Karyotypes in Species of *Eryngium* in Argentina

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Summary The objective of this study is to analyse the karyotype to determine the chromosomal asymmetry and its relationship with the evolution of species of *Eryngium*: *E. elegans* Cham et Schlecht, *E. ebracteatum* Lam., *E. horridum* Malme and *E. coronatum* Hook & Arn. in Argentina. *E. elegans*, *E. ebracteatum* and *E. horridum* have $2n=16$, and *E. coronatum* has $2n=48$, with relatively symmetric karyotypes. It is suggested that the studied species had great adaptation to diverse environmental conditions reaching a great invasive power and aggressiveness as weeds, keeping their karyotype through the evolution.

Key words Asymmetry, Evolution, *Eryngium*, Karyotype, Argentina.

Genus *Eryngium* (Apiaceae) has about 3000 species with an ample reproduction and adaptation capacity for prospering in warm subtropical and temperate climates in South America and Europe. The genus is presented in the South American countries of Argentina, Bolivia, Paraguay, Uruguay and Brazil. In the Argentinean northwest, especially in Tucumán province, several species of the genus acquired importance as weeds with great invasive power, affecting economically important crops, like sugarcane (Chaila and Sobrero 2009).

E. elegans Cham & Schlecht, *E. ebracteatum* Lam., *E. coronatum* Hook. & Arn. and *E. horridum* Malme are noted species because they represent important weeds for the Argentinean northwest (Rochi and Lallana, 1996, Nasif *et al.* 2001, 2003, Andrada *et al.* 2001a, Lorenzi 2000). From a phenotypic point of view, the 4 species have ample genetic variability indicated by means of isoenzyme markers which favour their ample invasive capacity (Andrada *et al.* 2001b, Pastoriza *et al.* 2009). From a cytogenetic point of view, Darlington and Wylie (1955) cited for the genus a basic number $x=8$ with diploid ($2n=16$), tetraploid ($2n=32$) and hexaploid ($2n=48$) species. Silveira Vianna and Irgang (1971) mentioned the existence of a high polyploidy and aneuploidy percentage for Apiaceae and proposed at least 3 basic numbers for Rio Grande do Sul (Brazil). Perdigo I Ariso (1981) reported the existence of diploid species with chromosome numbers varying from $2n=14$ to $2n=16$ and tetraploid species with $2n=28$ and $2n=32$. Further, Constance (1982) determined that the half of the genus chromosome numbers were multiples of $n=8$, with some species having $n=5-8$ and the possibility of inter specific hybridisations. Calviño *et al.* (2001) cited for *E. coronatum* and *E. horridum* the number $2n=16$, and also pointed that polyploidy and chromosome rearrangement would be important in the genus speciation. In the same sense, Nasif *et al.* (2000, 2001, 2003) reported for *E. elegans*, *E. ebracteatum* and *E. horridum* regular chromosome behaviour during the different meiosis stages with the presence of great quantities of open bivalents and also high pollen fertility. The same behaviour was observed in hexaploid species of *E. coronatum* showing characteristics of a diploid species (Andrada *et al.* 2001).

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Fig. 1. Mitotic chromosomes of 4 Argentinean *Eryngium* species. A, *E. elegans* Cham & Schlecht.; B, *E. ebracteatum* Lam.; C, *E. horridum* Malme.; D, *E. coronatum* Hook. & Arn. Scale bar=4 μ m.

The objective of this work is to analyse the chromosome complement, to realize the karyotype, to determine intra- and inter-specific chromosome asymmetry of 4 species of *Eryngium* genus that constitute the most common weeds of the Argentinean northwest: *E. elegans* Cham & Schlecht, *E. ebracteatum* Lam, *E. horridum* Malme and *E. coronatum* Hook. & Arn.

Materials and methods

The material of *E. elegans* and *E. ebracteatum* came from La Rinconada, located at the piedmont zone of Tucumán province (Argentina). The material of *E. coronatum* and *E. horridum* came from Santiago del Estero province (Argentina). All plants were maintained in pots in the greenhouse at the Facultad de Agronomía and Zootecnia of the Universidad Nacional de Tucumán. For the preparation of the samples, the root tips were pretreated with a saturated solution of paradichlorobenzene for 2.5 h. Then, they were fixed in a mixture of 3 : 1 (absolute ethylic alcohol : glacial acetic acid). Further, the root tips were hydrolysed in 1 N HCl at 58–60°C for 8 min and were conserved at 45% acetic acid. To finish the preparations, the root tips were squashed in a drop of 2% haematoxylin (Nasif *et al.* 2003). The karyotype of each species was taken from microphotographs in 3000 $\times$. The calculus of the intra and inter chromosome asymmetry (A1 and A2) was carried out according to Romero Zarco (1986).

Results and discussion

Chromosomal numbers determined for *E. elegans*, *E. ebracteatum* and *E. horridum* were $2n=16$, and for *E. coronatum* was $2n=48$, as shown in Figs. 1 and 2. In diploid species, morphology corresponds to that of metacentric and sub-metacentric chromosomes. Chromosome size was from small to medial and low values of asymmetry indexes were obtained. In *E. coronatum*, the karyotype was composed with medial and sub medial centromeres and the chromosome size was small and low asymmetry values. The karyotype formula of *E. elegans* is $8m+8sm$, that of *E. ebracteatum* and *E. horridum* is $10m+6sm$, and that of *E. coronatum* is $30m+18sm$. The comparative values of all considered parameters in the karyotype study and life cycles of all species are presented

Table 1. Comparative values of karyotypic parameters and their life cycle for the *Eryngium* species.

Species of <i>Eryngium</i>	Life cycle	2n	Karyotypic formula	Size (μm)	A1	A2
<i>E. elegans</i>	Perennial	16	8m+8sm	1.8–4	0.29	0.20
<i>E. ebracteatum</i>	Perennial	16	10m+6sm	1.6–3.6	0.27	0.28
<i>E. horridum</i>	Perennial	16	10m+6sm	1.3–2.4	0.20	0.55
<i>E. coronatum</i>	Perennial	48	30m+18sm	1.6–3.4	0.20	0.40

in Table 1. In all species the mean chromosome length was similar with a range from 1.3 to 4.0 μm . In relation to the chromosome morphology, the presence of SAT chromosomes in pair 2 was observed in all species.

E. elegans, *E. ebracteatum* and *E. horridum* species are considered diploid according to their basic number of $x=8$, established by Darlington and Wylie (1955). The chromosome size and their numbers constituted vantages for the confection of the respective karyotypes and permitted the establishment of differences at that level among the species.

The chromosome number $2n=48$ found in *E. coronatum* supports the notion that it is a hexaploid species (Andrada *et al.* 2001). These results differ from $2n=16$ for this species (Calviño *et al.* 2001).

In spite of the 3 diploid species having similarity in the cytogenetic parameters analysed, they present very different plant morphology, according to the reports by Rochi and Lallana (1996) and Nasif *et al.* (1999). These morphological differences in plants were also confirmed by the intra and inter population variability determined by means of isoenzymatic markers (Pastoriza *et al.* 2009). The similarities in the chromosomal number found and in the shape (metacentric and submetacentric) from diploid species suggest that they could come from a common ancestor. The low values of intra and inter asymmetry (A1 and A2) obtained in all studied species indicate the tendency of the chromosomes to present centromeres predominantly from medial to sub medial positions. According to this, the result is that they present relatively symmetric karyotypes, the value A2 of *E. horridum* (0.55) being slightly superior indicating a little asymmetry. It would therefore appear that these species have been able to achieve great adaptability to different environmental conditions, and also a great invasive and aggressive power as weeds, while keeping their karyotypes constant throughout their evolution.

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Fig. 2. Idiograms of 4 Argentinean *Eryngium* species. A, *E. elegans* Cham et Schlecht.; B, *E. ebracteatum* Lam., C, *E. horridum* Malme, D, *E. coronatum* Hook. et Arn. Dark region shows SAT chromosome. A scale bar = 4 μm .

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