Floriculture and Ornamental Biotechnology ©2012 Global Science Books

Mexican Geophytes III. Cytotypes and Meiotic Behavior in Mexican Populations of Species of *Echeandia* (Anthericaceae)

Guadalupe Palomino[1*] • Javier Martínez[1] • Rodrigo Barba-González[2] • Ignacio Méndez[3] • Benjamín Rodríguez-Garay[2]

[1] Instituto de Biología, Jardín Botánico, Universidad Nacional Autónoma de México, 04510 México D.F., Mexico
[2] Unidad de Biotecnología Vegetal, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., (CIATEJ), Av. Normalistas No. 800, 44270 Guadalajara, Jalisco, Mexico
[3] Instituto de Matemáticas Aplicadas y Sistemas, Universidad Nacional Autónoma de México, 01000 México D.F. Mexico

Corresponding author: * palomino@ibunam2.ibiologia.unam.mx

ABSTRACT

Echeandia Ortega includes about 85 perennial herbaceous species. The subgenus *Echeandia* is distributed from USA, to Argentina and Chile. Mexico is considered to be the genus center of origin and diversity. *Echeandia* is considered as a monobasic genus with x = 8. Diploid plants (2n = 16, n = 8, x = 8) have been reported for 35 species of *Echeandia*. Chromosome numbers for 22 polyploid species for the genus have been the reported (4x, 5x, 6x, 8x, 10x and 11x-4). These reports detail karyotype, meiotic chromosome behavior and, pollen fertility of 23 populations of eight species: *Echeandia echeandioides*, *E. hintonii*, *E. mexicana*, *E. montalbanensis*, *E. nana*, *E. pubescens*, *E. reflexa* and *E. tenuis.* All species of *Echeandia* were diploid (2n = 16, n = 8, x = 8). Each species had a distinctive karyotype that varied among populations of the same species. Spontaneous heterozygotic exchanges in species and cytotypes of *Echeandia* have a common behavior pattern in karyotype variation. The exchanges were observed in heteromorphic pairs of chromosomes with satellites, and, in metacentric, submetacentric and subtelocentric chromosomes. The origin of these rearrangements was evident in heteromorphic bivalents (IIs) and quadrivalents (IVs) observed in MI. Additional evidence for translocations and chromatid exchange comes from the low level of meiotic irregularities observed in anaphase I (AI), including U-type bridges, side arm bridges and lagging chromosomes. Populations of *E. nana*, display only two cytotypes. Based on these results, the translocations and chromatid exchange follow a behavior pattern common to species and cytotypes of *Echeandia*, and these chromosome aberrations have played a major role in evolution of the genus, providing a larger potential of colonization and distribution in new habitats.

Keywords: *Echeandia* karyotypes, cytotypes behavior of meiotic chromosomes, pollen fertility, heterozygotic exchanges, flow cytometry, nuclear DNA content

INTRODUCTION

Echeandia is a monocotyledonous genus of the Anthericaceae. It comprises about 85 species of which at least 60 species have been described from Mexico, and Central America, many of them are narrow endemics (**Table 1**) (World Checklist of Selected Family species 2011). It is a poorly known genus, though ornamental. The flowers of the different species are mainly in different shades of yellow and a few of them have white flowers. Despite the lack of color diversity there are different forms that are valuable as ornamentals (some of them are shown in **Fig. 1**), also, important is the wide distribution and the different habitats where they can be found. Some species are utilized as ornamentals, such as *E. luteola* Cruden (Arellano-Rodriguez *et al.* 2003); *E. flavescens* (Schult & Schult.) Cruden, *E. nana* (Baker) Cruden (Pérez-Escandón *et al.* 2003); *E. chandleri* (Greenm. & C.H. Thomps.) Cruden and *E. texensis* Cruden (Richardson and King 2010). Even though, up to 19 *Echeandia* species, distributed along the western Mexico have been proposed by Rodíguez-Contreras and Ortiz-Catedral (2003) as ornamentals owing to their ornamental traits. The genus has several polyploid species, in which different cytotypes are found in some cases.

Echeandia Ortega, includes about 85 perennial herbaceous species which are grouped in two subgenera: *Echeandia* and *Mscavea* (Cruden 1999, 2009). According to Cruden (1999) the two subgenera differ in time of flower opening, tepal shape, width and color, capsule shape, and altitudinal distribution. The two subgenera have endemic species distributed from Mexico to South America (Cruden 2009).

The subgenus *Echeandia* comprises nearly 59 species, 26 of them are endemic to Mexico (Cruden 1986, 1987, 1993, 1999). Most of the species (44) are yellow-flowered, and six include white-.flowered populations). The subgenus *Mscavea* comprises 26 species. Most of the species have white flowers (22/25), two have cream-colored flowers, one has orange flowers and one species includes orange or yellow-flowered populations. Most species in this subgenus occur in dry habitats with subtropical to warm-temperate climates, whereas most species of subgenus *Echeandia* occur in relatively mesic habitats with warm to cold temperate climates (Cruden 1999). They are commonly found in pine and pine/oak forests, grasslands, xerophytic shrublands, juniper forests, tropical deciduous vegetation and disturbed areas. These species are distributed between 0-100 masl such as *E. campechiana* Cruden (Cruden 1994), and 3600 masl such as *E. longipedicellata* Cruden (Cruden 1981, 1986, 1987; Cruden and McVaugh 1989; Cruden 1999, 2009).

Echeandia species are distributed from the USA, in the states of Arizona, New Mexico, Texas to Mexico, Central America, Venezuela, Colombia, Ecuador, Peru, Argentina and Chile (Cruden 1989; Cruden and McVaugh 1989; Cruden 2009).

More than 60 species have been described from México, and Central America, many of which are narrow endemics (Cruden 1986, 1987; Cruden and McVaugh 1989; Cruden 1993, 1994, 1999). México is considered to be the center of origin and diversity of this genus (Cruden RW, pers.

Received: 26 January, 2011. Accepted: 20 December, 2012.

Original Research Paper

Table 1 Mexican *Echeandia* species and their distribution to the World Check List of selected families (Cruden and Mc Vaugh, 1989. Cruden, 1981, 1986, 1987, 1989, 1993, 1994, 1999).

No	Species	Distribution	No	Species	Distribution
1	*E. albiflora* (Schlechtendal & Chamisso) Martins & Galeotti	México State, Veracruz	44	*E. mexicana* Cruden	México State, Chih, Mich.
2	*E. altipratensis* Cruden	Guatemala, Huehuetenango	45	*E. michoacensis* (Poelln.) Cruden	México State, Michoacán
3	*E. atoyacana* Cruden	México State, Guerrero (Gro)	46	*E. mirandae* Cruden	México State, Oax, Puebla
4	*E. attenuata* Cruden	México State, Sinaloa, Dgo	47	*E. molinae* Cruden	Guatemala
5	*E. bolivarensis* Cruden	Venezuela	48	*E. montealbanensis* Cruden	México State, Oaxaca
6	*E. breedlovei* Cruden	México State, Oaxaca, Chiapas	49	*E. nana* (Baker) Cruden	México State, Hidalgo
7	*E. campechiana* Cruden	México State, Campeche	50	*E. nayaritensis* Cruden	México State, Sinaloa, Nayarit
8	*E. chandleri* (Greenman & Thompson) Cruden	SE Texas to NE Mexico	51	*E. oaxacana* Cruden	México State, Oaxaca
9	*E. ciliata* (Kunth) Cruden	Peru, Guatemala, Venezuela	52	*E. occidentalis* Cruden	México State, Nayarit, Michoacán
10	*E. chiapensis* Cruden	México State, Oaxaca, Chiapas	53	*E. palmeri* Cruden	North Mexico
11	*E. coalcomanensis* Cruden	México State, Michoácan	54	*E. paniculata* Rose	México State, Jalisco, Michoacán, Morelos
12	*E. confertiflora* Cruden	México State, Oaxaca	55	*E. parra* Cruden	México State, Oaxaca, Puebla
14	*E. conzattii* Cruden	México State, Gro, Oaxaca.	56	*E. parva* Cruden	México State, Oaxaca
15	*E. denticulata* (Cruden)	Venezuela, Peru, Colombia	57	*E. parvicapsulata* Cruden	México State, Nayarit, Jal
16	*E. drepanoides* (Greenman) Cruden	México State, Oaxaca	58	*E. parviflora* Baker	Central Mexico to Guatemala
17	*E. durangensis* (Greenman) Cruden	México State, Dgo, Chih. Sin.	59	*E. petenensis* Cruden	Guatemala. Mexico, Yucatán
18	*E. echeandioides* (Schltdl.) Cruden	Mexico	60	*E. pihuamensis* Cruden	México State, Jalisco
19	*E. elegans* Cruden	México State, Morelos, Gro.	61	*E. pittieri* Cruden	Panamá, Colombia, Venezuela
20	*E. falcata* Cruden	México State, Guanajuato, Querétaro	62	*E. platyphylla* (Greenman) Cruden	México State, Puebla
21	*E. formosa* (Weatherby) Cruden= *E. macrocarpa*	SE Mexico to Central America	63	*E. pseudopetiolata* Cruden	México State, Guerrero
22	*E. flavescens* (Schult. & Schult.f.)= *E. leptophylla* Cruden	Arizona to SW Texas and Mexico	64	*E. pseudoreflexa* Cruden	México State, Chiapas
23	*E. flexuosa* Greenman	México State, Zacatecas	65	*E. pubescens* Cruden	Mexico
24	*E. gentryi* Cruden	México State, Sin, Dgo, Nay.	66	*E. ramosissima* (C.Presl) Cruden= *E. breviflolia* (Watson)= *E. haenkeana* (Kunth)= *E. nodosa* (Watson) Cruden	N & W Mexico
25	*E. gracilis* Cruden	México State, Mor, Ver.	67	*E. reflexa* (Cav.) Rose S.= *E. terniflora* (Ort.)	Texas to Honduras
26	*E. gramínea* Martins & Galeotti	México State, Oaxaca Puebla	68	*E. robusta* Cruden	México State, Jalisco
27	*E. grandiflora* Cruden	México State , Oaxaca	69	*E. sanmiguelensis* Cruden	México State, Guanajuato
28	*E. hallbergii* Cruden	México State, Oaxaca	70	*E. scabrella* (Bentham) Cruden	México State, Chihuahua to Michocán
29	*E. hintonii* Cruden	México State, Guerrero	71	*E. sinaloensis* Cruden	México State, Sinaloa to Jalisco
30	*E. hirticaulis* Cruden	México State, Gro, Mich	72	*E. skinneri* (Baker) Cruden	SE. Mexico to Central America
31	*E. herrerae*		73	*E. smithii* Cruden	México State, Oaxaca
32	*E. imbricata* Cruden	México State, Jal. Gro, Mich.	74	*E. tamaulipensis* Cruden	México State, Tamaulipas
33	*E. lehmannii= E. aequatoris* (Baker) Marais & Reilly	Ecuador	75	*E. taxacana* Cruden	Central & SW Mexico
34	*E. leucantha Klotzsch, Allg Gartenzeitun.= E. prolixa*	Central America, Venezuela	76	*E. taxcana*	México State, Guerrero
35	*E. longifolia* (Weatherby)= *E. macrophylla* Cruden	México State, Veracruz, Oaxaca	77	*E. tenuifolia* Cruden	México State, Oaxaca
36	*E. longipedicellata* Cruden	Mexico to Guatemala	78	*E. tenuis* (Weatherby) Cruden	México State, Guerrero
37	*E. luteola* Cruden	SE Mexico to Belize	79	*E. texensis* Cruden	USA, Texas
38	*E. llanicola* Cruden	México State, Oaxaca	80	*E. udipratensis* Cruden	México State, Jalisco
39	*E. macrophylla* Rose ex Weath	México State, S. L. Potosí	81	*E. vaginata* Cruden	México State, Oaxaca
40	*E. macvaughii* Cruden	México State, Nay, Jalisco	82	*E. venusta* Woodson	Panama
41	*E. magnifica* López. Espejo & Ceja	México State, Guerrero (Gro.)	83	*E. vestita* (Baker) Cruden	Mexico to Guatemala
42	*E. matudae* Cruden	SE Mexico to Central America	84	*E. weberbaueri* (Poelln) Cruden	Peru
43	*E. mexiae* Cruden	México State, Morelos, Gro.	85	*E. williamsii* Cruden	Honduras, Lempira

comm.).

Diploid plants (2n =16, n = 8, x = 8) have been reported for 35 species of *Echeandia* (Schnarf and Wunderlich 1939; Cruden 1981, 1986, 1987; Palomino and Romo 1987; Martínez 1988; Romero 1988; Cruden 1993; Palomino and Martínez 1994; Martínez and Palomino 1996; Cruden 1999; Martínez *et al.* 2000). Considering the reported chromosome numbers for 22 polyploid species for the genus (4x, 5x, 6x, 8x, 10x and 11x-4, Cruden 1986, 1987, 1993, 1994, 1999, **Table 2**) we agree with Palomino and Romo (1988) in considering *Echeandia* as a monobasic genus with x = 8.

Objectives

This study describes the karyotype, analyses of the meiotic chromosome behavior and pollen fertility of 23 populations of eight species: *Echeandia echeandioides* (Schltdl.) Cruden, *E. hintonii* Cruden, *E. mexicana* Cruden, *E. montealbanensis* Cruden, *E. nana* (Baker) Cruden, *E. pubescens* Cruden, *E. reflexa* Rose and, *E. tenuis* (Weath.) Cruden.

In *E. nana*, we found two cytotypes in nine populations analysed; we also report the number of fruits, seeds per fruit, and the total number of seeds after cross-pollination among and between these two cytotypes of *E. nana.* We studied the variation in DNA content in populations of *E. echeandioides*, *E. mexicana*, *E. nana* and *E. reflexa*. The results ob-

Fig. 1 Mexican species of the genus *Echeandia* with traits of ornamental importance. (**A**) *E. udipratensis*; (**B**) *E. albiflora*; (**C**) *E. attenuata*; (**D**) *E. breedlovii*; (**E**) *E. coalcomanensis*; (**F**) *E. durangensis*; (**G**) *E. echeandioides*; (**H**) *E. falcata*; (**I**) *E. flavescens*; (**J**) *E. flexuosa*; (**K**) *E. formosa*; (**L**) *E. gentry*; (**M**) *E. gracilis*; (**N**) *E. vestita*; (**O**) *E. hirticaulis*; (**P**) *E. imbricate*; (**Q**) *E. longipedicellata*; (**R**) *E. luteola*; (**S**) *E. matudae*; (**T**) E. *maxiae*; (**U**) *E. mexicana*; (**V**) *E. michoacensis*; (**W**) *E. montealbanensis*; (**X**) *E. nana*; (**Y**) *E. oaxacana*; (**Z**) *E. occidentalis*; (**A1**) *E. parva*; (**B1**) *E. parviflora*; (**C1**) *E. pringlei*; (**D1**) *E. ramossisima*; (**E1**) *E. reflexa*; (**F1**) *E. robusta*; (**G1**) *E. sanmiguelensis*; (**H1**) *E. sinaloensis*; (**I1**) *E. smithii*.

tained in this research provide an insight into the complex cytogenetics of the genera that must be taken into account in breeding programmes.

MATERIALS AND METHODS

Plant material

The species studied were: *Echeandia echeandioides*, an endemic species to the central region of Mexico (**Fig. 2A**), *E. hintonii* distributed in the State of Guerrero, and *E. mexicana*, distributed in Distrito Federal and the States of México, Michoacán, Morelos, Puebla, Jalisco and Chihuahua (**Fig. 2B**). These species are widely distributed in the Sierra Madre Occidental and the Mexican trans volcanic belt generally between 1900 and 2500 masl (Cruden 1999). *E. montealbanensis*is, considered endemic to the Monte Alban area in the State of Oaxaca (Cruden 1993). *E. nana* is located in the eastern region of Mexico, in the States of Tamaulipas, San Luis Potosi, Veracruz, Mexico, Hidalgo, Tabasco and Campeche (**Fig. 2D**). *E. pubescens* is distributed in the State of México, *E. reflexa* Cruden is located in the mountains from Texas (in the USA) to Chiapas (in Mexico) (**Fig. 2C**), and *E. tenuis* is distributed in the States of Guerrero, Mexico and Morelos, in Mexico (Cruden 1999). In all cases, plants were collected from wild populations in pine-oak forests (**Appendix 1**) and voucher specimens were deposited at the National Herbarium (MEXU), of the Universidad Nacional Autónoma de México (UNAM). For each species, six to 30 individual plants were collected from one or up to 23 populations from eight species of *Echeandia* (**Appendix 1**). Plants were taken to the Botanical Garden of the Univer-

Fig. 2 (**A**) *Echeandia echeandioides* 321 with: (**1**) Yellow flowers; (**2**) Free anthers; (**3**) Glabrous scape; (**B**) *E. mexicana* 357 with: (**1**) Yellow flowers; (**4**) Connate anthers; (**3**) Glabrous scape; (**C**) *E. reflexa* 290 with: (**1**) Yellow flowers; (**4**) Connate anthers; (**3**) Glabrous scape; (**D**) *E. nana* 009 with: (**5**) White flowers; (**2**) Free anthers; (**3**) Glabrous scape.

Table 2 Chromosome numbers in *Echeandia* (Anthericaceae).

No.	Species	Ploidy level							References
		n	2n	4n	5x	6x	8x	10x	
1	*E. atoyacana*	16							Cruden 1999
2	*E. altipratensis*	24							Cruden 1986
						48			Cruden 1994
3	*E. attenuata*	8							Cruden 1999
4	*E. campechiana*	24							Cruden 1986
						48			Cruden 1994
5	*E. chiapensis*	8							Cruden 1986
			16						Cruden 1994
6	*E. echeandioides*	8	16						Palomino and Martínez 1994; Martínez 1995
7	*E. elegans*	8							Cruden 1999
8	*E. falcata*	16							Cruden 1999
9	*E. formosa*							80	Cruden 1994
10	*E. gracilis*	8							Cruden 1981
11	*E. graminea*	8							Cruden 1999
12	*E. grandiflora*	8							Cruden 1993
13	*E. hallbergii*	16							Cruden 1993, 1999
		32							Cruden 1993, 1999
		8							Cruden 1999
14	*E. hintonii*	8	16						Martínez 1995; Martínez and Palomino 1996
15	*E. hirticaulis*	8							Cruden 1999
16	*E. leptophylla*		16						Palomino and Romo 1987
			16	32		48			Romero 1988
17	*E. longipedicellata*		16						Schnarf and Wunderlich 1939
		40							Cruden 1981
18	*E. luteola*				40				Cruden 1994
		32							Cruden 1986
							64		Cruden 1994
19	*E. llanicola*	16							Cruden 1993
20	*E. magnifica*	8							López-Ferrari *et al*. 2002
21	*E. matudae*	8							Cruden 1981
		16							Cruden 1986
				32					Cruden 1994
22	*E. mexiae*	8							Cruden 1999
23	*E. mexicana*	8							Cruden 1981
			16						Palomino and Romo 1987
		8	16						Palomino and Martínez 1994; Martínez 1995
24	*E. mcvaughii*	8							Cruden 1987
25	*E. mirandae*	8							Cruden 1993
26	*E. montealbanensis*	8							Cruden 1993
		8	16						Martínez 1995; Martínez and Palomino 1996
27	*E. nana*		16						Palomino and Romo 1988
		8	16						Martínez 1988; Martínez *et al*. 2000
28	*E. oaxacana*	8							Cruden 1993
29	*E. occidentalis*	8							Cruden 1987
30	*E. parva*	8							Cruden 1993
31	E. *parvicapsulata*	8							Cruden 1987
32	*E. parviflora*		16						Cruden 1994
33	*E. pihuamensis*	8							Cruden 1987
34	*E. pseudopetiolata*	8							Cruden 1999
35	*E. pseudoreflexa*	16							Cruden 1999
36	*E. pubescens*	8	16						Martínez 1995; Martínez and Palomino 1996
37	*E. reflexa*				32				Cruden 1994
		8	16						Martínez 1995; Martínez and Palomino 1996
38	*E. robusta*	8							Cruden 1987
39	*E. skinneri*						48		Cruden 1994
40	*E. smithii*	8							Cruden 1993
41	*E. taxacana*	8							Cruden 1999
42	*E. tenuifolia*	8							Cruden 1993
			16						Schnarf and Wunderlich 1939
43	*E. tenuis*	8	16						Palomino and Martínez 1994; Martínez 1995
44	*E. udipratensis*	40							Cruden 1987
45	*E. vaginata*	8							Cruden 1993
46	*E. venusta*							84	Cruden 1994
47	*E. vestita*						48		Cruden 1994

sidad Nacional Autónoma de México, where they were transplanted into pots containing a mixture of vermiculite and organic soil, and maintained under greenhouse conditions.

Mitotic chromosome analysis

Preparations were made from six to 30 individual plants from each of the 23 populations of eight species of *Echeandia* that were studied. Nine to ten cells from each population at mitotic metaphase were selected for examination. The cytological methods for the

analysis of mitotic chromosomes are detailed in Palomino and Martínez (1994) and Martínez *et al*. (2000). Elongating secondary root tips were placed in a saturated solution of paradichlorobenzene for 6 h at 4°C. They were stained following the Feulgen technique. For all populations, nine to ten of the best cells were photographed using a Zeiss Photomicroscope II. Idiograms were made using a Zeiss Drawing Apparatus. Chromosomes were classified according to Levan *et al*. (1964) terminology for centromere position.

Meiotic chromosome analysis

Meiotic behavior was studied in fresh anthers from young buds squashed in 1.8% aceto-orcein without prior fixation. A total of 107 to 998 MI (metaphase I) of pollen mother cells (PMC), and, of 329 to 1422 AI (anaphase I) PMC derived from 6 to 10 individual plants from each of the 23 populations of eight species of *Echeandia* were analyzed. For each population, the following information was recorded: for MI PMC the type of bivalents (IIs), chiasmata frequency (Fq), and recombination index (RI); and for AI PMC the occurrence of single and double bridges.

Pollen fertility

Estimates were made in samples of pollen stained with cotton blue in lacto-phenol. Percentages of well-filled stained grains were obtained from samples of 238 to 877 pollen grains, derived from four to ten plants from each of the populations and species of *Echeandia* studied.

Hybridization procedure

A total of 308 cross-pollinations were carried out in plants of nine populations of *Echeandia nana*. Of these cross-pollinations, 90 were made using plants with cytotype A (AxA); 112 with plants having cytotype B (BxB); and, 106 among plants with cytotypes A and B (AxB) (**Table 10**). Flowers were emasculated 24 h before the buds opened and then enclosed in gelatin capsules (Owens 1979). Cross pollinations were made the following morning, after which the capsules were replaced and coded with a colored tag. Due to the differences in the maturation process of each inflorescence, the number of flowers which were cross-pollinated varied. One month after making the experimental cross-pollinations records were taken about the numbers of pollinated flowers, number of fruits, seeds per fruit and total of produced seeds.

Seed germination

Random samples of seeds derived from AxA, BxB and AxB cross-pollination were tested for germination, as follows: Out of the 3002 seeds obtained from the AxA crosses, 85 were tested for germination, similarly, out of the 4104 seeds obtained from the BxB crosses, 94 were tested for germination. In the case of the AxB cross-pollinations all the 952 produced seeds were also tested for germination. For germination tests, seeds were scarified and placed in sterilized petri dishes lined with moist filter paper. The Petri dishes were placed in a culture chamber and kept at 25°C. The number of seedlings was recorded after 20 days of cultivation.

Determination of DNA content

Estimates of DNA content were made by using Feulgen micro spectrophotometry. 4C-DNA contents from 11 populations of five species of *Echeandia* were measured using a Zeiss Universal II scanning microscope (565 µm) with a Zeiss Zonax computer at the Department of Soil and Crop Sciences, Texas A&M University, USA. In all collections 4C DNA content was measured in root apex prophase nuclei of five plants obtained from each population. In all cases 60 mid-prophase nuclei were scanned, ten from each of two slides per plant in a sample of five to 10 plants for each population. The pictogram (pg) DNA content was estimated by using barley (*Hordeum vulgare* 'Sultan', 4C = 22.24 pg DNA; Bennett *et al*. 2000) as a standard. The method followed for DNA content determination was that of Price (1988). Root tips were fixed in ice-cold - 3:1 ethanol: glacial acetic acid for 24 h, and then transferred to cold 70% ethanol, and kept refrigerated until required. After fixation, root tips were placed in small four-compartment baskets made of plastic mesh and washed with distilled water for 30 min, hydrolyzed in 5 N HCl at 25°C for 40 min at room temperature, and stained with Schiff's reagent (pH 2.0) for 2 h. After three washes of 10 min each in SO_2 water and one in distilled water, root tips were placed on slides with a drop of enzyme solution (2% cellulysin, 0.5% macerase) for 30 min. The material was washed again with distilled water and squashed on slides in 45% acetic acid. The cover slip was removed after freezing with liquid nitrogen, and the slides were dried overnight at room temperature and mounted in Permount. The slides were kept for not more than one week in the dark before scanning.

Analysis of data

Differences between genomes of *Echeandia* species were analyzed. Inter- and intrapopulational variations of genome length were determined for populations of *Echeandia* species using one-way analysis of variance (ANOVA). Means were compared using the Tukey-Kramer's HSD method. A one-way ANOVA was performed using Box-Cox transformed values for the numbers of fruits, seeds per fruit derived from AxA, BxB and AxB cross-pollinations. Means were compared using the Tukey-Kramer's test. A complete nested unbalanced analysis of variance (ANOVA) was used to evaluate differences in 4C-DNA content within slides, and between plants of the same collection, plants of the same species from different localities, and among species. The Tukey's procedure, which uses the Studentized Range Distribution, was applied to detect differences in DNA content means. All statistical computations were performed using the JMP version 3.2.1 of the SAS software (SAS Institute, NC, USA).

RESULTS AND DISCUSSION

Chromosome number

All 23 populations of eight species of *Echeandia* were diploid with 2n = 16, n = 8 and x=8 (**Table 2, Figs. 3, 4**). Each species had a distinctive karyotype that varied among populations of the same species (**Table 3, Figs. 3, 4**).

Karyotypes and cytotypes of Echeandia

Each of the eight species of *Echeandia* analysed had a different karyotype with different numbers of metacentric, submetacentric and in some of them subtelocentric chromosome pairs. All of them had two pairs of chromosomes with satellite (**Table 3; Figs. 3, 4**). Each population of *Echeandia echeandioides*, *E. mexicana*, *E. nana*, *E. reflexa* and *E. tenuis* had a different cytotype, which was uniform within populations.

Cytotypes of *E. echeandioides* varied in number of metacentric (m) and submetacentric (sm) chromosomes. Population 321 had the least variable cytotype. All the chromosomes were homomorphic and had one pair of subtelocentric chromosomes (st) (**Table 3; Figs. 3A, 4A**), which were absent in the other populations. Population 359 had 3 pairs of heteromorphic chromosomes (**Figs. 3B, 4B**) whereas population 360 had a single heteromorphic pair of chromosomes (**Figs. 3C, 4C**).

Three populations of *E. mexicana* were examined. The population 236, 284 and 357 had different cytotypes and exhibited variation in number of m, sm and st chromosomes. Population 357 had only m and sm chromosomes (**Table 3**). None of the cytotypes of *E. mexicana* had heteromorphic pairs (**Table 7; Figs. 4E-G**).

Each population of *E. reflexa* had a different cytotype in the three populations analyzed. The population 292 had a cytotype with one pair of heteromorphic chromosomes in mitosis (**Table 7; Figs. 3L-N, 4L-N**). The two populations of *E. tenuis* had cytotypes that varied in quantity of m, sm, and st chromosomes (**Table 3**). Population 356 had a pair of heteromorphic chromosomes (**Table 7; Fig. 4O**) and population 484 had 2 pairs (**Table 7; Fig. 4P**). Samples of the

Table 3 Karyotypes and cytotypes of eight species of *Echeandia*.

Species and population	Cytotypes	Secondary constrictions	Range of chromosome length (µm)	Total chromatin length Mean ± SE	Index of asymmetry TF (%) Mean ± SE
E. echeandioides 321	8m+6sm+2st	2m+2sm	1.74-3.92	47.00±0.04	36.68±0.02
E. echeandioides 359	10m+6sm	2m+2sm	3.91-9.56	108.59±0.30	38.19±0.01
E. echeandioides 360	6m+10sm	2m+2sm	2.60-7.83	85.69±0.01	35.92±0.01
E. hintonii 308	10m+6sm	4m	1.73-3.47	22.99±0.30	38.97±0.01
E. mexicana 236	4m+8sm+4st	2m+2sm	3.48-5.66	69.60±0.02	32.47±0.02
E. mexicana 284	4m+10sm+2st	4m	3.02-5.23	66.96±0.08	35.85±0.01
E. mexicana 357	6m+10sm	2m+2sm	3.05-4.78	63.56±0.04	37.87±0.16
E. montealbanensis 4015	8m+6sm+2st	4m	1.74-5.65	57.90±0.04	35.85±0.01
E. nana 009	6m+8sm+2st	2sm	3.04-6.52	35.15±0.52	36.01±0.38
E. nana 009.3	6m+8sm+2st	2sm	2.61-4.35	27.39±0.46	35.74±0.37
E. nana 115	6m+8sm+2st	2sm	2.17-3.48	22.39±0.42	32.95±0.36
E. nana 242	6m+8sm+2st	2sm	2.02-5.92	28.46±0.47	34.12±0.39
E. nana 265	6m+8sm+2st	2sm	3.02-6.08	34.92±0.52	33.21±0.36
E. nana 277	10m+6sm	2m+2sm	2.60-5.65	28.61±0.47	39.43±0.39
E. nana 278	10m+6sm	2m+2sm	3.48-6.09	36.14±0.53	40.29±0.49
E. nana 282	10m+6sm	2m+2sm	3.02-5.46	35.00±0.52	38.27±0.38
E. nana 283	10m+6sm	2m+2sm	3.52-7.02	36.89±0.54	42.12±0.41
E. pubescens 482	6m+8sm+2st	4sm	3.47-8.70	97.34±0.43	35.71±0.01
E. reflexa 260	4m+8sm+4st	2m+2st	2.16-4.78	27.34±0.26	33.32±0.01
E. reflexa 290	8m+8sm	4m	1.74-3.90	22.12±0.04	37.25±0.01
E. reflexa 292	8m+6sm+2st	2m+2st	2.16-4.78	27.34±0.26	34.64±0.01
E. tenuis 356	10m+4sm+2st	4sm	3.04-5.22	58.13±0.28	40.52±0.01
E. tenuis 484	4m+10sm+2st	2m+2sm	2.07-6.95	75.93±0.06	34.07±0.02

Table 4 Results of Tukey's multiple range test on mean total haploid chromatin length (TCL) of seven species of *Echeandia*.

Species and population	Total chromatin length Mean ± SE	Tukey's grouping
E. echeandioides 359	53.24±0.30	a
E. pubescens 482	48.70±0.43	b
E. reflexa 292	48.66±0.30	b
E. echeandioides 360	41.70±0.01	c
E. tenuis 484	37.56±0.06	d
E. mexicana 236	34.73±0.02	e
E. mexicana 284	33.93±0.08	e
E. mexicana 357	30.97±0.04	f
E. tenuis 356	28.78±0.28	g
E. montealbanensis 4015	28.68±0.04	g
E. reflexa 260	27.37±0.26	g
E. hintonii 308	22.99±0.30	h
E. echeandioides 321	22.98±0.04	h
E. reflexa 290	22.15±0.04	h

Table 5 Results of Tukey's multiple range test on mean genome length of nine populations of *Echeandia nana* presenting two different cytotypes.

Populations	Total chromatin length Mean ± SE	Cytotype	Tukey's grouping
E. nana 283	36.89±0.27	A	a
E. nana 278	36.14±0.27	A	ab
E. nana 009	35.15±0.27	B	bc
E. nana 282	35.00±0.27	A	bc
E. nana 265	34.92±0.27	B	c
E. nana 277	28.61±0.27	A	d
E. nana 242	28.46±0.27	B	de
E. nana 009.3	27.39±0.27	B	e
E. nana 115	22.39±0.27	B	f

single population studied of each *E. hintonii* 308, *E. montealbanensis* 4015 and *E. pubescens* 482, had a different karyotype (**Table 3; Figs. 3D, 4D, 3H, 4H, 3K, 4K**).

Heteromorphic IIs in mitosis were absent from *E. mexicana* Nos. 284 and 357, *E. reflexa* Nos. 260 and 290, and *E. pubescens* No. 482 (**Table 7**), which may be due to one of the following causes: a) translocation was of a very small size, or b) translocation was equal for both chromosomes in which rearrangement took place.

High frequency of heteromorphic bivalents in MI of meiosis was observed in some populations of the species and cytotypes of *Echeandia* studied (**Tables 6, 7**). *Echeandia mexicana* populations 284 and 357 had two heteromorphic bivalents, such as *E. pubescens* 482 and cytotypes of *E. reflexa* populations 260 and 290. Some cytotypes had three or four heteromorphic bivalents such as *Echeandia echeandioides* populations 359 and 360, *E. tenuis* 484 and *E. reflexa* 292 (**Table 7; Fig. 5**). Cytotypes of *E. echeandioides* 359 and 360 also had heteromorphic quadrivalents (**Table 7; Figs. 5B, 5C**).

Heteromorphic bivalents and quadrivalents reflect heterozygotic exchange, 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 exchange in *Gibasis pulchella*, and in *Tradescantia cymbispatha* (Commelinaceae), respectively.

Similar intraspecific cytotype variation has been reported in other Liliaceae. Heteromorphic chromosome pairs resulting from asymmetrical exchanges were reported in *Scilla* (Sato 1942; Gimenez-Martin 1959; Haga and Noda 1976; Noda 1961), *Gloriosa superba* (Vijayavalli and Mathew 1990; and in tribe Aloineae (Brandham 1974, 1976). In *Scilla*, the heteromorphic chromosomes pairs were attributed to translocations and deletions. In *Scilla scilloides*, Noda (1961); Araki (1975, 1977, 1985); Araki *et al*. (1976) studied 46 natural populations, and reported diploid, polyploid and aneuploid cytogenetic types. Clusters of plants with the same karyotype were sexually unstable. The only effective mode of propagation was vegetative (Haga and Noda 1976). In *Smilacina* and *Daniella*, cytotype variation is supported by vegetative reproduction (Sen 1975). Polyploid and aneuploid cytotypes are known in many Liliaceae and *Polygonatum* (Tamura 1990).

Additional evidence for translocations and chromatid exchange was found in the low level of meiotic irregularities observed in anaphase I (AI), including U-type bridges associated with an acentric fragment, side arm bridges (SAB) without acentric fragments, and lagging chromosomes in the samples analysed of *Echeandia hintonii*, *E. montealbanensis*, *E. pubescens* and cytotypes of *E. reflexa*, as well as cytotypes of *E. echeandioides*, *E. mexicana* and *E. tenuis* (**Table 8; Figs. 6B-E**) (Palomino and Martínez 1994; Martínez and Palomino 1996). 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% (Brandham 1970).

Similar process of cytological and genical differentia-

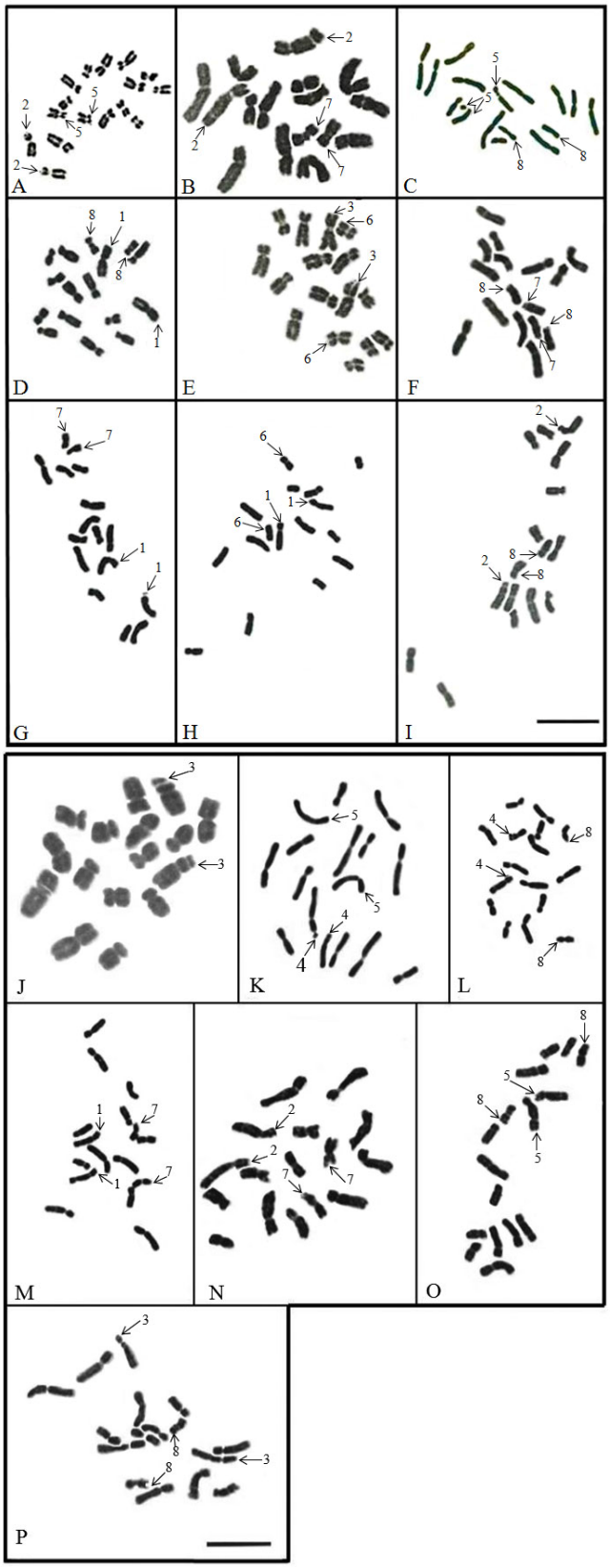

Fig. 3 Cytotypes for different *Echeandia* species. (**A**) *E. echeandioides* 321; (**B**) *E. echeandioides* 359; (**C**) *E. echeandioides* 360; (**D**) Karyotype of *E. hintonii* 308. Cytotypes for: (**E**) *E. mexicana* 236; (**F**) *E. mexicana* 284; (**G**) *E. mexicana* 357; (**H**) Karyotype of *E. montealbanensis* 4015. Cytotypes of: (**I**) *E. nana* 277; (**J**) *E. nana* 009; (**K**) Karyotype of *E. pubescens* 482. Cytotypes for: (**L**) *E. reflexa* 260; (**M**) *E. reflexa* 290; (**N**) *E. reflexa* 292; (**O**) *E. tenuis* 356; (**P**) *E. tenuis* 484. Numbers indicate chromosomes with satellites. Scale = 10 µm.

tion in the genomes of species and cytotypes which have their origin in heteromorphic bivalents and bridges with or without fragment that reflect structural changes such as heterozygous inversions, Robertsonian translocations, exchanges, deletions and duplications have been reported in several species of Liliaceae (Brandham 1970; Brandham and Johnson 1977), *Gibasis* (Kenton 1981, 1983, 1984; Kenton *et al*. 1987), populations of *Crotalaria incana* (Palomino and Vázquez 1991) and in some species of *Echeandia* (Palomino and Martínez 1994; Martínez and Palomino 1996; Martínez *et al*. 2000).

Total chromatin length (TCL)

Total chromatin length (TCL), or genome size, of 23 populations of eight species of *Echeandia* analyzed, showed significant inter- and intraspecific variation ($P < 0.01$) (**Tables 4, 5**). These facts corroborate the distinctiveness of the cytotypes of *E. echeandioides*, *E. mexicana*, *E. nana*, *E. reflexa* and *E. tenuis* (**Tables 3-5**). Intraspecific variation was evident in *E. echeandioides* cytotype No. 359, having a TCL of 53.24 µm, a genome size more than twice that of cytotype No. 321 (TCL = 22.98 µm). A similar case was observed in cytotype No. 292 of *E. reflexa* (TCL = 48.66 µm), having a genome size of more than twice that of cytotype No. 290 (TCL = 22.15 µm).

Pollen viability

The various types of meiotic irregularities observed during meiosis undoubtedly account for the high levels of shrunken, emptied, and/or small pollen grains observed in populations of *Echeandia* species (**Table 9**). The highest percentage of non-viable pollen was found in *Echeandia echeandioides* population 359 with 29.85% (Palomino and Martínez 1994; Martínez and Palomino 1996), and population 265 of *Echeandia nana* with 27.67% (Martínez *et al*. 2000). Likewise, in *Gibasis pulchella*, plant heterozygous for translocations exhibited a 50% reduction in pollen viability (Kenton *et al*. 1987).

Echeandia nana

Nine populations of *E. nana*, were diploids with 2n=16, n=8, x=8. The nine populations of *E. nana* displayed only 2 cytotypes. Cytotype A =10m+6sm, having two pairs of chromosomes with satellite, was observed in four populations from the eastern flank of the Pachuca mountain range. Cytotype B (6m+8sm+2st) having one pair of chromosomes with satellite, was found to be present in three populations from the western flanks of the Pachuca mountain range, and in two populations from the western flanks of the Sierra Nevada mountain range. In all cases, plants were collected from wild populations in pine-oak forests (**Table 3, Figs. 3I, 4I, 3J, 4J**). Intraspecific cytotype variation was apparent as hetromorphic bivalents in MI (**Table 7**), with three different heteromorphic chromosome pairs in each cytotype: chromosomes pairs No. 1, 2 and 5 in cytotype A; and chromosome pairs No. 3, 4 and 7 in cytotype B (**Figs. 4I, 4J**). Heteromorphic bivalents, and/or bridges with or without fragments, reflect structural changes such as heterozygous inversions, Robertsonian translocations, exchanges, deletions and duplications (Brandham 1970; Jones 1978; Kenton 1981; Palomino and Vázquez 1991). Additional evidence for chromatid exchange is found in the low level of meiotic irregularities during meiosis. These included side of arm bridges (SAB) without acentric fragment in AI. These SAB were observed in both cytotypes of *E. nana*; the highest frequency was recorded in cytotype B, as compared as to those in cytotype A (**Table 8**). SAB have long been recognized among the spontaneous meiotic irregularities caused by aberrant reunion at the sites of chiasmata formation. SAB aberrations are characterized by the formation of a bridge at anaphase I which connects two homologous chromatids and carries a pair of side arms approximately at its middle-point. SAB have been observed previously in several species of Liliaceae (Brandham 1970), populations of *Crotalaria incana* (Palomino and Vázquez 1991) and in some species of

Table 6 Type and frequency of bivalents, (IIs), quadrivalents, (IVs), chiasmata frequency and recombination index (RI) for eight species of *Echeandia*.

Species and populations	No. of PMC's	Ring IIs	Rod IIs	Ring IVs	Rod IVs	Chiasmata (%)	RI
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
E. echeandioides 321	168	5.12±0.21	2.88±0.21			13.21±0.08	21.21±0.06
E. echeandioides 359	168	4.69±0.09	1.82±0.11	0.47±0.04	0.23±0.03	13.76±0.47	21.76±0.06
E. echeandioides 360	168	4.61±0.20	3.11±0.22	0.14±0.03		12.89±0.40	20.89±0.05
E. hintonii 308	168	5.66±0.09	2.34±0.09	0.22±0.01		13.66±0.73	21.66±0.07
E. mexicana 236	168	3.02±0.15	4.98±0.15			11.02±0.98	19.02±0.18
E. mexicana 284	168	4.56±0.13	3.44±0.13			12.57±0.58	20.57±0.01
E. mexicana 357	168	4.58±0.14	3.42±0.14			12.58±0.57	20.58±0.09
E. montealbanensis 4015	107	6.48±0.26	1.50±0.12	0.04±0.01		14.50±0.37	22.49±0.04
E. nana 009	946	3.92±0.06	4.08±0.07			11.92±0.11	19.92±0.01
E. nana 009.3	957	3.79±0.06	4.21±0.07			11.79±0.11	19.79±0.01
E. nana 115	989	3.67±0.06	4.33±0.07			11.67±0.11	19.67±0.01
E. nana 242	998	3.65±0.06	4.35±0.07			11.65±0.11	19.65±0.01
E. nana 265	979	3.64±0.06	4.36±0.07			11.07±0.11	19.07±0.01
E. nana 277	219	3.53±0.13	4.47±0.14			13.79±0.07	21.79±0.75
E. nana 278	985	3.77±0.06	4.23±0.07			11.77±0.11	19.77±0.02
E. nana 282	902	3.40±0.06	4.60±0.07			11.40±0.11	19.49±0.19
E. nana 283	978	4.50±0.07	4.51±0.06			12.82±0.11	20.82±0.01
E. pubescens 482	168	5.05±0.15	2.90±0.15			13.06±0.13	21.06±0.01
E. reflexa 260	168	5.15±0.15	2.90±0.15			13.15±0.21	21.15±0.03
E. reflexa 290	168	4.67±0.17	3.33±0.17			12.67±0.27	20.67±0.02
E. reflexa 292	168	5.02±0.10	2.98±0.10			13.02±0.08	21.02±0.04
E. tenuis 356	168	3.32±0.12	4.68±0.12			11.32±0.91	19.32±0.04
E. tenuis 484	168	5.13±0.15	2.88±0.15			13.13±0.91	21.23±0.02

Echeandia (Palomino and Martínez 1994; Martínez and Palomino 1996) and *Gibasis schiedeana* (Martínez and Palomino 1997).

Chromosomal rearrangements may have played an important role in the evolution of *E. nana*. In consequence, we assume that the Pachuca mountain range is a geographical barrier that has isolated populations of *E. nana*, thus producing the observed intraspecific variation of both cytotypes described here. The differentiation of genomes, due to deletions, translocations and heterozygotic exchanges in the different cytotypes of *Echeandia echeandioides*, *E. mexicana*, *E. reflexa* and *E. tenuis* was previously reported by Palomino and Martínez (1994), and by Martínez and Palomino (1996). The occurrence of these chromosomal aberrations has a common behavior pattern in species and cytotypes of *Echeandia*, including *E. nana*. Observations of heteromorphic bivalents, and of low frequencies of bridges, both with fragments and without fragments, suggest that translocations and chromatid exchanges have played the mayor role in shaping the karyotype of populations and species of *Echeandia*. Total chromatin length (TCL) in nine populations of *E. nana* investigated ranged from 22.39 µm to 36.89 µm (**Tables 3, 5**). The smallest genome was represented by population No. 115 (cytotype B) and the largest, by population No. 283 (cytotype A). In general, the largest genomes were presented in populations having cytotype A, and the smallest with cytotype B ($P < 0.0001$) (**Table 5**). These results were corroborated when the DNA content value was compared in plants with cytotype A, that showed the highest genome size compared with plants of *E. nana* having cytotype B (**Table 12**).

Analysis of AI showed that sub-chromatid aberrations were more frequent in cytotype B than in cytotype A (**Table 8**). The intraspecific cytological and genetic differentiation of cytotypes A and B is probably the result of geographical isolation between populations of *E. nana.* The cytological and genetic differentiation between cytotypes A and B was evident in the significant differences of the low number of fruits and viable seeds produced after cross-pollination between cytotypes (AxB: 0-16 fruits; 0-448 abortive seeds), relative to the large values recorded after cross-pollination within cytotypes (AxA: 4-48; 152-1824) (BxB: 3-89; 114-3382, **Table 10**). The smallest percentage of shrunken or empty pollen grains (2.34 to 12.58%) was recorded in populations of cytotype B (**Table 9**); (Martínez *et al*. 2000).

Similar cytological and genetic differences to those obtained in plants that resulted from intraspecific cross-pollinations have been observed in F1 progenies of interspecific hybrids among four species of *Turnera* (Fernandez and Arbo 1996). Espinoza and Quarin (1998) observed low production of fruits and of abortive seeds between two diploid species of *Paspalum* (n=10). Likewise, Wulff (1992) observed chromosomal sterility (presence of heteromorphic bivalents) and low production of fruits in interspecific hybrids of *Hypochoeris*. The occurrence of chromosomal rearrangements having no effect on noticeable phenotypic changes, and in which this chromosomal remodelling is associated with processes of speciation, has been previously reported for several species (Grant 1981).

Genome size (nuclear DNA content) in species and cytotypes of *Echeandia*

Analysis of nuclear DNA content has revealed interspecific variation of genome size in different groups of plants (Bennett *et al*. 2000); intraspecific variation in other species has also been observed in *Microseris bigelovii* (Price *et al.* 1981a), in *M. douglasii* (Price *et al*. 1981b; Ohri 1998); and in *Lonchocarpus* (Palomino and Sousa 2000). A recent review has shown that genome size has been determined in 4427 species of angiosperms (Bennett and Leitch 2005). We found that genome size of *Echeandia echeandioides*, *E. hintonii*, *E. reflexa*, *E. mexicana* and *E. nana* were significantly different ($P < 0.05$, **Table 11**). Also, genome size in diploid cytotypes of *Echeandia echeandioides*, *E. mexicana*, *E. nana* and *E. reflexa* were significantly different ($P < 0.05$, **Table 12**). 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 the DNA content increase was reflected on the different ecological conditions in which the cytotypes live. The cytotypes had heteromorphic IIs and bridges and fragments resulted in heterozygotic inversions.

CONCLUSIONS

Based on these results, we can suggest that translocations and chromatid exchanges, 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 a larger potential of colo-

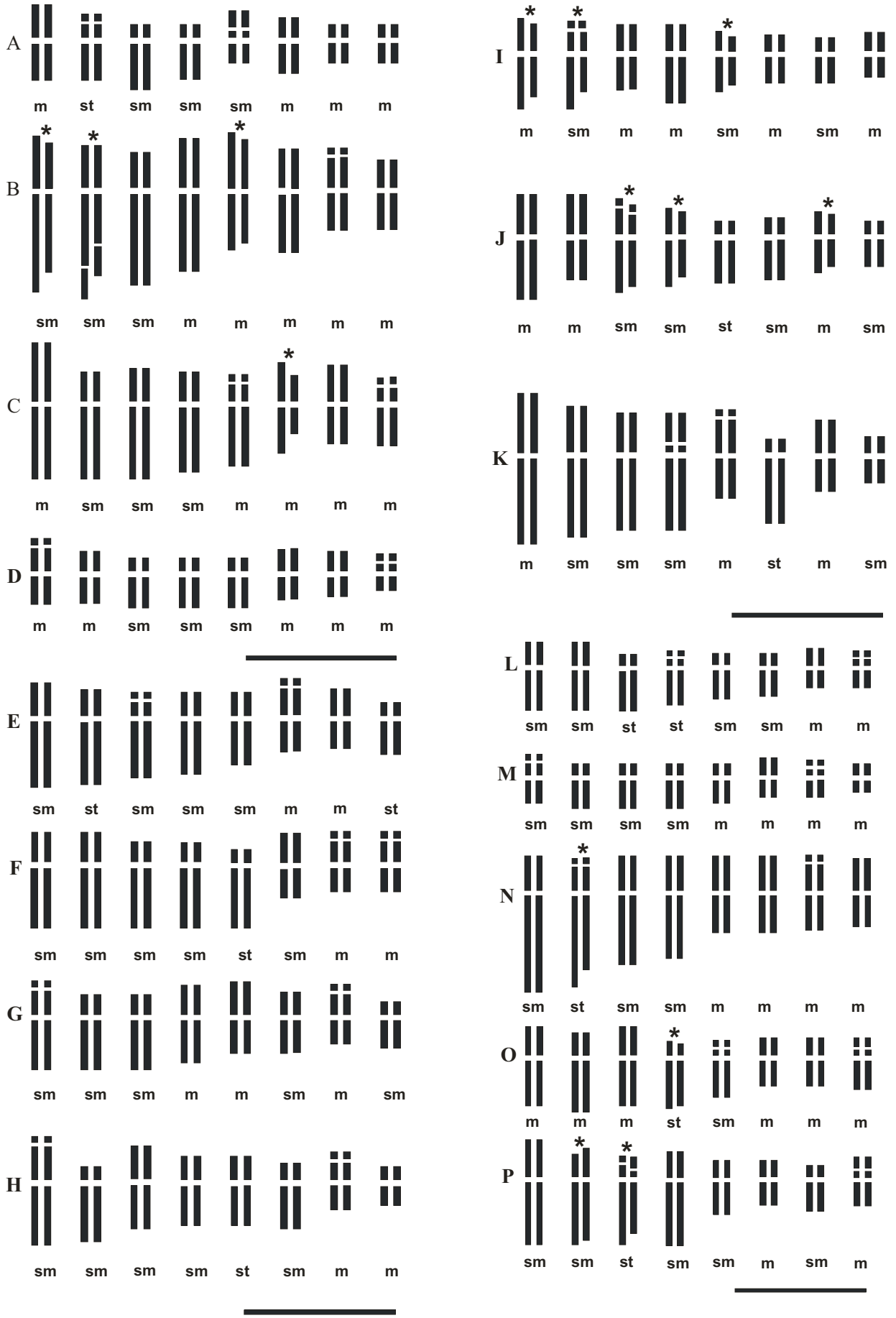

Fig. 4 Idiograms for different *Echeandia* species. (**A**) *E. echeandioides* 321; (**B**) *E. echeandioides* 359; (**C**) *E. echeandioides* 360; (**D**). *E. hintonii* 308; (**E**) *E. mexicana* 236; (**F**) *E. mexicana* 284; (**G**) *E. mexicana* 357; (**H**) *E. montealbanensis* 4015; (**I**) *E. nana* 277; (**J**) *E. Nana* 009; (**K**). *E. pubescens* 482; (**L**) *E. reflexa* 260; (**M**) *E. reflexa* 290; (**N**) *E. reflexa* 292; (**O**) *E. tenuis* 356; (**P**) *E. tenuis* 484. Asterisks indicate pairs of heteromorphic chromosomes. Scale = 10 µm.

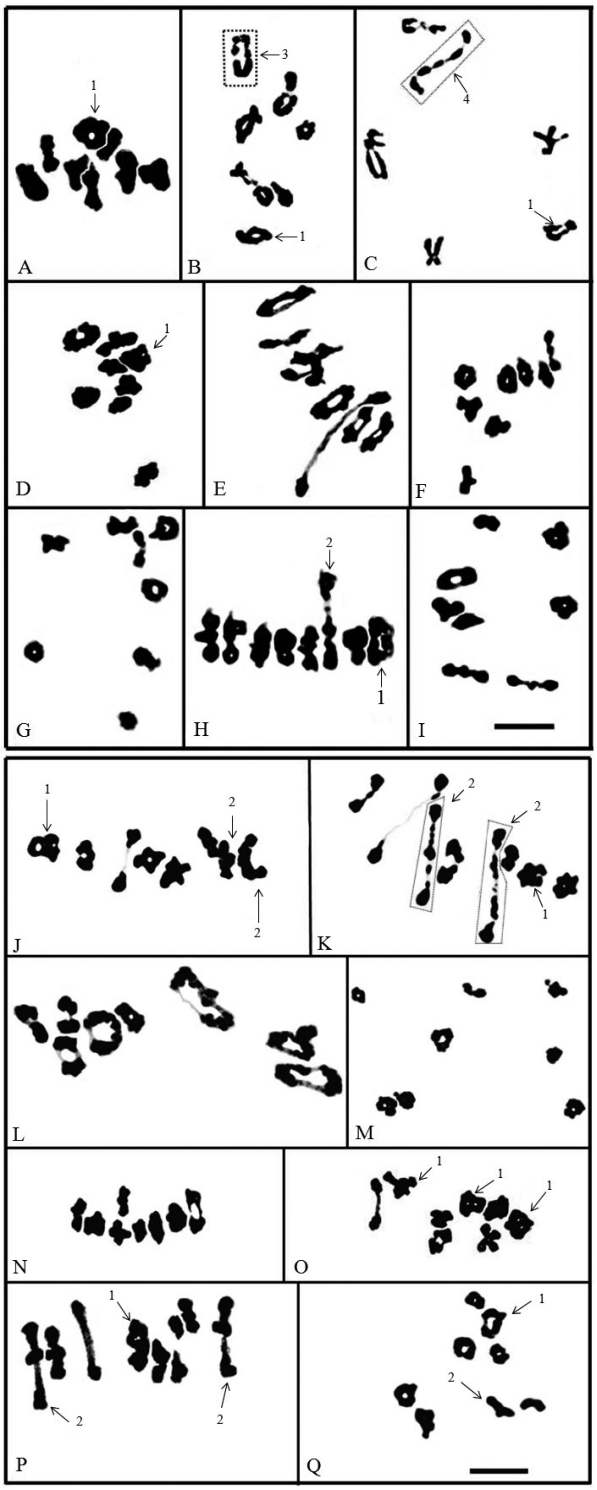

Fig. 5 PMC in Metaphase I of meiosis for different *Echeandia* species. (**A**) *E. echeandioides* 321 with 7 IIs+heteromorphic ring II; (**B**) *E. echeandioides* 359 with 5 IIs+heteromorphic ring II+heteromorphic ring IV; (**C**) *E. echeandioides* 359 with 5 IIs+heteromorphic ring II+heteromorphic chain IV; (**D**) *E. echeandioides* 360 with 7 IIs + heteromorphic ring II; (**E**) *E. hintonii* 308 with 8 IIs; (**F**) *E. mexicana* 236 with 8 IIs; (**G**) *E. mexicana* 284 with 8 IIs; (**H**) *E. mexicana* 357 with 6 IIs + heteromorphic ring II + heteromorphic rod II; (**I**) *E. montealbanensis* 4015 with 8 IIs; (**J**) *E. nana*277 with 5IIs + 1 heteromorphic ring II + 2 heteromorphic rod IIs; (**K**) *E. nana* 009 with 5 IIs+1 heteromorphic ring II +2 heteromorphic rod IIs; (**L**) *E. pubescens* 482 with 6IIs + 1heteromorphic ring II + heteromorphic rod II; (**M**) *E. reflexa* 260 with 8IIs; (**N**) *E. reflexa* 290 with 8IIs; (**O**) *E. reflexa* 292 with 5 IIs + 3 heteromorphic ring IIs; (**P**) *E. tenuis* 484 with 5 IIs + 1 heteromorphic ring II + 2 heteromorphic rod IIs; (**Q**) *E. tenuis* 356 with 6 IIs + 1 heteromorphic ring II + 1 heteromorphic rod II. Numbers indicate pairs of heteromorphic chromosomes. Numbers indicate: 1- heteromorphic ring IIs; 2- heteromorphic rod IIs; 3- heteromorphic ring IVs; 4- Heteromorphic chain IVs. Scale = 10 µm.

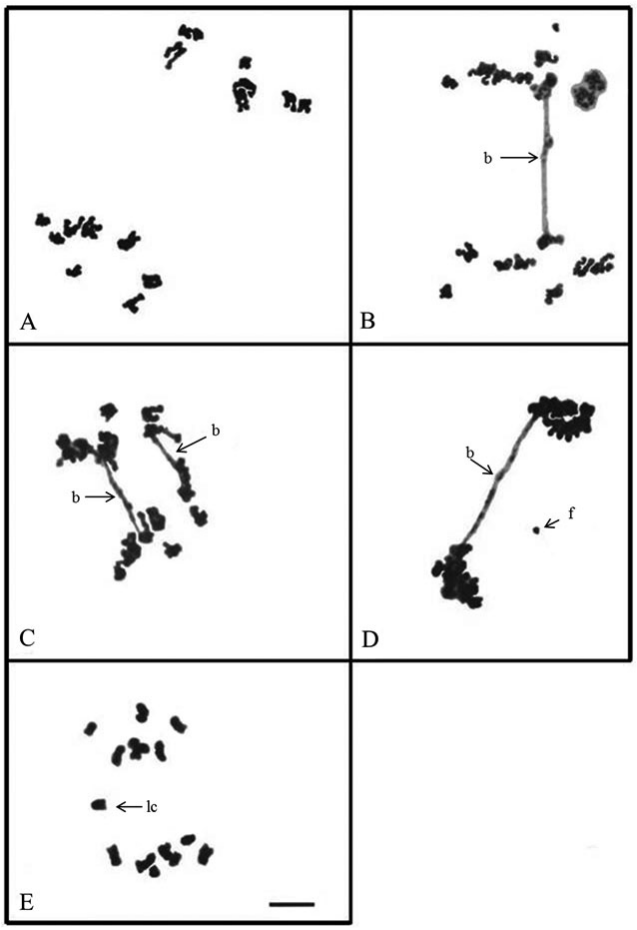

Fig. 6 PMC in AI of meiosis for different *Echeandia* species. (**A**) *E. tenuis* 484 showing regular AI (8:8). Irregular AI: (**B**) *E. mexicana* 357 AI with bridge; (**C**) *E. mexicana* AI with 2 bridges; (**D**) *E. montealbanensis* 4015 AI with bridge and fragment; (**E**) *E. pubescens* 482 AI with lagging chromosomes. b = bridges; f = fragment; lc = lagging chromosome. Scale = 10 µm.

Table 7 Heteromorphic chromosome pairs, and heteromorphic bivalents (IIs), and quadrivalents (IVs) of eight species of *Echeandia*.

Species and populations	Heteromorphic chromosome pairs	Heteromorphic	
		IIs	IVs
E. echeandioides 321		1	
E. echeandioides 359	3	3	1-2
E. echeandioides 360	1	3	1
E. hintonii 308			
E. mexicana 236			
E. mexicana 284		2	
E. mexicana 357		2	
E. montealbanensis 4015			
E. nana 009	3	3	
E. nana 009.3	3	3	
E. nana 115	3	3	
E. nana 242	3	3	
E. nana 265	3	3	
E. nana 277	3	3	
E. nana 278	3	3	
E. nana 282	3	3	
E. nana 283	3	3	
E. pubescens 482		2	
E. reflexa 260		2	
E. reflexa 290		2	
E. reflexa 292	1	4	
E. tenuis 356	1	2	
E. tenuis 484	2	3	

Table 8 Normal and irregular A1 for eight species of *Echeandia*.

Species and population	No. of PMCs	Regular A1 cells %	Cells with one bridge %	Cells with two bridges %	Cells with one bridge and fragment %	Cells with lagging chromosomes %
E. echeandioides 321	989	97.72	4.55	2.02		
E. echeandioides 359	998	65.72	9.92	5.81	3.91	1.80
E. echeandioides 360	913	68.28	8.54	5.04	2.74	1.31
E. hintonii 308	1099	94.18	5.10	0.55	0.18	
E. mexicana 236	996	89.13	1.91	1.10	0.30	
E. mexicana 284	978	93.60	12.78	4.09	1.23	
E. mexicana 357	969	88.74	9.60	3.82	0.72	
E. montealbanensis 4015	1244	95.34	4.42	0.24		
E. nana 009	495	57.37	41.62	1.01		
E. nana 009.3	446	52.47	44.84	2.69		
E. nana 115	485	51.96	44.74	3.30		
E. nana 242	479	54.70	44.26	1.04		
E. nana 265	499	57.72	39.88	2.41		
E. nana 277	425	68.47	31.53			
E. nana 278	329	91.79	8.21			
E. nana 282	385	97.14	2.86			
E. nana 283	404	79.46	20.55			
E. pubescens 482	1422	77.64	12.73	5.34	3.31	
E. reflexa 260	1184	84.38	9.29	3.46	2.45	
E. reflexa 290	1179	82.02	10.43	3.82	3.14	
E. reflexa 292	1384	78.47	12.57	4.99	3.18	
E. tenuis 356	923	81.23	5.96	3.47	1.41	0.87
E. tenuis 484	994	70.37	11.67	7.44	2.92	1.11

Table 9 Shrunken or empty pollen grain of eight species of *Echeandia*.

Species and populations	Total pollen grains	Shrunken or empty pollen grain%
E. echeandioides 321	238	2.94
E. echeandioides 359	644	29.85
E. echeandioides 360	589	20.37
E. hintonii 308	824	5.70
E. mexicana 236	633	13.74
E. mexicana 284	877	2.28
E. mexicana 357	502	14.14
E. montealbanensis 4015	692	3.61
E. nana 009	644	15.68
E. nana 009.3	618	16.02
E. nana 115	632	22.63
E. nana 242	720	26.67
E. nana 265	712	27.67
E. nana 277	644	12.58
E. nana 278	698	4.59
E. nana 282	684	2.34
E. nana 283	674	9.05
E. pubescens 482	865	22.54
E. reflexa 260	726	13.64
E. reflexa 290	817	16.52
E. reflexa 292	874	18.31
E. tenuis 356	361	13.85
E. tenuis 484	538	23.23

nization and distribution in new habitats. The intraspecific variation of genomes of two cytotypes of *Echeandia nana* described above is due to heterozygotic exchanges of chromosomes. Also, we suggest that since the populations having these two cytotypes are geographically isolated, an active process of speciation of this species is favoured. This cytological and genetic differentiation of cytotypes A and B was made further evident in the low number of fruits and of abortive seeds in plants derived from the cross-pollination between cytotypes A×B, as compared to the significantly higher number of fruits and of viable seeds produced by plants derived from the cross pollination of cytotypes AxA and BxB.

The results of this study form a basis for the application of biotechnology and the genetic improvement in species of *Echeandia* for ornamental purposes.

ACKNOWLEDGEMENTS

This study was supported by OEA: "Estudios Biosistemáticos en algunos géneros de Leguminosas, Liliaceae y Palmas de México" Citogenética 88-89 PRDCyT; CONACyT Project No. 1107P-N and Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México. We are grateful to Dr. Robert W. Cruden for the identification names of the plants used in this research. The authors are also grateful to Dr. Aaron Rodriguez-Contreras, of the Instituto de Botanica y Zoologia, Universidad de Guadalajara, Mexico for the pictures in **Fig. 1**. We thank M. Ladd for technical assistance, I. Brunner for help in maintaining of plants in greenhouse.

REFERENCES

Araki H (1975) Cytogenetics of *Scilla scilloides* complex V. The relationship between two adjacent natural population. *Nucleus* **18**, 1-6

Araki H (1977) Year-after-year occurrence of aneuploids in a natural population of the *Scillascilloides* complex. *Bulletin of the Fukuoka University of Education* **26**, 77-83

Araki H (1985) The distribution of diploids and polyploids of the *Scilla scilloides* complex in Korea. *Genetica* **66**, 3-10

Araki H, Hidaka S, Takahashi S (1976) Cytogenetics of the *Scilla scilloides* complex VI. The structures of natural population. *The Botanical Magazine (Tokyo)* **89**, 83-91

Arellano-Rodriguez JA, Flores-Guido JS, Tun-Garrido J (2003) Nomenclatura, forma de vida, uso, manejo y distribución de las especies vegetales de la península de Yucatán. Universidad Autónoma de Yucatán, Mexico, 393 pp

Bennett MD, Parmjit B, Leitch IJ (2000) Nuclear DNA amounts in angiosperms and their modern uses 807 new estimates. *Annals of Botany* **86**, 859-909

Bennett MD, Leitch IJ (2005) Plant DNA C-values Database. Available online: http://www.rbgkew.org.uk/cval/homepage.html

Brandham PE (1970) Chromosome behaviour in the *Aloineae* III. Correlations between spontaneous chromatid and sub-chromatid aberrations. *Chromosoma* **31**, 1-17

Brandham PE (1973) The chromosomes of the Liliaceae III: New cases of interchange hybridity in the in the *Aloineae*. *Kew Bulletin* **28**, 341-348

Brandham PE (1974) Interchange and inversion polymorphism among population of *Haworthia* var. *chalumnensis*. *Chromosoma* **47**, 85-108

Brandham PE (1976) The frequency of spontaneous structural change. In: Jones K, Brandham PE (Eds) *Current Chromosome Research* (1st Edn), Elsevier North Holland Biomedical Press Amsterdam, the Netherlands, pp 77-87

Brandham PE, Johnson MAT (1977) Population cytology of structural and numerical chromosome variants in the *Aloineae* (Liliaceae). *Plant Systematics and Evolution* **128**, 105-122

Cruden RW (1981) New *Echeandia* (Liliaceae) from México. *Sida* **9**, 139-146

Cruden RW (1986) New species of *Echeandia* (Liliaceae) from central America. *Phytologia* **59**, 373-380

Table 10 Number of flowers pollinated, fruits, seeds per fruit and total number of seeds produced after crossing of two cytotypes of *Echeandia nana*.

Cytotypes ♂ x♀	Flowers	Number of fruits		Number of seeds per fruit	Total number of seeds
		Total	%		
A x A					
277 x 277	52	48	53.33	38	1824
278 x 278	22	18	20.00	38	684
282 x 282	11	9	10.00	38	342
283 x 283	5	4	4.44	38	152
	Total = 90	Mean = 19.75			Total = 3002
B x B					
009 x 009	91	89	76.46	38	3382
009 x 265	4	3	2.68	38	114
265 x 265	17	16	14.29	38	608
	Total = 112	Mean = 36.00			Total = 4104
A x B					
277 x 265	25	8	7.55	28	224
277 x 009	46	16	15.09	28	448
278 x 265	8	3	2.83	28	84
278 x 242	3	0	0.00	0	0
278 x 009	7	2	1.89	28	56
282 x 009	15	5	4.72	28	140
282 x 242	2	0	0.00	0	0
	Total = 106	Mean = 4.86			Total = 952

Table 11 Results of Tukey's multiple range test on mean DNA content for five species of *Echeandia*.

Species	Mean DNA (pg)	Tukey's grouping
E. echeandioides	20.618	a
E. hintonii	19.962	b
E. reflexa	19.233	c
E. mexicana	18.089	d
E. nana	17.798	e

Letters indicate species that were significant different using α = 0.05

Table 12 Results of Tukey's multiple range test on mean DNA content for several populations of four species of *Echeandia*.

Species	Population numbers	Mean DNA (pg)	Tukey's grouping
E. echeandioides	361	23.003	a
	321	19.545	b
	460	19.308	c
E. mexicana	284	18.933	a
	466	17.244	b
E. nana	278	17.976	b
	242	17.619	b
E. reflexa	373	21.017	a
	290	19.244	b
	375	17.438	c

Letters indicate populations that were significant different using α = 0.05

Cruden RW (1987) New species of *Echeandia* (Liliaceae) from Nueva Galicia. *Contributions from the Michigan University Herbarium* **16**, 129-133

Cruden RW (1989) A new *Echeandia* (Liliaceae) from Venezuelan Guayana. *Annals of the Missouri Botanical Garden* **76**, 350

Cruden RW, McVaugh R (1989) *Echeandia* Ortega. In: Anderson WR (Ed) *Flora Novo-Galiciana, Bromeliaceae to Dioscoreaceae* (1st Ed, Vol 15) The University of Michigan Herbarium Ann Arbor, Michigan, USA, pp 178-197

Cruden RW (1993) New species of *Echeandia* (Liliaceae) from Oaxaca, México. *Phytologia* **74**, 128-137

Cruden RW (1994) 3. *Echeandia* Ortega. In: Davidse G, Sousa MS, Charter AO (Eds) *Flora Mesoamericana, Alismatácea a Ciperácea* (1st Edn, Vol 6), Universidad Nacional Autónoma de México, Instituto de Biología, México, pp 27-30

Cruden RW (1999) A new subgenus and fifteen new species of *Echeandia* (Anthericaceae) from Mexico and the United States. *Novon* **9**, 325-338

Cruden RW (2009) A sinopsis of south American *Echeandia* (Anthericaceae). *Annals of the Missouri Botanical Garden* **96**, 251-267

Espinoza F, Quarin CL (1998) Relación genómica entre citotipos diploides de *Paspalumsimplex* y *P. procurrens* (Poaceae, Paniceae) *Darwiniana* **36**, 59-63

Fernandez A, Arbo MM (1996) Relaciones genómicas entre las especies diploides de flores blanco-azuladas de *Turnera* (Serie Canaligerae). *Bonplandia* **9**, 95-102

Gimenez-Martin G (1959) Número cromosómico en especies de *Scilla*. *Genetica Iberica* **11**, 97

Grant V (1981) *Plant Speciation* (2nd Edn), Columbia University Press, New York, USA

Haga T, Noda S (1976) Cytogenetic population structure of *Scilla scilloides* complex. *Genetica* **46**, 161-176

Jones K (1978) Aspects of chromosome evolution in plants. *Advances in Botanical Research* **6**, 120-194

Kenton A (1981) Chromosome Evolution in the *Gibasis linearis* Alliance (Commelinaceae). I. The Robertsonian differentiation of *G. venustula* and *G. speciosa*. *Chromosoma* **84**, 291-304

Kenton A (1983) Qualitative and quantitative chromosome change in the evolution of *Gibasis*. In*: Proceedings of the Second Chromosome Conference held in Jodrell Laboratory, Royal Botanical Gardens*, 1-4 September, 1982, Kew, England, pp 273-281

Kenton A (1984) Chromosome evolution in the *Gibasis linnearis* group Commelinaceae III. DNA variation, chromosome evolution, and speciation in *G. venustula* and *G. heterophylla*. *Chromosoma* **90**, 303-310

Kenton A, Davies A, Jones K (1987) Identification of Renner complex and duplications in permanent hybrids of *Gibasis pulchella* (Commelinaceae). *Chromosoma* **95**, 424-434

Kenton A, Drakeford A (1990) Genome size and karyotype evolution in *Tradescantia* section *Cymbispatha* (Commelinaceae). *Genome* **33**, 604-610

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* **52**, 201-220

López-Ferrari AR, Espejo SA, Ceja RJ (2002) Una nueva especie de *Echeandia* (Anthericaceae) de Guerrero, México. *Novon* **12**, 77-79

Martínez J (1988) Estudio Cariotípico de la Especie *Echeandia nana* (Baker) Cruden de la Familia Liliaceae. BSc thesis, Facultad de Ciencias, Universidad Nacional Autonoma de México, Mexico, 46 pp

Martínez J (1995) Estudio Cromosómico de Citotipos en Siete Especies Mexicanas de *Echeandia* Ort. (Liliaceae). MSc thesis, Facultad de Ciencias, Universidad Nacional Autonoma de México, Mexico, 102 pp

Martínez J, Palomino G (1996) Karyotype analysis in three new species of *Echeandia* (Liliaceae) and cytotypes of *E. reflexa*. *Cytologia* **61**, 215-223

Martínez J, Palomino G (1997) Evidence of heterozygous chromosome interchange and chromatid exchange in autotetraploid cytotype of *Gibasisschiedeana* (Tradescantiae-Commelinaceae). *Cytologia* **62**, 275-281

Martínez J Méndez, I, Palomino G (2000) Cytological and genical differentiation between cytotypes of *Echeandia nana* (Anthericaceae). *Caryologia* **53**, 147-158

Noda S (1961) Chiasma studies in structural hybrids. VIII. Further evidences for chiasma formation by crossing over in reciprocal translocations of *Scilla scilloides*. *Japanese Journal of Genetics* **42**, 89-93

Ohri D (1998) Genome size variation and plants systematics. *Annals of Botany* **82**, 75-83

Owens SJ (1979) The use of empty hard gelatin capsules in controlled pollinations. *Euphytica* **28**, 609-610

Palomino G, Romo V (1987) IOPB chromosome numbers reports. *Taxon* **36**, 282-285

Palomino G, Romo V (1988) Karyotypic studies in two Mexican species of *Echeandia* Ort. (Liliaceae). *Southwest Naturalist* **33**, 382-384

Palomino G, Vázquez R (1991) Cytogenetic studies in Mexican population of species of *Crotalaria* L. (Leguminosae-Papiolonoideae). *Cytologia* **56**, 343-351

Palomino G, Martínez J (1994) Cytotypes and meiotic behavior in Mexican populations of three species of *Echeandia* (Liliaceae). *Cytologia* **59**, 295-304

Palomino HG, Sousa M (2000) Variation of nuclear DNA content in *biflorus* species of *Lonchocarpus* (Leguminosae). *Annals of Botany* **85**, 69-76

Pérez-Escandón BE, Villlavicencio-Nieto MA, Ramírez-Aguirre A (2003) Lista de las plantas útiles del Estado de Hidalgo. Universidad Autónoma del Estado de Hidalgo, Mexico, pp 23, 87

Price HJ, Chambers KL, Bachmann K (1981a) Genome size variation in diploid *Microseris bigelovii* (Asteraceae). *Botanical Gazette* **142**, 156-159

Price HJ, Chambers KL, Bachmann K (1981b) Geographic and ecological distribution of genomic DNA content variation in *Microseris douglasii* (Asteraceae). *Botanical Gazette* **142**, 415-426

Price HJ (1988) DNA content variation among higher plants. *Annals of the Missouri Botanical Garden* **75**, 1248-1257

Richardson A, King K (2010) *Plants of Deep South Texas. A Field Guide to the Woody and Flowering Species*, Everbest Printing Co., China, 26 pp

Rodríguez-Contreras A, Ortiz-Catedral L (2003) Algunas especies de plantas nativas con potencial ornamental del occidente de México. In: Carvajal S (Ed) *Avances en la Investigación Científica en el CUCBA* (1st Edn), Coordinación de Investigación. Centro Universitario de Ciencias Biológicas y Agropecuarias. Universidad de Guadalajara. México, pp 263-268

Romero AJ (1988) Estudio citogenético de *Echeandia leptophylla* Bentham. BSc thesis, Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico, 45 pp

Sato D (1942) Karyotype alteration and phylogeny in Liliaceae and allied families. *Japanese Journal of Botany* **12**, 57-161

Sen S (1975) Cytotaxonomy of Liliales. *Feddes Repertorium* **86**, 255-305

Schnarf K, Wunderlich R (1939) Zur vergleichenden embryologie der Liliaceae-Asphodeloideae. *Flora* **133**, 297-327

Tamura MN (1990) Biosystematic studies on the genus *Polygonatum* (Liliaceae). I. Karyotype analysis of species indigenous to Japan and its adjacent regions. *Cytologia* **55**, 443-466

Vijayavalli B, Mathew PM (1990) Karyomorphology of four morphotypes of *Gloriosa superba* L. from South India. *Cytologia* **55**, 531-533

World Checklist of Selected Plant Families (2011) The Board of Trustees of the Royal Botanic Gardens, Kew. Online: http://www.kew.org/wcsp/

Wulff AF (1992) Hibridación natural entre especies sudamericanas de *Hypochoeris* (Asteraceae). *Darwiniana* **31**, 167-171

Appendix

E. echeandioides. 1-México State, 43 km Toluca to Temascaltepec, 1850 masl. Palomino and Martínez 359 (MEXU). 2- México State, 2.6 km Temascaltepec to Valle de Bravo, 1800 masl. Palomino and Martínez 360 (MEXU). 3- Guerrero State, 33 km Chilpancingo to Chichihualco, 2350 masl. Palomino and Martínez 321 (MEXU). *E. mexicana.* 1- Mexico State, Cerro Tetcutzingo, 2650 masl. Palomino and Martínez 236 (MEXU). 2- Hidalgo State, 67 km Atotonilco to Molango, 2000 masl. Palomino and Martínez 284 (MEXU). 3- México State, 4 km Temascaltepec to Valle de Bravo, 2000 masl. Palomino and Martínez 357 (MEXU). *E. hintonii*.- Guerrero State, 4 km from the desviation to Agua de Obispo, 1100 masl. Palomino and Martínez 308 (MEXU). *E. montalbanensis*. Oaxaca State, Monte Albán, 2000 masl. A. García 4015 (MEXU). *E. nana*, 1- Hidalgo State, km 6 Pachuca-Tampico, 2700 masl. Martínez and Palomino 009 (MEXU). 2- Hidalgo State, km 6 Pachuca-Tampico, 2700 masl. Martínez and Palomino 009.3 (MEXU). 3- México State, La Siberia, 1.5 km to Huexotla, 2260 masl. Martínez and Palomino 115 (MEXU). 4- México State, La Siberia, 3.0 km to Huexotla, 2260 masl. Martínez and Palomino 242 (MEXU). 5- Hidalgo State, 10 km Zimapan to Pachuca, 2500 masl. Palomino and Kenton 265 (MEXU). 6- Hidalgo State, km 16.5 Pachuca to Tulancingo, 2700 masl. Palomino and Kenton 277 (MEXU). 7- Hidalgo State, km 8 Nopalillo to Real del Monte, 2708 masl. Palomino and Kenton, 278 (MEXU). 8- Hidalgo State, 57.5 km Atotonilco to Molango, 2000 masl. Palomino and Kenton, 282 (MEXU). 9- Hidalgo State, Atotonilco to Molango, 2000 masl. Palomino and Kenton, 283, (MEXU). *E. pubescens*. Mexico State, 4 km from Temascaltepec towards Valle de Bravo, 1750 masl. Martínez, 482. *E. reflexa*, 1- Hidalgo State, 8 km from Jacala towards Zimapán, 1610 masl. Palomino and Martínez 260 (MEXU). 2- Hidalgo State, 3 km from Molango to Zacualtipán, 1500 masl. Palomino and Martínez 290 (MEXU). 3- México State. 13.5 km Molango to Zacualtipán, 1500 masl. Palomino and Martínez 292 (MEXU). *E. tenuis*, 1- México State, 21.3 km Temascaltepec to Tejupilco, 2000 masl. Palomino and Martínez 356 (MEXU). 2- México State, 4 km Temascaltepec to Valle de Bravo, 1750 masl. Palomino and Martínez 484 (MEXU).