.* 1971). Furthermore, there is almost none information about chromosome morphology of this group, since

* Corresponding author, e-mail: seijo@agr.unne.edu.ar

only one karyotype for the genus was described so far (Reese 1969).

According to these facts, in this work we determine the chromosome numbers of Argentinian populations of *Eryngium* species, both in meiotic or mitotic cells, aiming to provide data for the resolution of taxonomic and phylogenetic problems as well as to contribute for the understanding of the role of chromosome change in the evolution of the genus.

Material and methods

Sources of the studied material are detailed in Table 1. Vouchers specimens were deposited at the herbaria of the Universidad Nacional de Misiones (MNES) and of the Instituto de Botánica del Nordeste (CTES).

Meiotic preparations were made from young floral buds fixed in absolute ethanol : lactic acid (5 : 1) for 24 h (Fernández 1973) and pollen mother cells were stained with aceto-orcein 2%. Mitotic studies were performed in root tips pretreated with a 0.002 M solution of 8-hydroxiquinoleine for

Table 1. Chromosome numbers and procedence of the studied material of *Eryngium* species

Species	2*n*		Localities, collectors and herbaria
	Meiosis	Mitosis	
Sect. Areata			
E. elegans Cham. and Schltdl.	8 II	16	Misiones, Dpto. Concepción de la Sierra, Barra Concepción. Almada 43. MNES, CTES
Sect. Ebracteata			
E. ebracteatum Lam.	8 II**		
		16**	Misiones, Dpto. Capital, Posadas. Almada 20. MNES, CTES
		16**	Misiones, Dpto. San Ignacio, Corpus. Almada 72. MNES
Sect. Foetida			
E. coronatum Hook. et Arn.	8 II*		Corrientes, Dpto. Capital, Corrientes. Seijo 2367. CTES
E. ekmanii H. Wolff.	8 II		Misiones, Dpto. Capital, Posadas. Almada 28. MNES
		16	Misiones, Dpto. Cainguas, Cuña Pirú. Almada 51. MNES, CTES
E. nudicaule Lam.	7 II		Misiones, Dpto. Capital, Posadas. Almada 32. MNES
		14	Misiones, Dpto. Capital, Posadas. Almada 36. MNES
E. floribundum Cham. et Schltdl.	8 II	16**	Misiones, Dpto. Capital, Posadas. Almada 94. MNES
Sect. Panniculata			
E. horridum Malme.		14**	Misiones, Dpto. Capital, Posadas. Almada 35. MNES
E. pandanifolium Cham. et Schltdl.		64**	Misiones, Dpto. Capital, Posadas. Almada 107. MNES
E. pristis Cham. et Schltdl.	8 II*	16*	Misiones, Dpto. Concepción de la Sierra, Barra Concepción. Almada 44. MNES, CTES
Sect. Sanguisorbiformia			
E. sanguisorba Cham. et Schltdl.	8 II		Misiones, Dpto. Candelaria, Cerro Corá. Almada 84. MNES

* First count for the species. ** Taxa with different chromosome numbers.

6 h, fixed in absolute ethanol : lactic acid (5 : 1) for 24 h and stained according to the Feulgen's technique. The meristems were macerated in a drop of aceto-orcein 2% and then squashed. Permanent slides were made using Euparal as mounting medium.

For karyotype description, chromosomes were arranged in groups according to the position of the centromere, and among each one by length according to Levan *et al.* (1964). Karyotype asymmetry was estimated using the Romero Zarco's (1986) intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indices, as well as the Stebbin's categories (Stebbins 1971).

Results and discussion

In this study, 10 out of the 30 species of *Eryngium* cited for Argentina were studied (Martínez and Calviño 2000) which, considering Wolff (1913) criteria, belong to 5 of the 7 sections reported for this country. Mitotic and meiotic counts are presented in Table 1.

Among species of section Areata, the $n=8$ (Fig. 1B) and $2n=16$ observed in *E. elegans* agree with those cited by Hunziker *et al.* (1985) for a population from Buenos Aires province and by Constance *et al.* (1971) for a population from Brazil.

In *E. ebracteatum* (section Ebracteata) our counts of $2n=16$ (Fig. 1A) found in Posadas and Corpus populations and the $n=8$ of Yabebiry's population are in accordance with previous counts from Brazil (Bell and Constance 1966, Constance *et al.* 1971) and from a southern Argentinian population (Hunziker *et al.* 1985). However, Vianna and Irgang (1971), found a $2n=18$ and $n=9$ in populations from Brazil. The data presented here together with those cited in the literature revealed constancy of $x=8$ in 4 populations and indicate that additional studies are necessary to confirm the number $x=9$, mainly considering that this number was reported only for this species of *Eryngium* and that it is very rare in the subfamily.

Within section Foetida, 3 species were studied. *E. coronatum* presented 8 bivalents at metaphase I (Fig. 1C), being the first report for the species. *E. ekmanii* showed a somatic number of $2n=16$ (Fig. 1D) and regular meiotic behaviour with 8 bivalents at metaphase I. These counts confirm the $n=8$ published by Constance *et al.* (1976) for another Argentinian population. *E. nudicaule* presented $2n=14$ and $n=7$ (Fig. 1E), numbers that are coincident with those cited for a population from Rio Grande do Sul, Brazil (Vianna and Irgang 1971).

The $n=8$ and $2n=16$ of *E. floribundum* are in accordance with the number published for a Brazilian population (Vianna and Irgang 1971). However, the last authors also reported $n=7$ and $2n=14$ for a population which lives just 20 km far from the later population. From a morphological point of view, this is a highly variable taxa and 4 varieties were described by Urban (1879), however, due to the absence of discrete differences, Irgang (1974) did not maintained this infraspecific organization. Chromosome data, specially the presence of 2 basic chromosome numbers, suggest that different entities may be included under the name of *E. floribundum sensu* Irgang (1971) and support wider biosystematic studies.

Three species of the Panniculata section were studied. Our counts of $2n=16$ (Fig. 1F) and $n=8$ in *E. pristis* constitute the first report for the species. On the other hand, the $2n=14$ (Fig. 1G) chromosomes of *E. horridum* disagrees with the $n=8$ and $2n=16$ reported for Brazilian populations (Constance *et al.* 1971, Vianna and Irgang 1971), indicating the presence of taxa with different basic chromosome number under this name.

The other analysed species of this section corresponds to *E. pandanifolium* according to the criteria of Mathias *et al.* (1972). These authors have subordinated *E. decaisneanum* Urb., *E. lasseauxii* Dcne. and *E. chamissonis* Urb. as varieties of *E. pandanifolium*, however, this criteria is not shared by other authors, which have treated most of these entities as independent species (Pedersen 1997, Martínez 1999). Considering *E. pandanifoliun* in the amply sense of Mathias *et al.* (1972) our material could not be attributed certainly to any of the varieties by them recognized,

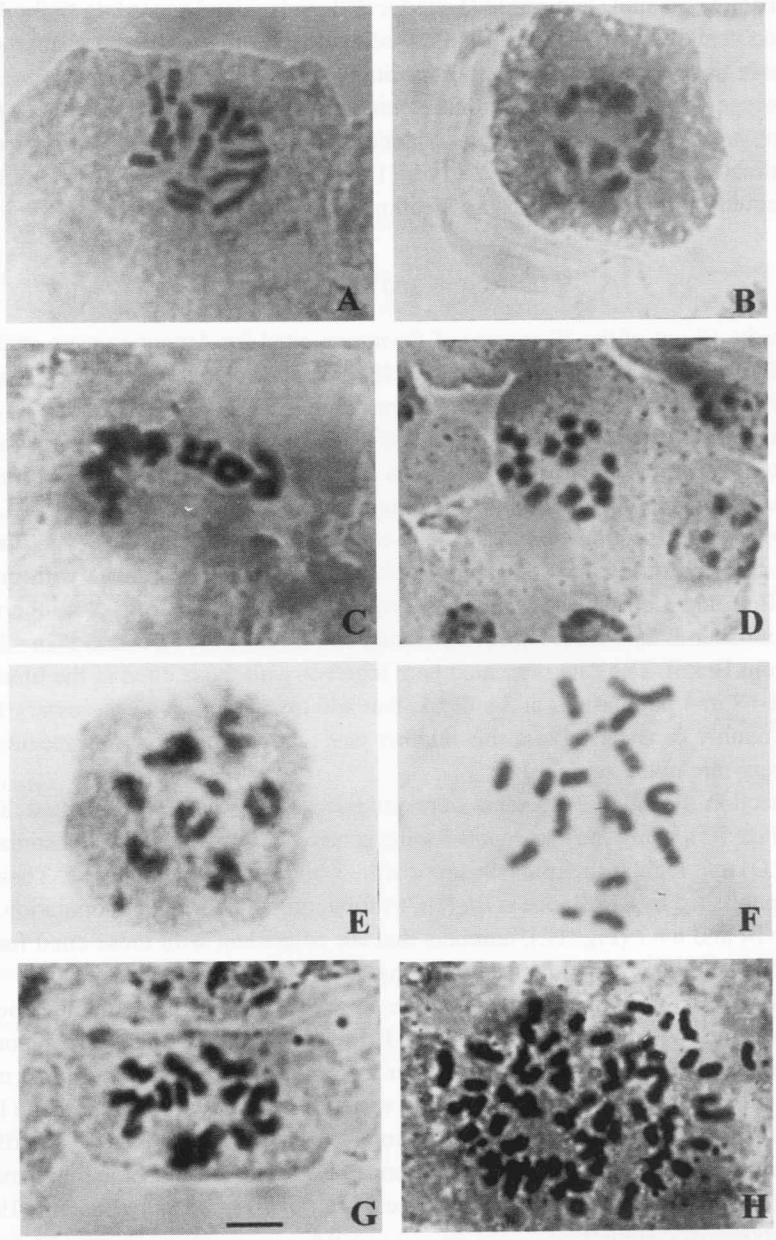

Fig. 1. Mitotic and meiotic chromosomes of some *Eryngium* species. A) *E. ebracteatum*, B) *E. elegans* , C) *E. coronatum*, D) *E. ekmanii*, E) *E. nudicaule*, F) *E. pristis*, G) *E. horridum*, H) *E. pandanifolium*. Bar indicates 2 μm.

however it is closer to var. *chasmisonis* and var. *lasseauxii* than to the other varieties. This is true since the plants showed involucral bracts with entire margin like the former, but the spination of leaves and fruits are more similar to the latter.

Our count of $2n=64$ (Fig. 1H) were cited neither for *E. pandanifoliun* var. *chasmisonis* nor for *E. pandanifolium* var. *lasseauxii*, for which only counts of $n=24$ and $n=48$ for Brazilian populations were reported (Constance *et al.* 1971). However, the $2n=64$ agrees with the report of $n=32$ of Vianna and Irgang (1971) for Brazilian collections of *E. pandanifolium* var. *pandanifolium*.

E. pandanifolium and related entities were considered by Constance *et al.* (1971) as a polyploid complex since different chromosome numbers $n=16$, 24, 32, 48 have been reported for these taxa and $x=8$ has been proposed as basic number (Constance *et al.* 1971, Hunziker *et al.* 1985, Vianna and Irgang 1971). Chromosome variation reflects the variability encountered in morphological characters since in many instances individuals show intermediate characters. Moreover, as was remarked by Constance in a letter to Pedersen "The problem is that the character of size and colour of heads and spination of leaves vary quite independently of one another" (Pedersen 1997). Consequently, in order to understand the taxonomy and evolution of this group of species, amply biosystematic studies are required.

Within section Sanguisorbiformia, *E. sanguisorba* shows $n=8$, which is coincident with previous counts made in a Brazilian population (Constance *et al.* 1971, Vianna and Irgang 1971).

The chromosomes of all the species analysed belong to the small size in the Lima de Faria's classification (1980) and, in general, there is a predominance of m and sm chromosomes while st-type are very rare. In order to increase the karyological knowledge of this genus, we have analysed the karyotype of *E. ebracteatum*, which was comprised by 12 metacentric and 4 submetacentric chromosomes (Fig. 2). Quantitative parameters of each chromosome pair and karyotype features are presented in Tables 2 and 3, respectively. Romero Zarco's asymmetry indices showed that this species has a symmetric karyotype and, in the Stebbins's classification, it lays in the 2A category. The total length of the complement is 16 μm, with a mean chromosome length of 2 μm. This description constitutes the first report on chromosome morphology for the New World species of *Eryngium*.

Chromosome analysis showed that *E. pristis*, *E. ekmanii*, *E. ebracteatum*, *E. elegans*, *E. coronatum* and *E. floribundum* are diploids and *E. pandanifolium* is octoploid with $x=8$, while *E. horridum* and *E. nudicaule* are diploids with $x=7$. These data confirm that the genus is at least dibasic and suggest that rare reported basic chromosome numbers need to be confirmed. When basic chromosome numbers were analysed considering sections, growth habit and ecological preferences no relationship was found (Table 4), therefore it seems that chromosome change may have been independent of morphological and ecological evolution.

Changes in basic chromosome number may have occurred by dysploidy, since as $x=7$ and $x=8$ appeared in diploids, changes by aneuploidy may have imply the loose of great

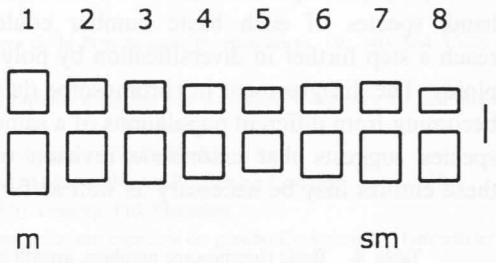

Fig. 2. Idiogram of *Eryngium ebracteatum*. Bar indicates 1 μm.

Table 2. Quantitative parameters of the *E. ebracteatum* karyotype

Type	Par	c (μm)	s (μm)	l (μm)	i	r	RL
m	1	2.628±0.042	1.074±0.112	1.554±0.348	40.87	1.45	16.42
m	2	2.083±0.009	0.849±0.047	1.234±0.109	40.76	1.45	13.01
m	3	2.083±0.195	0.785±0.035	1.298±0.237	37.69	1.65	13.01
m	4	1.875±0.009	0.705±0.053	1.170±0.305	37.60	1.66	11.71
m	5	1.618±0.040	0.626±0.068	0.993±0.272	38.63	1.59	10.11
m	6	1.843±0.152	0.705±0.047	1.138±0.128	38.25	1.61	11.51
sm	7	2.035±0.018	0.753±0.073	1.282±0.309	37.00	1.70	12.71
sm	8	1.843±0.133	0.593±0.053	1.250±0.163	32.18	2.11	11.51

c=mean chromosome length, s=mean length of the short arm, l=mean length of the long arm, I=centromeric index, r=arm ratio and RL=relative length of each chromosome pair.

amount of genetic information that generally is not withstand by individuals. Considering this hypothesis numerical changes through disploidy probably have happened independently in several times. Arguments in favour of this hypothesis arise from the fact that both basic chromosome numbers can be found in 3 out of the 5 sections here studied. Otherwise, if it occurred once, this phenomenon may have taken place in a basal section, for instance Section Foetida. From this group, species with both basic numbers, probably by convergent evolution, originated the sections of *Eryngium* with more advanced characters. Furthermore, if the rare basic numbers $x=5$, 6 and 9 cited for few *Eryngium* species (Moore 1971, Vianna and Irgang 1971) proved true, their origin could be also explained by dysploid events.

The chromosome numbers here obtained, together with those cited in the literature, show that polyploidy is not a frequent phenomenon in the genus. In this sense, polyploidy seems to have played a less important role in the diversification of the genus, except for the section Panniculata, which has a relatively high percentage of polyploid species. The $2n=64$ here observed for *E. pandanifolium* together with the $n=ca.$ 24 cited by Hunziker *et al.* (1985) for this species, are the only reports of polyploidy for Argentinean populations. If $x=8$ is considered as the basic chromosome number for this species, our counts of $2n=64$ indicates that this population would be an octoploid cytotype, that of $n=ca.$ 24 would be an hexaploid and the $n=16$ (Constance and Irgang 1971) a tetraploid. These numbers suggest that this species may constitute a polyploid series with the diploid cytotype still unknown.

In summary, available chromosome data suggest that at least 2 mechanisms of chromosome variation have occurred in *Eryngium*. On the one hand, basic chromosome number variation by dysploid processes, and on the other hand, species of each basic number could reach a step further in diversification by polyploidy. The disagreement in chromosome data becoming from different populations of a same species suggests that taxonomic revision of these entities may be necessary as well as fur-

Table 3. Chromosome features of *E. ebracteatum*

$2n$	16, 8 II
Karyotype formula	12 m+4 sm
TCL (μm)	16.01
Chromosome length range (μm)	1.62–2.63
ic	37.87
r	1.62±0.07
R	0.62
A_1	0.39
A_2	0.14
Stebbin's asymmetry type	2A

TCL=total chromosome length, i=(s/c) mean centromeric index, r±S.E.=mean arm ratio±standard error, R=largest/smallest chromosome ratio. A_1: intrachromosomal asymmetry index, A_2: interchromosomal asymmetry index.

Table 4. Basic chromosome numbers, growth habit and environment of the analysed species of *Eryngium*

Species	Basic number	Growth habit and environment
Sect. Ebracteata		
E. ebracteatum Lam.	8	herb, grows in sandy and moistly soils.
Sect. Foetida		
E. ekmanii H. Wolff.	8	herb, develops on rocks and moist soils.
E. nudicaule Lam.	7	herb, heliophytic and hydrophilic.
E. coronatum Hook. et Arn.	8	herb, grows in sand and modified soils.
Sect. Areata		
E. elegans Cham. et Schltdl.	8	herb, mesophytic invader.
E. floribundum Cham. et Schltdl.	8	robust perennial, soils with permanent water.
Sect. Panniculata		
E. horridum Malme.	7	robust herb, lives in modified dry fields.
E. pristis Cham. et Schltdl.	8	xerophyitic and heliophytic, grows in basaltic soil.
E. pandanifolium Cham. et Schltdl.	8	gigantic plant, hydrophyilic and heliophytic.
Sect. Sanguisorbiformia		
E. sanguisorba Cham. et Schltdl.	8	herb, inhabit sandy soils, heliophytic.

ther cytogenetics studies. Moreover, further investigations on karyotype analysis will provide new features on species relationship and taxonomic position.

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