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Cytological and genical differentiation between cytotypes of *Echeandia nana* (Anthericaceae)

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Abstract — The analysis of 9 populations of *Echeandia nana* showed all to be diploid, with *2n* = 16, *n* - 8 (x = 8). The analyzed populations displayed two cytotypes. Cytotype A — 10m+6sm, having two pairs of chromosomes with a satellite, was observed in four populations from the eastern flanks of the Pachuca mountain range. The five remaining populations from the western flank of the Pachuca and Sierra Nevada mountain ranges (Mexico) showed cytotype B = 6m+8sm+2st, having one pair of chromosomes with a satellite. The analysis of meiosis revealed heterozygotic exchanges. Analysis of MI showed three heteromorphic bivalents in cytotypes A and B. Analysis of AI showed sub-chromatid aberrations, (side arm bridges = SAB), which were more frequent in cytotype B (39.88-44.84%) than in cytotype A (2.86-31-53%). Cells with two bridges (SAB aberrations) were observed in cytotype B. The intraspecific cytological and genical differentiation of cytotypes A and B is probably the result of geographical isolation between populations of *E. nana.* This suggests that this species is undergoing through a major process of genomic differentiation involving heterozygotic chromosomal rearrangements; which favors a process of speciation between both cytotypes without the occurrence of significant morphological changes. This cytological and genical 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 among cytotypes (AxB: 0-16 fruits; 0-448 abortive seeds), relative to the larger values recorded after cross-pollination within cytotypes (AxA: 4-48; 152-1824) (BxB: 3-89; 14-3382).

Key words: cytotype, *Echeandia nana*, heterozygotic exchanges, sub-chromatid aberrations

INTRODUCTION

Echeandia Ort. includes about 78 perennial herbaceous species which are grouped in two subgenera: Echeandia and Mscavea (CRUDEN 1999). The subgenus *Echeandia* is distributed from the USA, in the states of Arizona, New Mexico and Texas, to Argentina and Chile (CRUDEN and McVAUGH 1989). More than 60 species have been described from Mexico and Central America, many of which are narrow endemies (CRUDEN 1986, 1987, 1993, 1994, 1999; CRUDEN and McVAUGH 1989). Mexico is considered to be the genus center of origin and diversity (R.W. Cruden, pers. comm.). *Echeandia nana* (Baker) Cruden subgenus Echeandia (= *Anthericum flavescens* Schultes & Schultes) is found in pine and pine-oak forests from the Mexican state of Durango to Guatemala (CRUDEN 1981), including the Pachuca and Sierra Nevada mountain ranges located in the Mexican transvolcanic belt.

Diploid plants (2*n* = 16, *n* =8, x = 8) have been reported for 32 species of *Echeandia* (SCHNARF and WUNDERLICH 1939; CRUDEN 1981, 1986, 1987, 1993, 1999; PALOMINO and ROMO 1987; PALOMINO and MARTINEZ 1994;

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MARTINEZ and PALOMINO 1996). Given the above, and considering the reported chromosome numbers for 22 polyploid species of the genus (4x, 5x, 6x, 8x, l0x and 11x-4) (CRUDEN 1986, 1987, 1993, 1994, 1999), we agree with PALOMINO and ROMO (1988) in considering *Echeandia* as a monobasic genus with x = 8.

The karyotypes of seven species of *Echeandia* have been shown to display interspecific variation. Likewise, the karyotypes of the studied populations of these same species displayed distinctive cytotypes (PALOMINO and MARTINEZ 1994; MARTINEZ and PALOMINO 1996).

Spontaneous heterozygotic exchanges in species and cytotypes of *Echeandia* have a common behavior pattern in karyotype variation. These exchanges were observed in heteromorphic pairs of chromosomes with satellites, and, of metacentric, submetacentric and subtelocentric chromosomes. The origin of these rearrangements was evidenced in heteromorphic bivalents (IIs) and quadrivalents (IVs) observed in metaphase I (MI). Additional evidence for translocations and chromatid exchanges comes from the low level of meiotic irregularities observed in anaphase I (AI), including U-type bridges, side arm bridges (SAB) and lagging chromosomes (PALOMINO and MARTINEZ 1994; MARTINEZ and PALOMINO 1996).

This study describes the karyotype, analyzes the meiotic chromosome behaviour and pollen fertility of nine populations of *Echeandia nana* with two different cytotypes. Also reported here are the number of fruits, seeds per fruit, and the total number of seeds after cross-pollination among and between these two cytotypes of *Echeandia nana.*

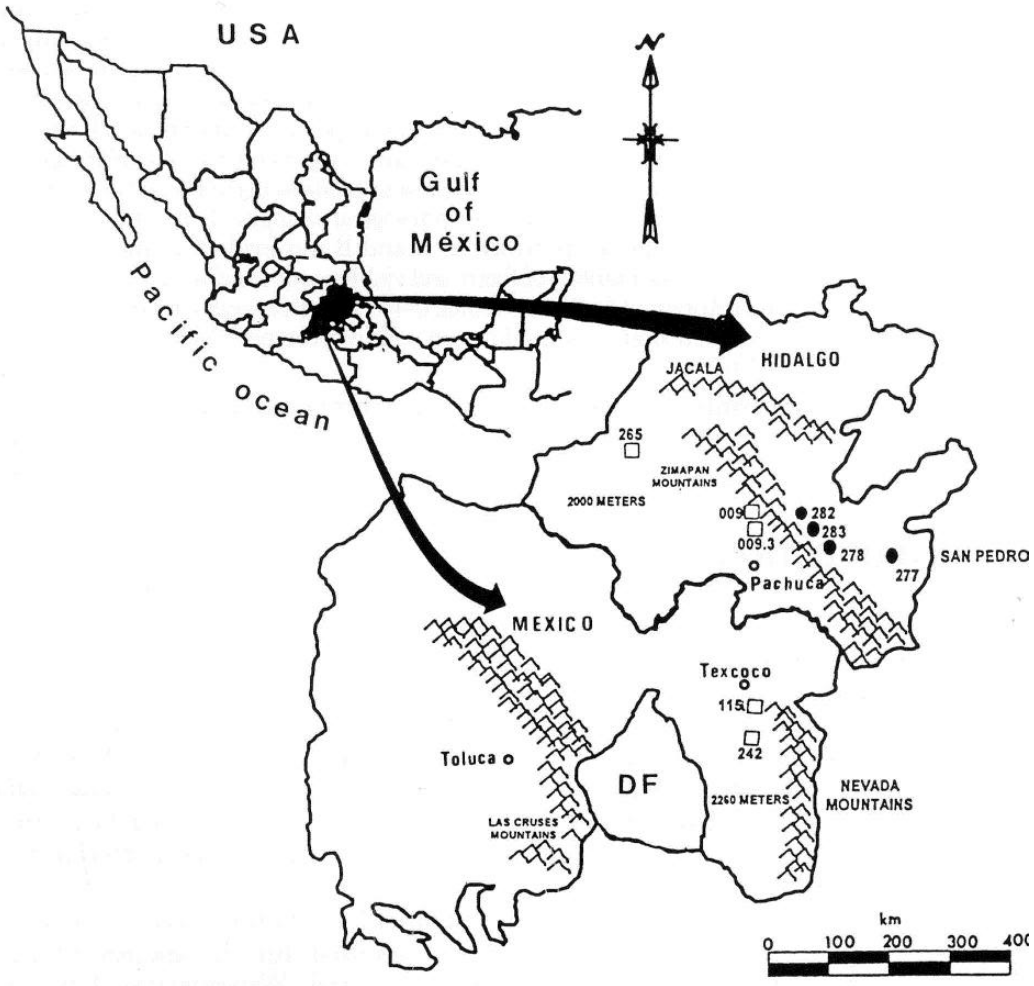

Fig. 1 — Geographical location and collection number of the nine studied populations of *Echeandia nana* with cytotypes A ( ) B( ).

MATERIALS AND METHODS

Plant material

Samples of nine populations of *Echeandia nana* were analyzed, collected from the Mexican states of Hidalgo and Mexico (Fig. 1). Populations no. 277, 278, 282, 283 were located on the eastern flanks of the Pachuca mountain range. Populations no. 009, 009.3, and 265 were located on the western flanks of the Pachuca mountain range. Two additional populations, no. 115 and 242, were located on the western flanks of the Sierra Nevada mountain range. The Pachuca and Sierra Nevada mountain ranges belong to the Mexican transvolcanic belt mountain system (Table 1; Fig. 1). In all cases, plants were collected from wild populations in pine-oak forests (Table 1) and voucher specimens were deposited in the National Herbarium (MEXU) of the Universidad Nacional Autonoma de Mexico (UNAM). From each population, 20 or 30 individual plants were collected to be transported to the Jardin Botanico, Institute de Biologia, UNAM, where they were transplanted in pots containing a mixture of vermiculite and organic soil, and maintained in a greenhouse.

Mitotic chromosome analysis

Preparations were made from 20 to 30 plants from all nine populations were studied and from each one, 116 to 159 cells at mitotic metaphase were selected for examination. For all populations, five to ten of the best cells from each plant 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. Index of asymmetry (TF%) was obtained following GUPTA and GUPTA (1978).

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 219 to 998 MI pollen mother cells (PMC), and, of 329 to 499 AI PMC derived from 10 plants from each of the nine populations 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) (WHITE 1973); for AI PMC the occurrence of single and double bridges.

Pollen fertility

Estimates were made in samples of pollen stained with cotton blue in lactophenol. Percentages of well-filled stained grains were obtained from samples of 618 to 720 pollen grain, derived from four to ten plants from each one of the populations 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

Table 1 — Provenance and cytotype of nine populations of *Echeandia nana* collected by Martinez and Palomino, all with $2n = 16$.

Locality and collection number	NC	Cytotype	Secondary constrictions	Range of chromosome length (µm)	Genome length (µm) $\bar{X}$ ± SE		Index of Asymmetry TF (%) $\bar{X}$ ± SE	
Cytotype A								
México. Hidalgo. Pachuca. *277*	10	10m + 6sm	2m + 2sm	2.60 - 5.65	28.61	0.27	39.43	0.12
México. Hidalgo. Nopalillo. *278*	10	10m + 6sm	2m + 2sm	3.48 - 6.09	36.14	0.27	40.29	0.12
México. Hidalgo. San Pedro. *282*	10	10m + 6sm	2m + 2sm	3.02 - 5.46	35.00	0.27	38.27	0.12
México. Hidalgo. Atotonilco. *283*	10	10m + 6sm	2m + 2sm	3.52 - 7.02	36.89	0.27	42.12	0.12
Cytotype B								
México. Hidalgo. Pachuca. *009*	10	6m + 8sm + 2st	2sm	3.04 - 6.52	35.15	0.27	36.01	0.12
México. Hidalgo. Pachuca. *009.3*	5	6m + 8sm + 2st	2sm	2.61 - 4.35	27.39	0.27	35.74	0.12
México. México state. Huexotla. *115*	10	6m + 8sm + 2st	2sm	2.17 - 3.48	22.39	0.27	32.95	0.12
México. México state. Huexotla. *242*	10	6m + 8sm + 2st	2sm	2.02 - 5.92	28.46	0.27	34.12	0.12
México. Hidalgo. Zimapan. *265*	10	6m + 8sm + 2st	2sm	3.02 - 6.08	34.92	0.27	33.21	0.12

NC = Number of cells measured per population

A and B (AxB) (Table 6). Flowers were emasculated 24 h before the buds opened and they were enclosed in gelatin capsules (OWENS 1979, 1981). 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 of the numbers of pollinated flowers, fruits produced, seeds produced per fruit, and total seeds produced.

Seed germination

Random samples of the seeds derived from AxA and BxB cross-fertilizations were tested for germination, as follows: Of the 3002 seeds obtained from the AxA crosses, 85 were tested for germination; of the 4104 seeds obtained from BxB crosses, 94 were tested for germination. All of the 952 seeds produced after the AxB cross-pollinations were 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 ± 1°C. The number of seedlings was recorded after twenty days of cultivation.

Data analysis

Differences between nine genomes of *Echeandia nana* were analyzed. Inter and intrapopulational variation of genome length and recombination index (RI) were determined for nine populations of *E. nana* using one way analysis of variance (ANOVA). Means were compared using Tukey-Kramer's HSD method. Differences between couplet mean on karyotype asymmetry index (TF%) were assessed by the Kruskal-Wallis non parametric test. A one way ANOVA was performed using Box-Cox transformed values of the numbers of fruits and of seeds per fruit which derived from the AxA, BxB and AxB cross-fertilizations. Means were compared using the Tukey-Kramer test. All statistical computations were performed using the JMP version 3.2.1 of the SAS company program, using a Pentium PC.

RESULTS

Chromosome number

All the plants from the nine populations of *Echeandia nana* with the two cytotypes were found diploid with $2n = 16$, ($x = 8$) (Table 1).

Cytotypes

Two cytotypes were observed in a total of 1279 cells of *Echeandia nana* of which 85 were selected to measure the chromosomes (Table 1). First cytotype, designated as cytotype A (A = 10m+6sm), had two pairs of chromosomes with satellites (Table 1. Fig. 2A) and three pairs of heteromorphic chromosomes (No. 1, 2 and 5, Fig. 2B). It was present in four populations (No. 277, 278, 282 and 283) from the eastern flanks of the Pachuca mountain range. The remaining five populations (No. 009, 009.3, 115, 242 and 265), located on the western flanks of the Pachuca and Sierra Nevada mountain ranges, displayed the second cytotype, designated as B (B = 6m + 8sm + 2st), with one pair of chromosomes with satellite (Table 1. Fig. 2C) and three pairs of heteromorphic chromosomes (No. 3, 4 and 7, Fig. 2D).

Genome length

The genome length in the nine populations of *Echeandia nana* investigated ranged from 22.39 m to 36.89 m (Table 1). The smallest genome was represented by population No. 115 (cytotype B) and the largest, by the population No, 283 (cytotype A). In general the largest genomes were present in populations having cytotype A (populations No. 283 and 278) and the smallest in populations with cytotype B ($P < 0.0001$) (Table 2).

Table 2 — Results of Tukey's multiple range test on mean genome length of nine populations *of Echeandia nana* presenting two different cytotypes.

Populations no.	Genome length $\bar{X}$ (µm)	Cytotype	Tukey's grouping
283	36.89	A	a
278	36.14	A	a b
009	35.15	B	b c
282	35.00	A	b c
265	34.92	B	c
277	28.61	A	d
242	28.46	B	d e
009.3	27.39	B	e
115	22.39	B	f

Same letters indicate no statistical difference using $\alpha = 0.05$, with an ANOVA $P < 0.0001$.

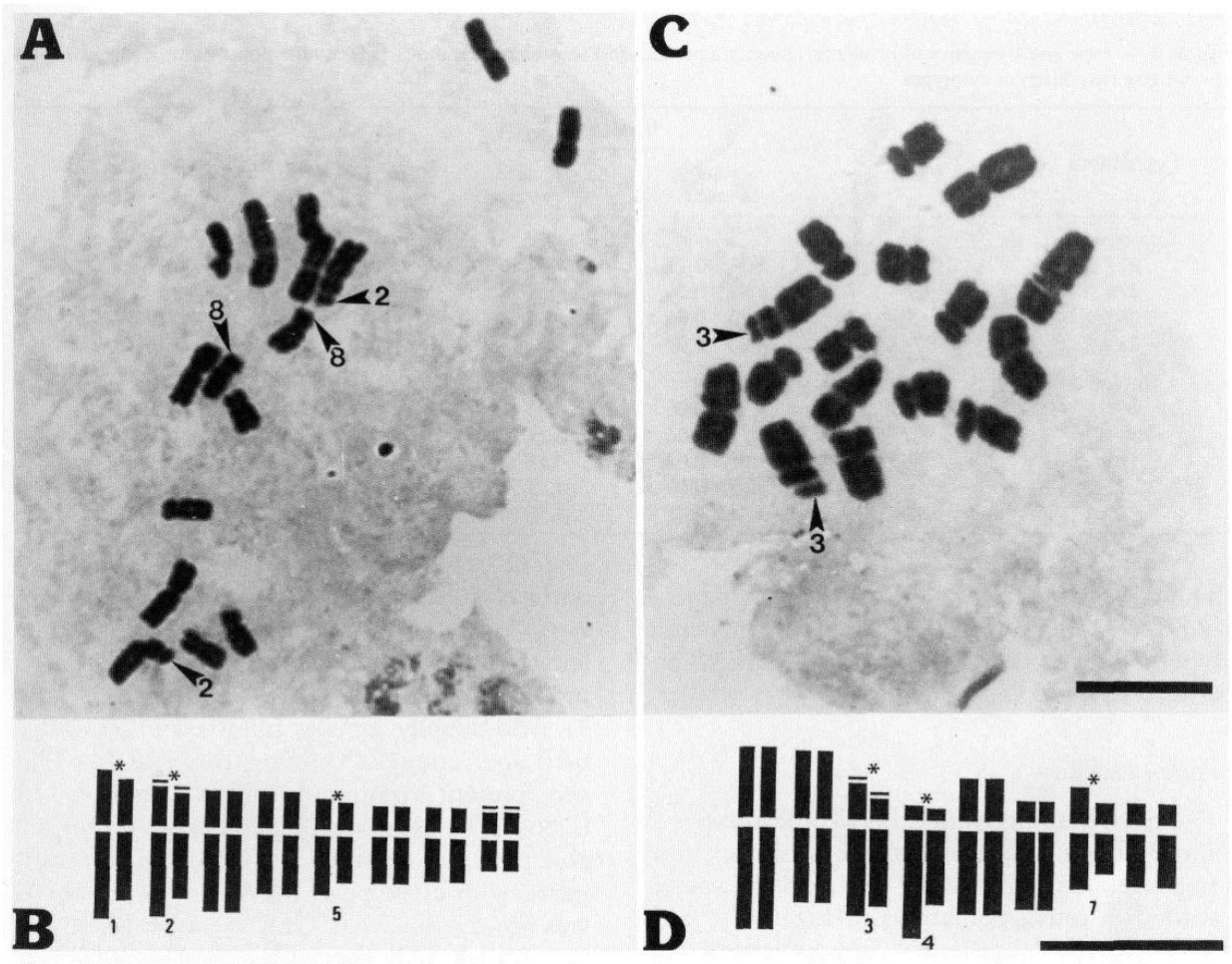

Fig. 2 — Cytotypes of *Echeandia nana 2n* = 16. (A) Somatic chromosomes of cytotype A. (B) Idiogram of cytotype A showing ten metacentrics (m) and six submetacentrics (sm) with four satellites. (C) Somatic chromosomes of cytotype B. (D) Idiogram of cytotype B showing six metacentrics, eight submetacentrics and two subtelocentrics with two satellites. (Arrows and numbers indicate satellites). Asterisks and numbers showed heteromorphic chromosome pairs. Scale 10μm.

Karyotype asymmetry

Populations of *Echeandia nana* with cytotype B showed more asymmetric karyotypes than did populations with cytotype A ($P < 0.0001$) (Table 3).

Meiotic chromosome behavior

Meiotic chromosome analysis in MI of PMC of *Echeandia nana* showed different proportions of eigth ring and rod IIs (Table 4). Cytotypes A and B presented 3 heteromorphic biva-lents which were observed in chromosome pairs 1, 2 and 5 in cytotype A; and, in chromosome pairs 3,4 and 7 in cytotype B (Figs. 2 A, B, C, D; 3 A and B). The RI was not significantly different ($P < 0.144$) in the nine populations *of Echeandia nana* studied, regardless of their cytotype (Table 4). All populations studied exhibited SAB aberrations at AI with variable frequencies. Cells with one bridge (Fig. 3C) were more frequent in populations with cytotype B (39.88-

Table 3 — Results of Kruskal-Wallis test on mean Index of asymmetry (TF%) of nine populations *of Echeandia nana* presenting two different cytotypes.

Population no.	TF (%) $\bar{X}$	Cytotype	Tukey's grouping
283	42.12	A	a
278	40.29	A	b
277	39.43	A	c
282	38.27	A	d
009	36.01	B	e
009.3	35.74	B	e
242	34.12	B	f
265	33.21	B	g
115	32.95	B	g

Letters indicate group of taxa that were not significantly different using $\alpha = 0.05$, with an ANOVA $P < 0.0001$.

Table 4 — Type and frequency of bivalents, chiasmata per cell and recombination index (RI) of nine populations *Echeandia nana* presenting two different cytotypes.

Populations	Observed PMC	Bivalents		Chiasmata/cell	RI
		Ring $\bar{X}$ ± SE	Rod $\bar{X}$ ± SE	$\bar{X}$ ± SE	$\bar{X}$ ± SE
Cytotype A					
277	219	3.53 0.13	4.47 0.14	13.79 0.70	21.79 0.75
278	985	3.77 0.06	4.23 0.07	11.77 0.11	19.77 0.02
282	902	3.40 0.06	4.60 0.07	11.40 0.11	19.40 0.19
283	978	4.50 0.07	3.51 0.06	12.82 0.11	20.82 0.01
Cytotype B					
009	946	3.92 0.06	4.08 0.07	11.92 0.11	19.92 0.01
009.3	957	3.79 0.06	4.21 0.07	11.79 0.11	19.79 0.01
115	989	3.67 0.06	4.33 0.07	11.67 0.11	19.67 0.01
242	998	3.65 0.06	4.35 0.07	11.65 0.11	19.65 0.01
265	979	3.64 0.06	4.36 0.07	11.07 0.11	19.07 0.01

44.84%), compared to the same in cytotype A (2.86 to 31.53%) (Fig. 3C; Table 5). Only cytotype B showed cells with two bridges (SAB aberrations, Fig. 3D; Table 5).

Pollen viability

Pollen viability varied between cytotypes A and B. The smallest percentage of shrunken or empty pollen grains (2.34 to 12.58%) was recorded in four populations of *Echeandia nana* with cytotype A; the largest, in populations with cytotype B (15.68-27.67%) (Table 5).

The analysis of karyological parameters in *Echeandia nana* showed that chromosome complements of cytotype B have undergone to a major number of chromosome rearrangements of those of cytotype A. This is due to the higher number of submetacentric chromosomes (8) and two subtelocentric in comparision to cytotype A. Cytotype B showed smaller genome length than cytotype A, the first also presented more asymmetric karyotypes (Tables 1, 2, and 3). Additionally, a major number of one bridge SAB aberrations in cytotype B (39.88-44.84%) was present compared with the cytotype A (2.86-31.53%). A major percentage of non viable pollen in cytotype B (15.68-27.67%) compared with cytotype A (2.34-12.58%, Table 5) was observed as well. Only cytotype B showed two bridge SAB aberrations (1.02-3.30%, Table 5).

Hybridization procedure

The mean of fruits produced from cross-pollination within cytotypes (AxA = 19.75; BxB —

Table 5 — Shrunken or empty pollen grains, and irregular AI of nine populations *of Echeandia nana* presenting two different cytotypes.

Populations	Total pollen grains	Shrunken or empty pollen grain %	PMC			
			Total	regular AI cells (%)	Cells with one bridge (%)	Cells with two bridges (%)
Cytotype A						
277	644	12.58	425	68.47	31.53	
278	698	4.59	329	91.79	8.21	
282	684	2.34	385	97.14	2.86	
283	674	9.05	404	79.46	20.55	
Cytotype B						
009	644	15.68	495	57.37	41.62	1.01
009.3	618	16.02	446	52.47	44.84	2.69
115	632	22.63	485	51.96	44.74	3.30
242	720	26.67	479	54.70	44.26	1.04
265	712	27.67	499	57.72	39.88	2.41

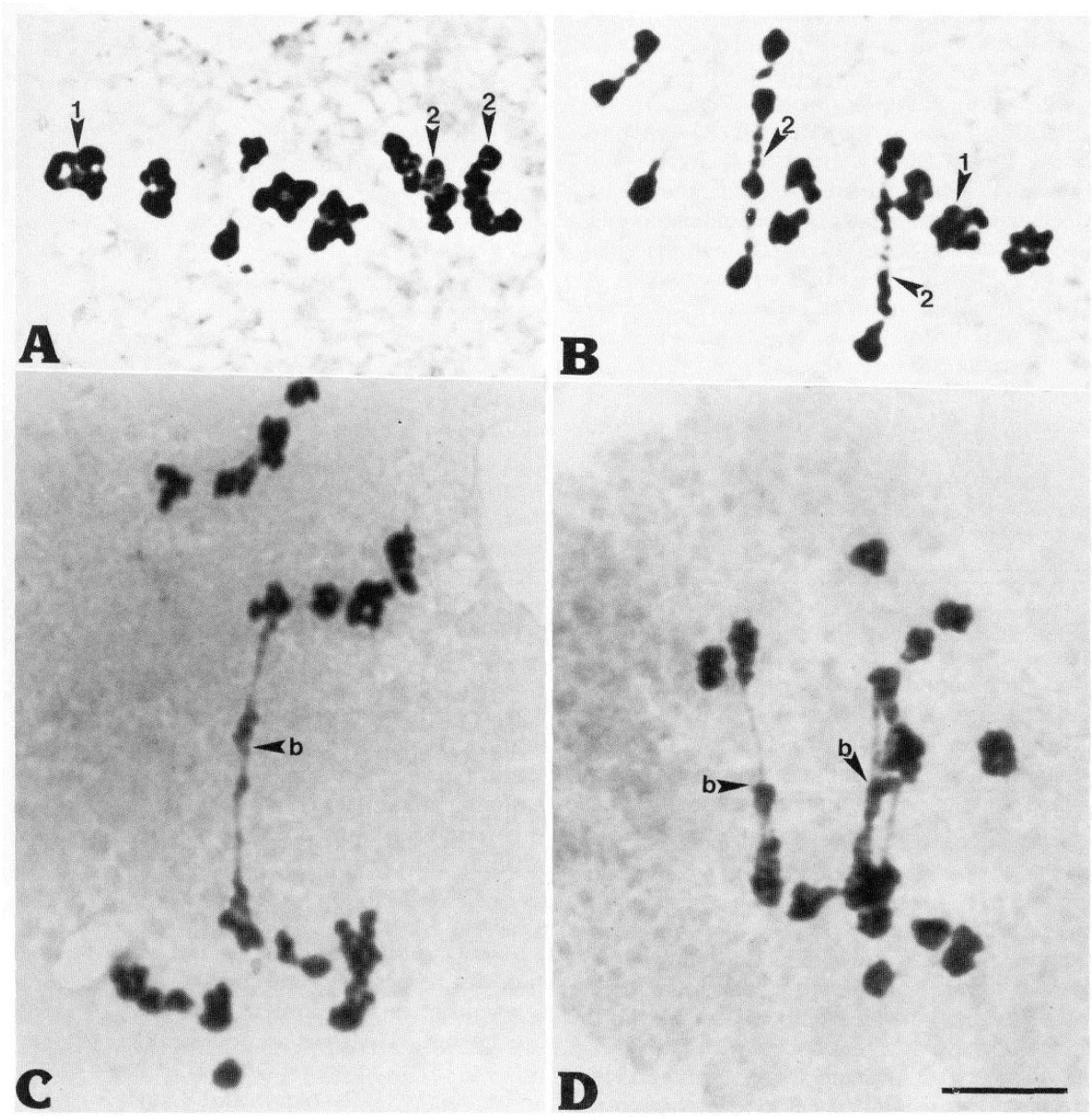

Fig. 3 — PMC's showing MI and irregular AI in *Echeandia nana* cytotypes. (A) MI with 8 IIs of cytotype A. (B) MI with 8 IIs of cytotype B. (C) AI with one bridge (b) of cytotype A. (D) AI with two bridge (b) of cytotype B. Numbers indicate: 1. heteromorphic ring IIs. 2. heteromorphic rod IIs. Scale 10µm.

36.00), differed significantly from those produced after the cross-pollinations among cytotypes (AxB = 4.86) ($P < 0.0001$) (Tables 6 and 7). Cross-fertilizations within cytotypes (AxA and BxB) produced an average of 38 seeds per fruit, a number which did not differ significantly from the 28 seeds per fruit produced in cross-pollinations among cytotypes A and B. The total number of seeds produced after cross-pollination within cytotypes, 3002 in AxA; 4104 in BxB, differed significantly from the total of 952 seeds produced in the AxB cross-pollinations ($P < 0.001$) (Table 6).

Seed Germination

Of the 85 seeds derived from AxA crosses which were tested for germination, a 96.47%

Table 6 — Number of fruits, seeds per fruit and total number of seeds produced after crossing two cytotypes of *Echeandia nana.*

Cytotypes ♂ x ♀	Flower pollination	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	$\bar{X}$ = 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	$\bar{X}$ = 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	$\bar{X}$ = 4.86			Total = 952

produced seedlings. Similarly, of 94 seeds derived from the BxB crosses which were tested for germination, 93.62% produced seedlings. In contrast, of the 952 seeds produced in the AxB crosses, all of which were tested for germi nation, only 7.56% initiated germination but in these, seedling development was limited to the formation of a radicle.

DISCUSSION

The chromosome numbers observed in the populations of *Echeandia nana* with cytotypes A and B *(2n* = 16, *n* = 8, x = 8) agree with previous reports for this same species of PALOMINO and ROMO (1987, 1988), and, for other 32 diploid species in the genus (CRUDEN 1981, 1986, 1987, 1993,1994,1999; CRUDEN and McVAUGH 1989; PALOMINO and MARTINEZ 1994; MARTINEZ and PALOMINO 1996).

The karyotypes of the nine studied populations displayed two different cytotypes. Cytotype A (10m+6sm) was observed in four populations from the eastern flanks of the Pachuca mountain range. Cytotype B (6m+8sm+2st) 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 (Figs. 2A, B, C and D; Table 1).

Intraspecific cytotype variation was apparent as heteromorphic bivalents in MI (Figs. 3A and 3B), with three different heteromorphic chromosome pairs in each cytotype: chromosome pairs no. 1, 2 and 5 in cytotype A; and, chromosome pairs no. 3, 4 and 7 in cytotype B (Figs. 2A and B;). Also, both cytotypes showed different numbers of chromosome pairs with satellites (Figs. 2C and D). 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 *et al.* 1975; JONES 1978; KENTON 1981; PALOMINO and VAZQUEZ 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 at AI. These SAB were observed in both cytotypes of *E. nana;* the highest frequency was recorded in cytotype B, to those in cytotype A (Table 5). SAB have long been recognized among the spontaneous meiotic irregularities caused by aberrant reunion at the sites of chias-mata formation. SAB aberrations are caracter-ized by the formation of a bridge at anaphase I which connects two homologous chromatids

and carries a pair of side arms approximately at its midle-point. SAB are more common than was generally supposed (BRANDHAM 1970); SAB have been observed previously in *Podophyllum* (NEWMAN 1967), in several species of Liliaceae (BRANDHAM 1970), populations of *Crotalaria incana* (PALOMINO and VAZQUEZ 1991) and in some species of *Echeandia* (PALOMINO and MARTINEZ 1994, MARTINEZ and PALOMINO 1996).

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 the populations *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 MARTINEZ (1994), and by MARTINEZ 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 IIs and of low frequencies of bridges, both with fragments and without fragments, suggest that translocations and chromatid exchanges have played a mayor role in shaping the karyotype of populations and species of *Echeandia* (PALOMINO and MARTINEZ 1994; MARTINEZ and PALOMINO 1996).

Similar processes of cytological and genical differentiation in the genomes of cytotypes which have their origin in deletions, translocations and heterozygotic exchanges, have been reported for a large number of species in the following genera: *Dianella* (SEN 1975), *Crocus* (BRIGHTON 1976), *Scilla* (ARAKI 1985), *Gloriosa* (VIJAYAVALLI and MATHEW 1990), *Polygonatum* (TAMURA 1990), *Alopecurus* (KUMAR and GOHIL 1990), *Barleria* (RANGANATH and KRISHNAPPA 1990), *Crotalaria* (PALOMINO and VAZQUEZ 1991), PATIL and CHENNAVEERAIAH 1975), *Sabal* (PALOMINO and QUERO 1992), *Glandularia* (POGGIO *et al.* 1993), *Myrtillocactus* (CID and PALOMINO 1996), *Gibasis* (KENTON 1981, 1983, 1984), KENTON *et al.* (1987), MARTINEZ and PALOMINO (1997) and, *Tradescantia* (KENTON *et al.* 1988).

Additional evidence for the cytological and genical differentiation between the cytotypes of *E. nana* described above was found in the low number of fruits (0-16) and of abortive seeds (0-448) resulting from cross-pollinations among cytotypes (AxB), relative to crosses within cytotypes (AxA: 4-48 fruits; 152-1824 seeds. BxB: 3-89 fruits; 114-3382 seeds) (Tables 6 and 7). The progenitors used in these cross-pollinations had high percentages of fertile pollen (cytotype A, 97%; cytotype B, 72%) (Table 5), and the progeny of cross-pollinations within the same cytotype produced abundant fertile seeds which germinated readily and formed seedlings. In contrast the seeds produced in the AxB crosses were abortive and unable to produce seedlings in the Fl generation.

Table 7 — Results of Tukey's multiple range test on the average number of fruits produced after crossing two cytotypes of *Echeandia nana.*

Number of fruits $\bar{X}$	Cross cytotype	Tukey's grouping
19.75	AxA	a
36.00	BxB	a
4.86	AxB	b

Same letters indicate no statistical difference using $\alpha = 0.05$, with an ANOVA $P < 0.0001$.

The genical divergence between cytotypes A and B is the result of geographic isolation of the populations of *Echeandia nana.* The disjoint distribution of populations and the differences in chromosomal behavior observed between them suggests that this species is going through a mayor process of differentiation due to heterozygotic chromosomal rearrangements. This isolation favors the occurrence of an active process of speciation, despite no significant morphological changes may be detected between plants of *E. nana* having different cytotypes (R.W. Cruden, pers. comm.). The occurrence of chromosomal rearrangements having no effect on noticeable phenotypic changes, and in which this chromosomal remodeling is associated with processes of speciation, has been previously reported for several species (GRANT 1989).

GRANT and GRANT (1960) showed that, in experimental cross-pollinations of diploid *(n* = 8) cytotypes of *Gilia capitata,* compatibility decreases proportionally to the increase of the

genie divergence between genomes. Similar results were reported by KRUCKEBERG (1957) in *Streptanthus glandulosus,* regarding the inverse correlation of fertility in the Fl progeny derived from 334 cross-pollination combinations, with the geographical distance separating the populations of the progenitors. The highly sterile Fl progeny of cross-pollinations of plants from different populations of *Gibasis venustula* subsp. *venustula* revealed divergence and speciation mechanisms with originated in heterozygotic chromosomic and chromatidic exchanges. These exchanges evidenced errors in all stages of meiotic division including differences in the sizes of genomes (DNA content in pg) (KENTON 1983,1984). While the cytological and genetical differentiation of allopatric populations of this subspecies may not be further associated with phenotypic changes, it is an indicator of the existence of a process of speciation (KENTON 1984). A similar example of speciation arising from cytological and genical differentiation of the genomes of cytotypes is seen in *Echeandia nana.* The genomic divergence between cytotypes observed at the intraspecific level in *G. venustula* subsp. *venustula* was the same as that observed in interspecific hybrids of this subspecies with G. *venustula* subsp. *robusta,* in which KENTON (1984) finds the taxonomic distinction to be based exclusively on morphological and ecological criteria.

Similar cytological and genical differences to those obtained in plants resulting from intraspecific cross-pollinations have been observed in Fl progenies of interspecific hybrids between four species of *Turnera (n* = 10). These hybrid Fl progenies showed a low frequency of IIs, univalents (Is), a high percentage of non viable pollen, and low proportions of immature fruits (1-17%) and of abortive seeds (0-130) (FERNANDEZ and ARBO 1996). ESPINOZA and QUARIN (1998) observed low proportions of immature fruits (0-10.4%) and of abortive seeds (0.1%) in interspecific hybrids between two diploid species of *Paspalum (n* = 10). Likewise, WULFF (1992) observed chromosomal sterility (presence of heteromorphic IIs and of Is) and low production of immature fruits in interspecific hybrids of *Hypochoeris.*

Thus, we conclude that the intraspecific variation of the genomes of the 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 in this species is favored. This cytological and genical differentiation of cytotypes A and B was made further evident in the low number of fruits and of abortive seeds in plants derived from cross-pollination between cytotypes (AxB), compared to the significantly higher number of fruits and of viable seeds of plants derived from cross-pollination within cytotypes (AxA and BxB).

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