Karyotype Analysis in Three New Species of *Echeandia* (Liliaceae) and Cytotypes of *E. reflexa*

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In a previous paper we (Palomino and Martínez 1994) reported different cytotypes in three diploid populations ($2n=16$) of *Echeandia* analyzed: *E. echeandioides*, *E. tenuis* and *E. mexicana*. Cytotype variation was observed in heteromorphic pairs of metacentric, sub-metacentric and subtelocentric chromosomes, and in chromosomes with satellite. Meiotic analysis showed spontaneous heterozygotic exchanges. Metaphase I (MI) analysis showed heteromorphic bivalents (IIs) and quadrivalents (IVs). Analysis of anaphase I (AI) displayed U-type chromatid exchange and subchromatid aberrations (SAB).

This report details karyotypes, meiotic chromosome behavior and pollen fertility of four additional Mexican species of the genus: *Echeandia hintonii*, *E. montealbanensis*, *E. pubescens*, and three cytotypes of *E. reflexa*.

Material and methods

Plant material. The species studied were: *Echeandia hintonii* Cruden, distributed in Guerrero State (Cruden 1993), *E. montealbanensis* Cruden, considered to be endemic to the Monte Albán area in Oaxaca State (Cruden 1993), *E. pubescens* Cruden, distributed in México State (R. W. Cruden, personal communication); and *E. reflexa* Cruden (= *Anthericum reflexum* (Cav.) Rose, which is distributed in the mountains from Texas to Chiapas, one of the diversification centers for species of the genus (R. W. Cruden, personal communication).

Plant material was collected from wild populations in pine-oak forests (Appendix 1). Voucher specimens were deposited at the National Herbarium of the Universidad Nacional Autónoma de México (MEXU).

For each species, three to six individual plants were collected from one or three populations. Plants were taken to the Jardín Botánico of the Universidad Nacional Autónoma de México, where they were transplanted into pots containing a mixture of vermiculite and organic soil, and maintained in a greenhouse.

Mitotic and meiotic chromosome analyses. The cytological methods for the analysis of mitotic chromosomes, types of bivalents in MI, abnormalities in AI, and pollen grain fertility, are detailed in Palomino and Martínez (1994).

For karyotype and total haploid chromatin length (TCL) analysis, we observed 9 cells of three to six plants for each population. For MI analysis, 168 pollen mother cells (PMCs) were analyzed for each population. For AI analysis we recorded 1179 to 1422 PMCs. To estimate pollen fertility, 692 to 874 pollen grains were analyzed for each population.

Data analysis. Differences between fourteen genotypes of seven species of *Echeandia* were analyzed based on the data published by us (Palomino and Martínez 1994), and on the results reported here. Inter and intraspecific variation of total haploid chromatin length was determined for fourteen populations of *Echeandia* using analysis of variance (ANOVA); means were compared using Tukey's method. The relation between unviable pollen grain number and

presence of anaphase irregularities was determined for fourteen populations of seven species of *Echeandia* using both Pearson's correlation analysis and regression analysis. Unviable pollen percentage was transformed to probit units, and percentage of irregularities during anaphase was transformed using the logarithm of probit units (Fisher and Yates 1963). Statistical analysis was performed in the computer using Statgraphics 4.0 software.

Results

Samples from the single population studied of each *E. hintonii*, *E. montealbanensis* and *E. pubescens*, as well as those collected from three population of *E. reflexa*, were diploid with $2n =$

Table 1. Karyotypes of *Echeandia hintonii*, *E. montealbanensis*, *E. pubescens* and cytotypes of *E. reflexa*

Species and populations	Karyotypes	Secondary constrictions	Range of chromosome length (μm)	Total haploid chromatin length (μm) $\bar{x} \pm \text{EE}$		TF (%) $\bar{x} \pm \text{EE}$	
<i>E. hintonii</i> 308	10m + 6sm	4m	1.73–3.47	22.99	0.30	38.97	0.01
<i>E. montealbanensis</i> 4015	8m + 6sm + 2st	4m	1.74–5.65	28.68	0.04	35.85	0.01
<i>E. pubescens</i> 482	6m + 8sm + 2st	4sm	3.47–8.70	48.70	0.43	35.71	0.01
Cytotypes							
<i>E. reflexa</i> 260	4m + 8sm + 4st	2m + 2st	2.16–4.78	27.37	0.26	33.32	0.01
<i>E. reflexa</i> 290	8m + 8sm	4m	1.74–3.90	22.15	0.04	37.25	0.01
<i>E. reflexa</i> 292	8m + 6sm + 2st	2m + 2st	4.34–8.69	48.66	0.30	34.64	0.01

Fig. 1. Karyotypes of: (A) *Echeandia hintonii* (308), (B) *E. montealbanensis* (4015), (C) *E. pubescens* (482), and of cytotypes: (D) *Echeandia reflexa* (260), (E) *E. reflexa* (290), and (F) *E. reflexa* (292). Numbers indicate chromosomes with satellites. Asterisk indicates pair of heteromorphic chromosomes. Scale equals 10 μm .

16 and $n=8$. Each species had a different karyotype (Table 1, Figs. 1, 2) with different numbers of metacentric, submetacentric and subtelocentric chromosome pairs. All of them presented two pairs of chromosomes with satellite. Each population of *E. reflexa* had a different cytotype, which was uniform within populations (Table 1). Cytotype of *E. reflexa* sample no. 292 showed one pair of heteromorphic chromosomes in mitosis (Figs. 1, 2F). Meiotic behavior of the six population of four species of *Echeandia* sampled was studied by determination of the average number of chiasmata per cell, and by calculation of the recombination index. Ring and rod bivalents at MI were found with variable frequencies (Table 2). Both in *Echeandia hintonii* and in *E. montealbanensis* all bivalents were homomorphic. *Echeandia pubescens* showed three heteromorphic bivalents. Cytotypes of *E. reflexa* Nos. 260 and 290 had 2 heteromorphic bivalents, while cytotype No. 292 showed four of these (Table 4).

All population studied exhibited variable frequencies of aberrations at AI (Table 3). *Echeandia hintonii*, *E. montealbanensis*, *E. pubescens* and the three cytotypes of *E. reflexa* had cells with one or two bridges, (subchromatid aberrations, side arm bridges or SAB), and cells with bridges with a fragment (U-type chromatid exchange). In addition, cytotypes of *Echeandia reflexa* and *E. pubescens* showed lagging chromosomes (Table 3). Compared to *E. hintonii* and *E. montealbanensis*, *E. pubescens* and the three cytotypes of *E. reflexa* displayed more structural chromosomal aberrations, and more non-viable pollen grains (Table 3).

Fig. 2. Idiograms of: (A) *Echeandia hintonii* (308), (B) *E. montealbanensis* (4015), (C) *E. pubescens* (482), and of cytotypes: (D) *E. reflexa* (260), (E) *E. reflexa* (290), and (F) *E. reflexa* (292). Asterisk indicates pair of heteromorphic chromosomes. Scale equals 10 μm .

Table 2. Type and frequency of bivalents (IIs), chiasmata frequency and recombination index (RI) for *Echeandia hintonii*, *E. montealbanensis*, *E. pubescens* and cytotypes of *E. reflexa*

Species and populations	No. of PMCs	Ring IIs $\bar{x} \pm EE$	Rod IIs $\bar{x} \pm EE$	Chiasmata per cells $\bar{x} \pm EE$	RI $\bar{x} \pm EE$
<i>E. hintonii</i> 308	168	5.66 0.09	2.34 0.09	13.66 0.73	21.66 0.07
<i>E. montealbanensis</i> 4015	168	4.98 0.15	3.02 0.15	12.07 0.86	20.07 0.01
<i>E. pubescens</i> 482	168	5.05 0.15	2.90 0.15	13.06 0.13	21.06 0.01
<i>E. reflexa</i> 260	168	5.15 0.15	2.90 0.15	13.15 0.21	21.15 0.03
<i>E. reflexa</i> 290	168	4.67 0.17	3.33 0.17	12.67 0.27	20.67 0.02
<i>E. reflexa</i> 292	168	5.02 0.10	2.98 0.10	13.02 0.08	21.02 0.04

Table 3. Shrunk or empty pollen grains and irregular A1 for *Echeandia hintonii*, *E. montealbanensis*, *E. pubescens* and cytotypes of *E. reflexa*

Species and populations	Shrunk or empty pollen grain %	No. of PMCs	Irregular A1 cells %	Cells with one bridge and fragment %	Cells with one bridge %	Cells with two bridges %	Cells with lagging chromosomes %
<i>E. hintonii</i> 308	5.70	1099	5.83	0.18	5.10	0.55	
<i>E. montealbanensis</i> 4015	3.61	1244	4.66		4.42	0.24	
<i>E. pubescens</i> 482	22.54	1422	22.36	3.31	12.73	5.34	0.98
<i>E. reflexa</i> 260	13.64	1184	15.62	2.45	9.29	3.46	0.42
<i>E. reflexa</i> 290	16.52	1179	17.98	3.14	10.43	3.82	0.59
<i>E. reflexa</i> 292	18.31	1384	21.53	3.18	12.57	4.99	0.79

Discussion

All populations of the four species examined were diploid with $2n=16$, and $n=8$. These results agree with reports from other authors for 23 species of *Echeandia* (Schnarf and Wunderlich 1939, Palomino and Romo 1987, Cruden 1987, 1993, 1994, Cruden and McVaugh 1989, Palomino and Martínez 1994). Given this, and chromosome numbers for 16 polyploid species of the genus (4x, 5x, 6x, 8x, 10x and 11x-4) (Palomino and Romo 1987, Cruden 1981, 1986, 1993, 1994), we agree with Palomino and Romo (1987) in considering *Echeandia* as a monobasic genus with $X=8$.

Echeandia hintonii, *E. montealbanensis*, *E. pubescens* and *E. reflexa*, had distinctive karyotypes. All three populations of *E. reflexa* analyzed displayed intraspecific cytotype variation.

High frequency of heteromorphic bivalents in MI was observed in the eleven populations of the five species and cytotypes of *Echeandia* studied (Table 4). Some cytotypes had three or four heteromorphic bivalents (*Echeandia echeandioides* Nos. 359 and 360, *E. tenuis* No. 484, and *E. reflexa* No. 292), most of the cytotypes showed two of these (*Echeandia mexicana* Nos. 357 and 284, *E. reflexa* Nos. 260 and 290, and *E. tenuis* No. 356), and cytotypes of *Echeandia echeandioides* Nos. 359 and 360 presented heteromorphic quadrivalents (Table 4).

Heteromorphic bivalents and quadrivalents reflect heterozygotic exchanges, as demonstrated in several species of the tribe Aloineae by Brandham (1973, 1974, 1976), and by Brandham and Johnson (1977). Kenton *et al.* (1987) and Kenton and Drakeford (1990) have reported heterozygotic exchanges in *Gibasis pulchella*, and in *Tradescantia cymbispatha* (Comelinaceae), respectively.

Heteromorphic IIs in mitosis were absent from *E. mexicana* Nos. 284 and 357, *E. reflexa* Nos. 260 and 290, and *E. pubescens* (Table 4), which may be due to one of the following causes: 1.—translocation was of a very small size, or 2.—translocation was equal for both chromo-

Table 4. Heteromorphic chromosome pairs, and heteromorphic bivalents (IIs), and quadrivalents (IVs) of seven species of *Echeandia*

Species and cytotypes	Heteromorphic chromosome pairs	Heteromorphic	
		IIs	IVs
* <i>E. echeandioides</i> 359	3	3	2
* <i>E. echeandioides</i> 360	1	3	1
* <i>E. echeandioides</i> 321		1	
<i>E. hintonii</i> 308			
* <i>E. mexicana</i> 236			
* <i>E. mexicana</i> 284		2	
* <i>E. mexicana</i> 357		2	
<i>E. montealbanensis</i> 4015			
<i>E. pubescens</i> 482		3	
<i>E. reflexa</i> 260		2	
<i>E. reflexa</i> 290		2	
<i>E. reflexa</i> 292	1	4	
* <i>E. tenuis</i> 356	1	2	
* <i>E. tenuis</i> 484	2	3	

* Reported by Palomino and Martínez (1994).

somes in which rearrangement took place.

Additional evidence for translocations and chromatid exchange is found in the low level of meiotic irregularities observed during AI, including U-type bridges associated with an acentric fragment, SAB without acentric fragments, and lagging chromosomes. Samples analyzed of *Echeandia hintonii*, *E. montealbanensis*, *E. pubescens* and cytotypes of *E. reflexa* (Table 3), as well as cytotypes of *E. echeandioides*, *E. tenuis* and *E. mexicana* (Palomino and Martínez 1994), showed 1 or 2 SAB in variable proportion, and more frequently than U-type chromatid exchanges, the latter were absent from *E. montealbanensis*. These U-type chromatid exchanges were observed in 186 plants belonging to 167 taxa in the tribe Aloineae, in proportions ranging from 1 to 20%. In 22% of these plants, U-type exchange occurred alone, and chromatid and subchromatid aberrations (SAB) occurred together in 56% of them. No plants produced subchromatid errors alone (Brandham 1970).

Heteromorphic bivalents and bridges with or without fragment reflect structural changes such as heterozygous inversions, Robertsonian translocations, exchanges, deletions and duplications (Brandham 1970, Brandham and Johnson 1977, Kenton *et al.* 1987, Kenton and Drakeford 1990, Palomino and Vázquez 1991, Palomino and Martínez 1994).

Total chromatin length (TCL), or genome size, of 14 populations of the seven species of *Echeandia* analyzed both in this paper and in Palomino and Martínez (1994), showed significant inter and intraspecific variation (at $P < 0.01$ level) (Table 5). This fact corroborates the distinctiveness of the cytotypes of *E. echeandioides*, *E. tenuis* and *E. reflexa*. Intraspecific variation was evident in *E. echeandioides* cytotype No. 359, having a TCL of $53.24 \mu\text{m}$, which is equivalent to more than twice the genome size of cytotype No. 321 (TCL = $22.98 \mu\text{m}$). A similar case was observed in cytotype No. 292 of *E. reflexa* (TCL = $48.66 \mu\text{m}$), having a genome size of more than twice that of cytotype No. 290 (TCL = $22.15 \mu\text{m}$) (Table 5).

In some cases, interspecific variation was demonstrated, such as in *E. pubescens* with TCL of $48.70 \mu\text{m}$, which is more than twice that observed in *E. hintonii* (TCL = $22.90 \mu\text{m}$) (Table 5).

Despite that a better estimate of the genome size of species (Price 1988) and of cytotypes (Price *et al.* 1981a, b, Kenton 1983) is obtained by the amount of DNA in picograms (which is done through scanning spectroscopic measurement of the light absorbency of Feulgen stained nuclei), the variation in the TCL of some cytotypes of *Echeandia echeandioides*, *E. tenuis* and

Table 5. Results of Tukey's multiple range test on mean total haploid chromatin length (TCL) of seven species of *Echeandia*

Species and populations	TCL (μm) $\bar{x} \pm \text{EE}$	Tukey's grouping
* <i>E. echeandioides</i> 359	53.24 0.30	a
<i>E. pubescens</i> 482	48.70 0.43	b
<i>E. reflexa</i> 292	48.66 0.30	b
* <i>E. echeandioides</i> 360	41.70 0.01	c
* <i>E. tenuis</i> 484	37.56 0.06	d
* <i>E. mexicana</i> 236	34.73 0.02	e
* <i>E. mexicana</i> 284	33.93 0.08	e
* <i>E. mexicana</i> 357	30.97 0.04	f
* <i>E. tenuis</i> 356	28.78 0.28	g
<i>E. montealbanensis</i> 4015	28.68 0.04	g
<i>E. reflexa</i> 260	27.37 0.26	g
<i>E. hintonii</i> 308	22.99 0.30	h
* <i>E. echeandioides</i> 321	22.98 0.04	h
<i>E. reflexa</i> 290	22.15 0.04	h

Lines indicate species that were not significantly different using $\alpha=0.01$. *Reported by Palomino and Martinez (1994).

E. reflexa is evident (Table 5).

The estimation of DNA content often provides a distinctive parameter of species differentiation (Bennett and Smith 1976, 1991), any many examples are reported where differences within species are comparable, or even greater, than differences between species. Intraspecific variation in DNA content of 25% and 20% has been reported in *Microseris bigelovii* (Price *et al.* 1981a) and in *M. douglasii* (Price *et al.* 1981b). Cavallini *et al.* (1986), found 58.16% variation of DNA content among cultivars of *Helianthus annuus*. Variation in DNA content between plants of the same species and the same ploidy level are due to the generation, dispersion and deletion of repeated sequences in there genome (Price 1991). This variation has an effect on several cell attributes, such as the chromosome size, the nuclear and cell volumes, and the length of the mitotic and meiotic cycles, the nucleotypic effect (Bennett 1972, Price 1988). The study of inter and intraspecific DNA content variation patterns, suggest that these variations have an adaptive value, in response to environmental changes, and that they are a subject of natural selection (Price 1991).

Kenton (1983), found 60% variation in DNA content of 13 diploid cytotypes ($2n=12$) of *Gibasis venustula* subsp. *venustula*. She also noticed that these changes in DNA content have an adaptive nature, given that DNA content increase was reflected on the different ecological conditions in which the cytotypes live, as well as on their phenotypes (flower color and size, and leaf dimensions). These cytotypes had heteromorphic IIs (during MI), and bridges and fragments (during AI), both of which resulted in heterocytotic inversions. Those cytotypes showing greater values of TCL (genome size), *Echeandia echeandioides* Nos. 359 and 360, *E. tenuis* No. 484, *E. reflexa* No. 292, y *E. pubescens* 482, were also those displaying larger numbers of structural modifications in their chromosomes (Palomino and Martinez 1994, results in this paper. See Tables 3, 4, 5 and 6). Morphologic variation, such as that observed in *Gibasis venustula* subsp. *venustula* cytotypes (Kenton 1983), was not seen in *Echeandia* cytotypes.

In cytotypes of *E. reflexa* Nos. 260, 290 and 292, and of *E. pubescens* abnormal segregation of a lagging chromosome was observed. These same observation was made by Palomino and Martinez (1994) in two cytotypes of *E. echeandioides* and of *E. tenuis*. Lagging chromosomes are rarely observed in nature and have been recorded in the meiotic anaphase of pollen grains

Table 6. Shrunken or empty pollen grains and irregular A1 of seven species of *Echeandia*

Species and populations	No. of pollen grains	Shrunken or empty pollen grains %	No. of PMCs A1	Irregular A1 %
* <i>E. echeandioides</i> 359	644	29.85	998	21.44
* <i>E. echeandioides</i> 360	589	20.37	913	17.63
* <i>E. echeandioides</i> 321	238	2.94	989	6.57
<i>E. hintonii</i> 308	824	5.70	1099	5.83
* <i>E. mexicana</i> 236	633	13.74	996	3.31
* <i>E. mexicana</i> 284	877	2.28	678	18.10
* <i>E. mexicana</i> 357	502	14.14	969	14.14
<i>E. montealbanensis</i> 4015	692	3.61	1244	4.66
<i>E. pubescens</i> 482	865	22.54	1422	22.36
<i>E. reflexa</i> 260	726	13.64	1184	15.62
<i>E. reflexa</i> 290	817	16.52	1179	17.98
<i>E. reflexa</i> 292	874	18.31	1384	21.53
* <i>E. tenuis</i> 356	361	13.85	923	11.71
* <i>E. tenuis</i> 484	538	23.23	994	23.14

* Reported by Palomino and Martínez (1994).

from diploid clones coming from several populations of *Tradescantia commelinoides* (Kenton *et al.* 1988). Johnson (1980), reported lagging chromosomes, bridges and fragments during AI and AII in polyploid species of *Mammillaria prolifera*, these aberrations resulted in abnormal segregation and, thus, in pollen grains of reduced size and in an increase in the proportion of unviable pollen.

Those 14 populations from 7 species of *Echeandia* showing larger numbers of both chromatid exchanges and heterocytotic inversions, also had larger proportion of unviable pollen ($r=0.80$) (Table 6). These unviable pollen grains were derived from chromatid exchanges, inversions, and lagging chromosomes. In *Gibasis pulchella*, diploid plants being heterocytotic for a chromatid exchange also had an increased proportion of unviable pollen (Kenton *et al.* 1987). The presence of the same cytotype in different populations of *Echeandia* species suggests, these were originated from a single plant by vegetative reproduction, which is common among the species of the genus. It can be assumed that several aberrant cytotypes confer advantages to those individuals carrying them in the colonization of new habitats.

In sum, chromosomal rearrangements may have played an important role in the evolution of *Echeandia*. The occurrence of these chromosomal aberrations has a common behavior pattern in species and cytotypes of *Echeandia*. Observation of bivalents and of low frequency of quadrivalents and bridges, both with and without fragments, as well as the presence of heteromorphic chromosome pairs, suggests that translocations and chromatid exchange have played a major role in shaping the karyotypes of populations and species.

Summary

Karyotypic analysis of *Echeandia hintonii*, *E. montealbanensis*, *E. pubescens* and three populations of *E. reflexa*, showed that all of them were diploid with $2n=16$, $n=8$ and $X=8$. Each species had a distinctive karyotype. Also, each one of the populations of *Echeandia reflexa* analyzed presented a distinct karyotype. Cytotype variation was observed in heteromorphic chromosome pairs. Meiotic analysis showed heteromorphic exchanges. Analysis of MI showed heteromorphic IIs, while AI analysis showed U-type chromatid exchanges and sub-chromatid aberrations. Lagging chromosomes were recorded in AI of *E. pubescens* and in cytotypes of *E. reflexa*. Those cytotypes with a larger proportion of chromosomal aberrations,

were also those with greater proportion of unviable pollen and a larger value of TCL (genome size). Based on these results and on previous reports, we can suggest that translocations and chromatid exchange, follow a behavior pattern common to species and cytotypes of *Echeandia*, and that these chromosome aberrations have played a major role in the evolution of the genus, providing with a larger potential for colonization and distribution in new habitats.

Appendix 1

E. hintonii. Guerrero state, 4 Km. from the deviation to Agua de Obispo, *Palomino and Martínez* 308 (MEXU).

E. montealbanensis. Oaxaca state, Monte Albán, *Abisai García* 4015 (MEXU).

E. pubescens. México state, 4 Km. from Temascaltepec towards Valle de Bravo, *Javier Martínez* 482 (MEXU).

E. reflexa. 1. Hidalgo state, 8 Km. from Jacala towards Zimapán, *Palomino and Martínez* 260 (MEXU). 2. Hidalgo state, 3 Km. from Molango towards Zacualtipán, *Palomino and Martínez* 290 (MEXU). 3. México state, 13.5 Km. Molango to Zacualtipán, *Palomino and Martínez* 292 (MEXU).

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