

Karyology, phytogeography, and the origin of intraspecific karyotypic variation in *Zamia paucijuga* and *Z. polymorpha* (Zamiaceae)

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Abstract. A karyological study on *Zamia paucijuga* and *Z. polymorpha* (Zamiaceae), two species from Mexico and Belize, has resulted in the description of both new chromosome numbers ($2n=19$ in *Z. paucijuga* and $2n=23, 28$ in *Z. polymorpha*) and previously recorded numbers ($2n=23, 25, 27, 28$ in *Z. paucijuga* and $2n=22, 24, 25, 26, 27$ in *Z. polymorpha*). Comparative analysis of distribution and ecology in the two species as well as in several morphologically and/or geographically related species of *Zamia* from North and Central America has shown that intraspecific karyotypic variation in *Zamia paucijuga* and *Z. polymorpha* is associated to their wide distribution, unstable ecological conditions and high morphological variation. The analysis also showed that $2n=18$, the diploid number occurring in the species resulted as the most closely related to *Zamia paucijuga* and *Z. polymorpha* in distribution and morphology, could be one of the putative ancestral karyotype that gave rise to the cytotypes in the two latter species.

Riassunto. Lo studio cariologico di *Zamia paucijuga* e *Z. polymorpha* (Zamiaceae), due specie originarie del Messico e del Belize, è risultato nella descrizione sia di nuovi numeri cromosomici ($2n=19$ in *Z. paucijuga* e $2n=23, 28$ in *Z. polymorpha*) sia di numeri cromosomici già noti ($2n=23, 25, 27, 28$ in *Z. paucijuga* e $2n=22, 24, 25, 26, 27$ in *Z. polymorpha*). L'analisi comparata di dati ecologici e distributivi delle due specie, nonché di specie di *Zamia* del Nord e Centro America riferibili alle due specie per morfologia e distribuzione geografica, ha mostrato che la variazione cariotipica intraspecifica di *Zamia paucijuga* e *Z. polymorpha* è associata all'ampia distribuzione geografica, alle condizioni ecologiche instabili degli habitat e all'alta variabilità morfologica delle due specie. Lo studio ha anche mostrato che $2n=18$, il numero diploide presente nelle specie più affini a *Zamia paucijuga* e *Z. polymorpha* per distribuzione e morfologia, potrebbe essere uno dei cariotipi ancestrali che hanno dato origine ai citotipi delle due specie.

Key words: Centric fission, Cycads, Karyology, Phytogeography, Zamiaceae, *Zamia paucijuga*, *Zamia polymorpha*

INTRODUCTION

Zamia L. (Zamiaceae, Cycadales) is a cycad genus composed of about 60 widespread species in Florida, Mexico, West Indies, and Central and South America (STEVENSON 1990; STEVENSON & OSBORNE 1993; STEVENSON *et al.* 1995; HILL *et al.* 2004). Owing to the wide distribution and intra- and interspecific morphological variation, several taxonomic and systematic problems are still debated in *Zamia* (SABATO 1990; STEVENSON 1990; STEVENSON & OSBORNE 1993; STEVENSON *et al.* 1995). Karyological studies have contributed to clarify the systematics of some species, because *Zamia* is unique among cycads in having inter-

specific karyotypic variation. Chromosome numbers ranging from $2n=16$ to 28, with the exception of $2n=19$ and 20, are reported for the genus (MARCHANT 1968; MORETTI 1990; MORETTI & SABATO 1984; MORETTI *et al.* 1991; NORSTOG 1980; VOVIDES 1983; VOVIDES & OLIVARES 1996). Intraspecific chromosome variation also occurs in six species of the genus (MORETTI 1990; MORETTI & SABATO 1984; MORETTI *et al.* 1991; NORSTOG 1980, 1981; VOVIDES & OLIVARES 1996).

Zamia paucijuga Wieland and *Z. polymorpha* D. Stevenson *et al.*, two species with intraspecific chromosome variation and with a wide distribution, are of special interest in this regard. *Zamia paucijuga* occurs in sporadic

populations for approx. 1500 km along the Pacific coast of Mexico and is $2n=23, 24, 25, 26, 27, 28$ (MORETTI 1990; MORETTI & SABATO 1984). In contrast, *Z. polymorpha* occurs in sporadic populations for approx. 1000 km along the coast of the Yucatan Peninsula of Mexico and along the coast of Belize and is $2n=17, 22, 24, 25, 26, 27$ (MORETTI *et al.* 1991; STEVENSON *et al.* 1995-1996a; VOVIDES & OLIVARES 1996). In the two species, occurrence of morphological and ecological adaptive traits in plants with higher chromosome numbers suggested both that higher numbers are derived (CAPUTO *et al.* 1996; MORETTI & SABATO 1984; MORETTI *et al.* 1991; VOVIDES & OLIVARES 1996) and that centric fission of metacentric chromosomes is involved in the increasing disloid series (CAPUTO *et al.* 1996; MORETTI 1990; MORETTI & SABATO 1984; MORETTI *et al.* 1991). Interbreeding between plants of the same species with different chromosome numbers coupled with the ability for meiotic alignment of two telocentric chromosomes produced by centric fission of a metacentric chromosome to pair have been suggested as the mechanism for chromosome number variation within the two species and to account for the observed infrapopulational variation

(CAPUTO *et al.* 1996; MORETTI *et al.* 1991).

However, no information is available on the causes of such high karyotypic variations and on the putative ancestral karyotype that gave rise to the cytotypes in the two species. Moreover, systematic problems have been long debated for the two species. *Zamia polymorpha*, before its description (STEVENSON *et al.* 1995-1996a), had been included in *Z. loddigesii* Miq., a species from Veracruz, with extensions in Oaxaca and Tabasco, Mexico, with which indeed shares many morphological characters (CAPUTO *et al.* 1996; NORSTOG 1980; SCHUTZMAN 1987; VOVIDES & OLIVARES 1996). Furthermore, *Z. paucijuga*, *Z. polymorpha*, and *Z. loddigesii* as well as two additional species, *Z. furfuracea* L. from Veracruz, Mexico and *Z. spartea* A. DC from Oaxaca, Mexico, are all regarded as related on morphological and geographical grounds (CAPUTO *et al.* 1996; SCHUTZMAN 1987; SCHUTZMAN *et al.* 1988). Hybrid events have been also hypothesised to have been involved in the origin of *Z. loddigesii*, with *Z. furfuracea* and *Z. spartea* being the putative parental species and with *Z. paucijuga* involved in such events (SCHUTZMAN 1987).

New chromosome numbers in *Z. paucijuga*

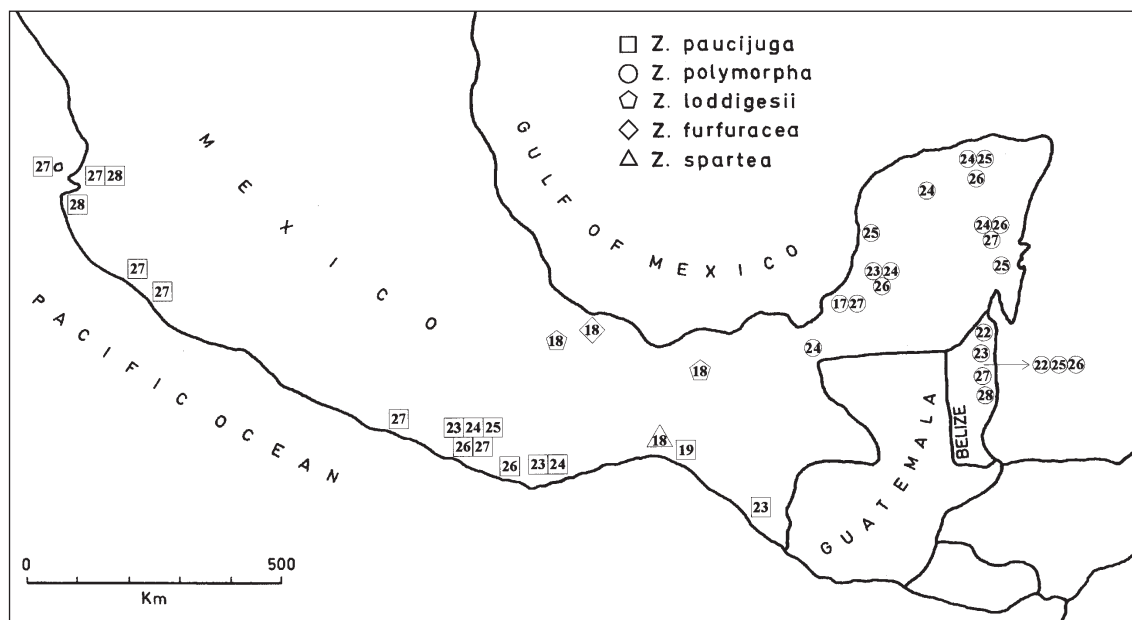


Fig. 1- Collecting localities of plants of *Z. paucijuga*, *Z. polymorpha* and of the related species *Z. loddigesii*, *Z. furfuracea* and *Z. spartea*. Both presently described and previously reported chromosome numbers are indicated. Numbers in symbols correspond to diploid chromosome numbers. Abutting symbols refer to plants from the same locality.

Table 1 - Collection data and karyotypes of examined plants of *Zamia*

Species	Place of cultivation ^a and Accession no.	Origin	2n	Karyotype				Figure
				M	S	A	T	
<i>Z. furfuracea</i> x <i>spartea</i> <i>Z. paucijuga</i>	FTG80/164	Cultivated	18	10	2	2	4	2A,3A
	NAP928/74	Oaxaca, Mexico	19	9	2	2	6	2B,3B
	NAP928/63	Oaxaca, Mexico	23	5	2	2	14	2C,3C
	NAP928/24	Oaxaca, Mexico	25	3	2	2	18	2D,3D
	NAP928/M1	Nayarit, Mexico	27	1	2	2	22	
	NAP928/M11	Nayarit, Mexico	27	1	2	2	22	2E,3E
	NAP928/46	Nayarit, Mexico	27	1	2	2	22	
	NAP928/M5	Nayarit, Mexico	28	2	2	24	2F,3F	
<i>Z. polymorpha</i>	NY1/89/11	Northern Belize	22	6	2	2	12	4A,5A
	NAP1606/M293	Northern Belize	23	5	2	2	14	
	NY101/91	Northern Belize	23	5	2	2	14	4B,5B
	NAP2285/0	Campeche, Mexico	23	5	2	2	14	
	NAP1606/9	Quintana Roo, Mexico	24	4	2	2	16	4C,5C
	NAP2288/0	Campeche, Mexico	24	4	2	2	16	
	NAP846/0	Northern Belize	25	3	2	2	18	
	NAP1606/10	Northern Belize	25	3	2	2	18	4D,5D
	NAP2292/0	Campeche, Mexico	26	2	2	2	20	
	NAP2290/0	Campeche, Mexico	26	2	2	2	20	
	NAP2286/0	Campeche, Mexico	26	2	2	2	20	
	NAP2284/0	Campeche, Mexico	26	2	2	2	20	
	NAP2282/0	Campeche, Mexico	26	2	2	2	20	4E,5E
	NAP1606/11	Yucatan, Mexico	26	2	2	2	20	
	NAP1606/M292	Central Belize	27	1	2	2	22	4F,5F
	NAP833/0	Central Belize	27	1	2	2	22	
	NAP1606/M300	Central Belize	27	1	2	2	22	
	NAP1606/4	Central Belize	27	1	2	2	22	
	NAP1606/17	Central Belize	28	2	2	24	4G,5G	
	NAP1606/1	Central Belize	28	2	2	24		

^a FTG: Fairchild Tropical Garden, Florida; NAP: Botanical Garden of Naples, Italy; NY: New York Botanical Garden, New York.

and *Z. polymorpha* as well as the karyotype of a hybrid plant synthesised by NORSTOG (1987, 1990) by crossing *Z. furfuracea* and *Z. spartea* are described in the present paper. The new karyological findings, together with phytogeographical data mainly deriving from authors' field investigations, are discussed for their implications in the karyo-systematics and phytogeography of *Z. paucijuga* and *Z. polymorpha* as well as of other species of *Zamia* in North and Central America.

MATERIALS AND METHODS

Plants of *Z. paucijuga*, *Z. polymorpha* and a hybrid *Z. furfuracea* x *Z. spartea* that were examined are listed in Table 1. Collecting localities of the plants are reported in Table 1

and are also shown on the map in Fig. 1.

Plants of *Z. paucijuga* and *Z. polymorpha* were field collected by the authors as well as by M. Vázquez Torres (Universidad Veracruzana, Mexico), D. W. Stevenson (The New York Botanical Garden, New York), S. Cozzolino, L. Gaudio (University of Naples, Italy) and G. Vallariello (Botanical Garden of Naples, Italy). Plants were then transferred to the Botanical Garden of Naples, to The New York Botanical Garden, and to the Fairchild Tropical Garden, Miami, Florida. The hybrid *Z. furfuracea* x *Z. spartea*, synthesised by NORSTOG (1990), is cultivated at the Fairchild Tropical Garden. The present research was conducted at all three institutions (Table 1).

Chromosome observations were based on root tip mitosis. Root tips were collected from 10:00 am to 1:00 pm, because a decrease in

mitotic frequency has been noted in roots collected at other hours. Root tips were pre-treated in isopropyl-N-phenylcarbamate (IPC), which proved to be very efficacious in shortening the very large chromosomes of cycads. IPC was prepared as suggested by NORSTOG (1980) with the technique modified by extending the pre-treatment from 12 to 24 h. This extension of pre-treatment resulted in a greater reduction in chromosome size and in a more reliable count in plants with odd chromosome numbers. Root tips were fixed in ethanol: acetic acid (3:1) for 30 min and stained following the Feulgen technique. Hydrolysis was carried out in 1N HCl at 58 °C for 8 min. Staining in leucobasic fuchsin lasted 20 min. Tips were squashed in 45% acetic acid before observation.

In order to eliminate bias with respect to the origin of the samples, a code number was given to each sample when it was collected. Each chromosome number was proposed after the observation of at least twenty metaphase plates. Data from the specimen of *Z. paucijuga* NAP 928/74 (Table 1) were obtained from observations conducted over a three year period involving three different vegetative cycles (i.e., three annual leaf flushes) of the plant in order to eliminate perturbations that might occur in any given growth cycle.

RESULTS AND DISCUSSION

All the chromosome numbers and karyotypes reported are listed in Table 1. Metaphase plates and karyograms are shown in Figs. 2-5.

The hybrid plant *Z. furfuracea* x *Z. spartea* is $2n=18$ with ten metacentric (M) chromosomes, two submetacentric (S) chromosomes, two acrocentric (A) chromosomes and four telocentric (T) chromosomes (Table 1; Figs. 2A, 3A). The same number and karyotype are already known for the parental species (MORETTI 1990; NORSTOG 1980).

Both *Z. paucijuga* and *Z. polymorpha* have more than one chromosome number.

In *Z. paucijuga*, one plant is $2n=19$ with $9M+2S+2A+6T$ (Table 1; Figs. 2B, 3B); one

plant is $2n=23$ with $5M+2S+2A+14T$ (Table 1; Figs. 2C, 3C); one plant is $2n=25$ with $3M+2S+2A+18T$ (Table 1; Figs. 2D, 3D); three plants are $2n=27$ with $1M+2S+2A+22T$ (Table 1; Figs. 2E, 3E); and one plant is $2n=28$ with $2S+2A+24T$ (Table 1; Figs. 2F, 3F). The cytotype $2n=19$ is new for the species. The cytotypes $2n=23, 25, 27, 28$ have been previously recorded as well as two others, $2n=24$ and 26 , not reported here for *Z. paucijuga* (MORETTI 1990; MORETTI & SABATO 1984).

In *Z. polymorpha*, one plant is $2n=22$ with $6M+2S+2A+12T$ (Table 1; Figs. 4A, 5A); three plants are $2n=23$ with $5M+2S+2A+14T$ (Table 1; Figs. 4B, 5B); two plants are $2n=24$ with $4M+2S+2A+16T$ (Table 1; Figs. 4C, 5C); two plants are $2n=25$ with $3M+2S+2A+18T$ (Table 1; Figs. 4D, 5D); six plants are $2n=26$ with $2M+2S+2A+20T$ (Table 1; Figs. 4E, 5E); four plants are $2n=27$ with $1M+2S+2A+22T$ (Table 1; Figs. 4F, 5F); and two plants are $2n=28$ with $2S+2A+24T$ (Table 1; Figs. 4G, 5G). The cytotypes $2n=23$ and 28 are new for the species. MORETTI *et al.* (1991) have also previously published $2n=22$ and 26 . Additionally, VOVIDES & OLIVARES (1996) observed $2n=17, 24, 25, 26$, and 27 in *Z. polymorpha* (as *Z. loddigesii*).

Secondary constrictions, knobs and very small satellites are observable in some preparations from all species. Because these structures are inconsistent, even in metaphase plates from the same root tip, their occurrence, number, and significance in each plant remains enigmatic. Therefore, no discussion on the meaning of these structures will be provided here.

The number of A-chromosomes seems to be variable in preparations from different plants in each species and sometimes from the same individual, ranging from a minimum of two to a maximum of eight. The increase in A-chromosome number is correlated with a parallel decrease in T-chromosome number. Very short arms are observable on the putative A-chromosomes (see, as examples, Figs. 3D and 5B). The short arms also appear heterochromatic. The distinction between A- and T-chromosomes have always represented an intriguing problem in karyological studies of *Zamia*

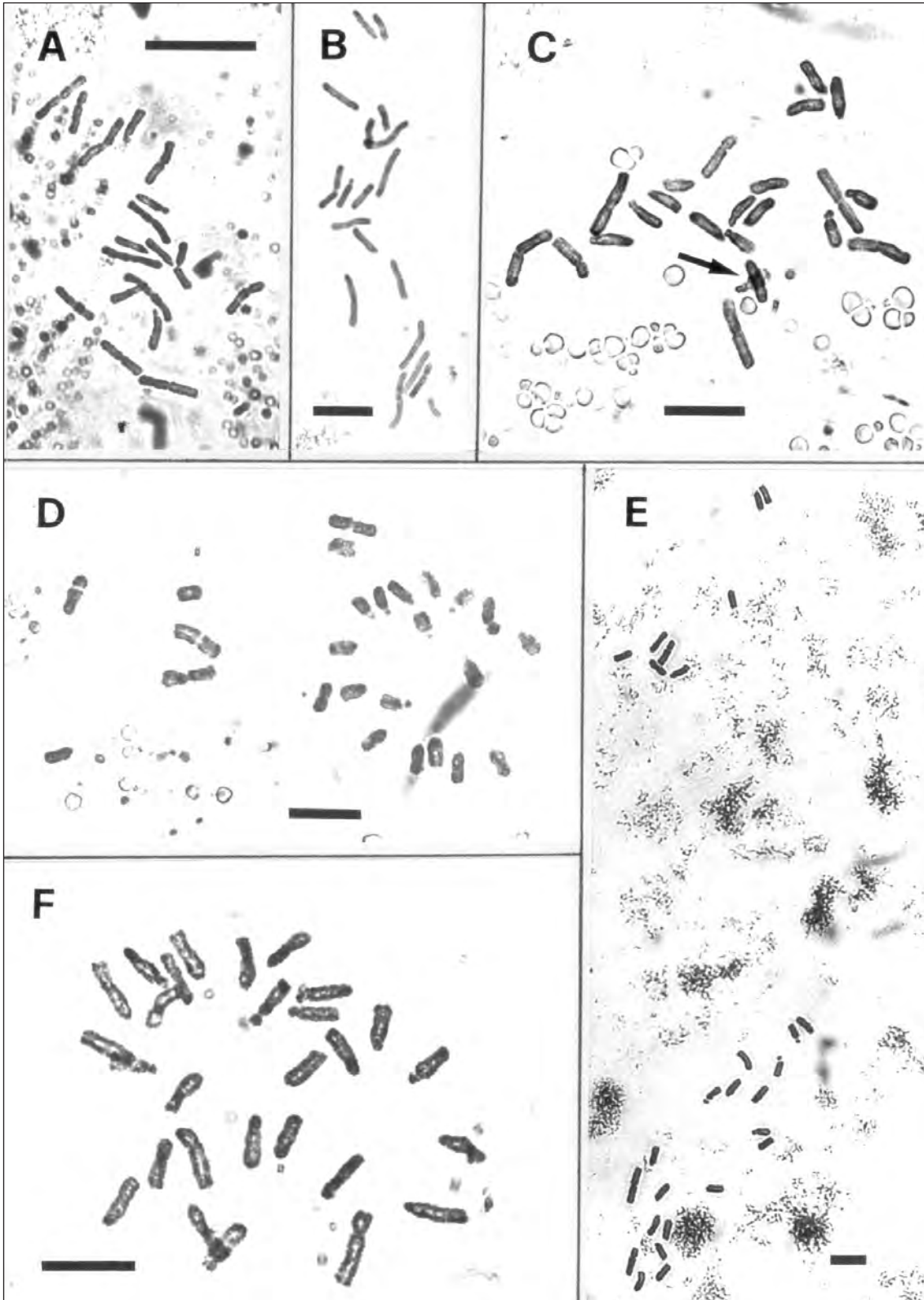


Fig. 2- Metaphasic plates of *Z. furfuracea* x *Z. spartea* and of plants of *Z. paucijuga*. - **A.** *Z. furfuracea* x *Z. spartea* ($2n = 18$ with $10M+2S+2A+4T$). - **B.** *Z. paucijuga* ($2n = 19$ with $9M+2S+2A+6T$). - **C.** *Z. paucijuga* ($2n = 23$ with $5M+2S+2A+14T$) (Two overlapping chromosomes arrowed). - **D.** *Z. paucijuga* ($2n = 25$ with $3M+2S+2A+18T$). - **E.** *Z. paucijuga* ($2n = 27$ with $1M+2S+2A+22T$). - **F.** *Z. paucijuga* ($2n = 28$ with $2S+2A+24T$). (Bar = $15\mu\text{m}$)

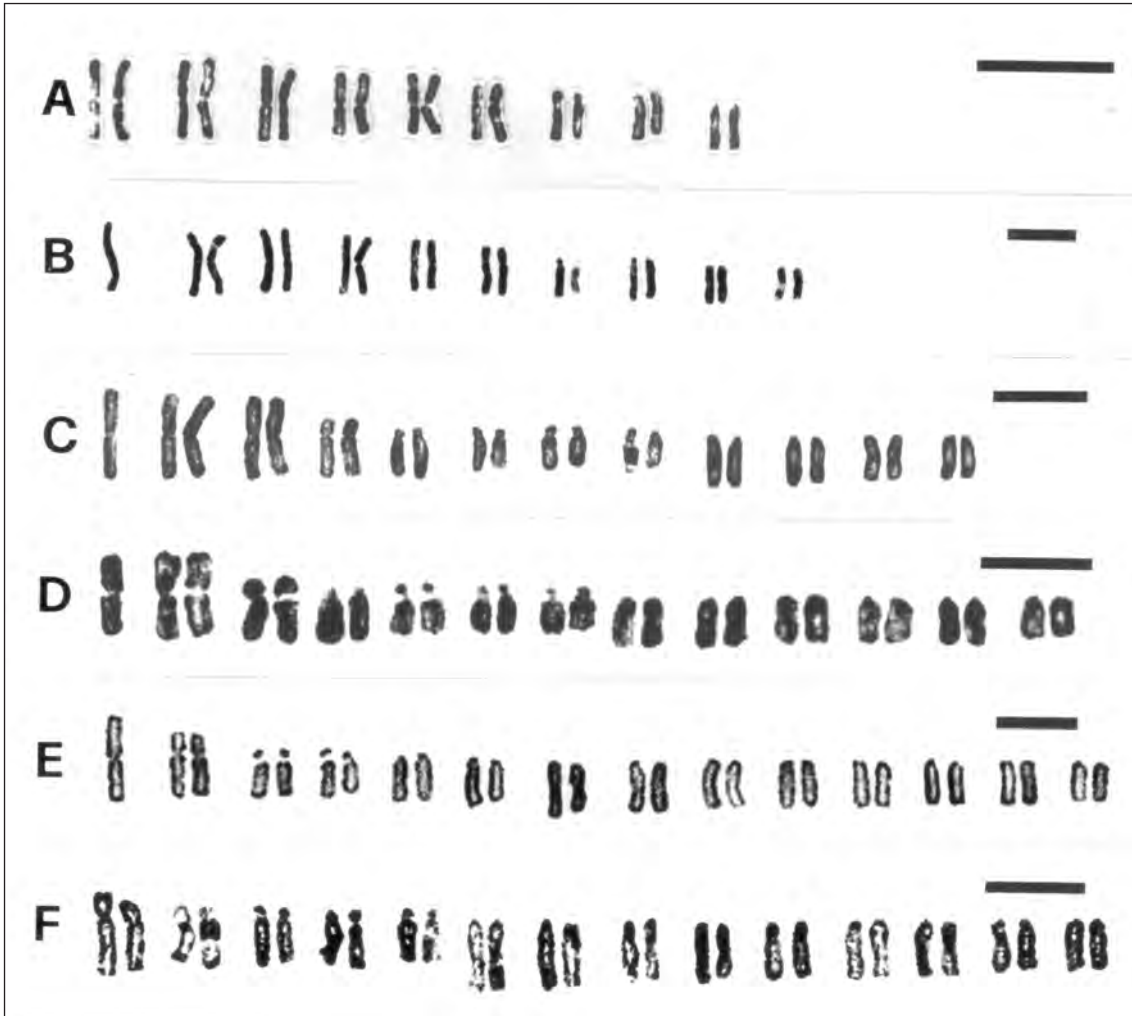


Fig. 3- Karyograms of *Z. furfuracea* x *Z. spartea* and of plants of *Z. paucijuga*. - A. *Z. furfuracea* x *Z. spartea* ($2n = 18$ with $10M+2S+2A+4T$). - B. *Z. paucijuga* ($2n = 19$ with $9M+2S+2A+6T$). - C. *Z. paucijuga* ($2n = 23$ with $5M+2S+2A+14T$). - D. *Z. paucijuga* ($2n = 25$ with $3M+2S+2A+18T$). - E. *Z. paucijuga* ($2n = 27$ with $1M+2S+2A+22T$). - F. *Z. paucijuga* ($2n = 28$ with $2S+2A+24T$). (Bar = $15\mu\text{m}$)

(MORETTI *et al.* 1991, and references therein). Because the products of centric fission may involve both T- and A-chromosomes with very reduced short arms (LIMA-DE-FARIA 1983, and references therein), centric fission has been regarded as the cause of the variable counts for the two types of chromosomes in *Zamia* (CAPUTO *et al.* 1996; MORETTI 1990; MORETTI *et al.* 1991; VOVIDES & OLIVARES 1996). Interbreeding between plants with different chromosome numbers would contribute to the variability of the relative numbers of A- and T-chromosomes by affecting their distribution in progeny. Because of the possible random acquisition or loss of acrocentric-like chromo-

somes in these plants via centric fission and/or interbreeding mechanisms, we considered only two A-chromosomes (the minimum number always observed in all preparations) as present in all complements (Table 1), in order to give more uniformity to the data. For these reasons, we reconsidered previous data reported for *Z. paucijuga* (MORETTI & SABATO 1984), and reduced to two the number of eight A-chromosomes previously recorded in some plants of this species. We also adapted the terminology used by VOVIDES & OLIVARES (1996) in the description of chromosomes of *Z. polymorpha* (as *Z. loddigesii*) to our chromosome nomenclature. By incorporating these modifications,

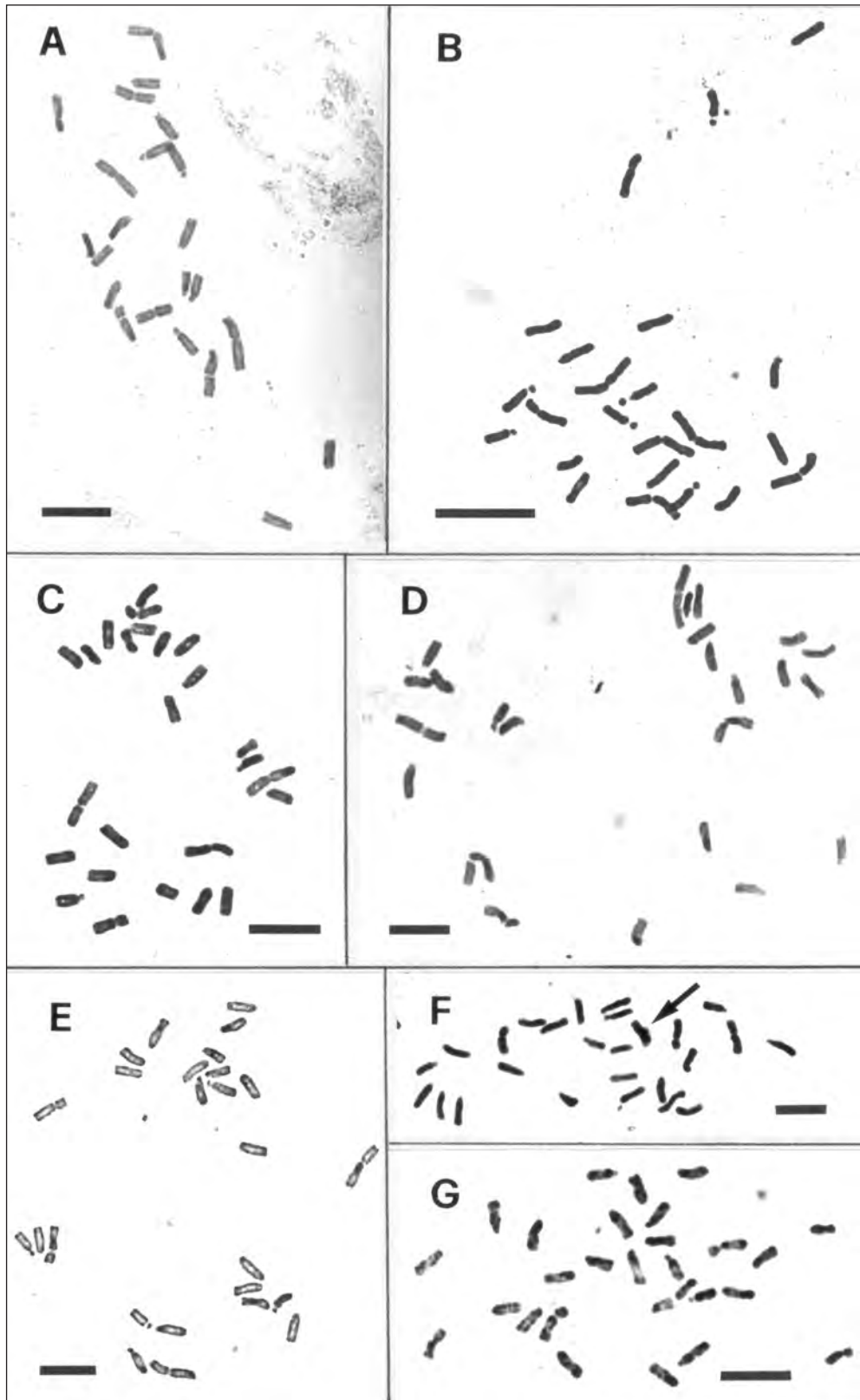


Fig. 4- Metaphasic plates of plants of *Z. polymorpha*. - **A.** *Z. polymorpha* ($2n = 22$ with $6M+2S+2A+12T$). - **B.** *Z. polymorpha* ($2n = 23$ with $5M+2S+2A+14T$). - **C.** *Z. polymorpha* ($2n = 24$ with $4M+2S+2A+16T$). - **D.** *Z. polymorpha* ($2n = 25$ with $3M+2S+2A+18T$). - **E.** *Z. polymorpha* ($2n = 26$ with $2M+2S+2A+20T$). - **F.** *Z. polymorpha* ($2n = 27$ with $1M+2S+2A+22T$) (Two overlapping chromosomes arrowed). - **G.** *Z. polymorpha* ($2n = 28$ with $2S+2A+24T$). (Bar = 15μ)



Fig. 5- Karyograms of plants of *Z. polymorpha*. A. *Z. polymorpha* ($2n = 22$ with $6M+2S+2A+12T$). - B. *Z. polymorpha* ($2n = 23$ with $5M+2S+2A+14T$). - C. *Z. polymorpha* ($2n = 24$ with $4M+2S+2A+16T$). - D. *Z. polymorpha* ($2n = 25$ with $3M+2S+2A+18T$). - E. *Z. polymorpha* ($2n = 26$ with $2M+2S+2A+20T$). - F. *Z. polymorpha* ($2n = 27$ with $1M+2S+2A+22T$). - G. *Z. polymorpha* ($2n = 28$ with $2S+2A+24T$). (Bar = 15μ)

the cytotypes described here for *Z. paucijuga* and *Z. polymorpha* (see Table 1) have chromosome formulae which are equivalent to those already reported in the two species (see Table 2).

Centric fission, taken into consideration by the authors who previously studied *Z. paucijuga* and *Z. polymorpha* (CAPUTO *et al.* 1996; MORETTI & SABATO 1984; MORETTI *et al.* 1991; VOVIDES & OLIVARES 1996), is proposed also here as the mechanism involved in the newly reported chromosome variation and odd diploid numbers (Table 1). As in previous studies, the mechanism of centric fission is suggested on the observation that variation in M- and T-chromosome numbers occurs in an inverse ratio in the cytotypes. That is, for every decrease in one metacentric chromosome there is a concomitant increase of two telocentric chromosomes (Table 1). Our choice of centric fission is an alternative to that of centric fusion as proposed by NORSTOG (1980) and was thor-

oughly discussed in CAPUTO *et al.* (1996).

Further, still undiscovered chromosome numbers may occur and indeed may be predicted via Robertsonian changes in *Z. paucijuga* and *Z. polymorpha*. That is, one would predict that those numbers included in the range $2n=19-28$, but unreported as yet for *Z. paucijuga* should occur, namely $2n=20-22$. Similarly, for *Z. polymorpha* one would predict the occurrence of diploid numbers between $2n=18-21$.

In order to understand the origin of karyological variability within these morphologically and geographically diverse species, a comparison of their ecological situations and distribution with other related species of *Zamia* which also occur in Mexico, Guatemala, and Belize is necessary. These species and their cytological data are listed in Table 2.

Zamia paucijuga and *Z. polymorpha* are geographically well separated (Fig. 1).

Table 2 - Karyotypic data of the species of *Zamia* discussed in the text

Species	Origin of plants ^a	2n	Karyotype ^b				Source
			M	S	A	T	
<i>Z. amblyphyllidia</i>	Puerto Rico	16	12	2	2		c
<i>Z. cremnophila</i>	Mexico	16	12	2	2		c, d
<i>Z. fischeri</i>	Mexico	16	12	2	2		e, f, g
<i>Z. inermis</i>	Mexico	16	12	2	2		h
<i>Z. integrifolia</i>	U.S.A.	16	12	2	2		c
<i>Z. portoricensis</i>	Puerto Rico	16	12	2	2		c, i
<i>Z. pumila</i>	Dominican Republic	16	12	2	2		c
<i>Z. purpurea</i>	Mexico	16	12	2	2		h
<i>Z. pygmaea</i>	Cuba	16	12	2	2		c, i
<i>Z. soconuscensis</i>	Mexico	16	12	2	2		d
<i>Z. splendens</i>	Mexico	16	12	2	2		c, j
<i>Z. polymorpha</i>	Mexico	17	11	2	2	2	k
<i>Z. furfuracea</i>	Mexico	18	10	2	2	4	c, f, i
<i>Z. loddigesii</i>	Mexico	18	10	2	2	4	c, f, i
<i>Z. spartea</i>	Mexico	18	10	2	2	4	c, l
<i>Z. vazquezii</i>	Mexico	18	10	2	2	4	m, n
<i>Z. paucijuga</i>	Mexico	19	9	2	2	6	q
<i>Z. variegata</i>	Mexico	21	7	2	2	10	o
<i>Z. variegata</i>	Mexico	22	6	2	2	12	o
<i>Z. polymorpha</i>	Belize	22	6	2	2	12	p, q
<i>Z. paucijuga</i>	Mexico	23	5	2	2	14	c, f, q
<i>Z. polymorpha</i>	Mexico, Belize	23	5	2	2	14	q
<i>Z. paucijuga</i>	Mexico	24	4	2	2	16	c
<i>Z. polymorpha</i>	Mexico	24	4	2	2	16	k, q
<i>Z. paucijuga</i>	Mexico	25	3	2	2	18	c, f, q
<i>Z. polymorpha</i>	Mexico, Belize	25	3	2	2	18	k, q
<i>Z. paucijuga</i>	Mexico	26	2	2	2	20	c, f
<i>Z. polymorpha</i>	Mexico, Belize	26	2	2	2	20	k, p, q
<i>Z. paucijuga</i>	Mexico	27	1	2	2	22	c, f, q
<i>Z. polymorpha</i>	Mexico, Belize	27	1	2	2	22	k, q, r
<i>Z. paucijuga</i>	Mexico	28		2	2	24	c, f, q
<i>Z. polymorpha</i>	Belize	28		2	2	24	q

^aCollecting localities of plants are indicated in Fig. 1. ^bAuthors' interpretation of the chromosomes morphology in some karyotypes previously described is given (see the text for details). ^cMORETTI 1990; ^dSCHUTZMAN *et al.* 1988; ^eMARCHANT 1968; ^fMORETTI and SABATO 1984; ^gMORETTI *et al.* 1991; ^hVOVIDES 1983; ⁱNORSTOG 1980; ^jSCHUTZMAN 1984; ^kVOVIDES and OLIVARES 1996 (as *Z. loddigesii*); ^lVOVIDES 1983 (as *Z. loddigesii* var. *angustifolia*); ^mMORETTI *et al.* 1991 (as *Z. fischeri* from Veracruz); ⁿSTEVENSON *et al.* 1995-1996b; ^oMORETTI *et al.* 1991 (as *Z. picta*); ^pMORETTI *et al.* 1991 (as *Zamia* sp. nov.); ^qPresent report; ^rNORSTOG 1980 (as *Z. loddigesii* from Yucatan).

Morphologically, the two species are similar but differ in leaflet serration and male cone characters such as colour of pubescence and shape of the microsporophylls. General similarities include a wide range of leaf shape; leaflet number, shape, and texture; cytotypes; geographic distribution. Both species have a preference for open disturbed areas with poor soils and, in fact, individual plants do poorly in well-developed secondary vegetation. For example, the plasticity of leaf and leaflet characters such as number, shape, and texture are directly correlated. The adaptative morphological plasticity consists in both species of “sun” forms, characterised by narrow, coriaceous, acute leaflets and reduced leaflet numbers per leaf growing in open xeric habitat, and “shade” forms, with broad, papyraceous, acuminate leaflets and more leaflets per leaf growing in understory humid areas of secondary vegetation. In fact some plants can be found with both types of leaves on the same plant where one leaf is in full sun and the other in shade (STEVENSON *et al.* 1995-1996a).

Zamia paucijuga and *Z. polymorpha* form a clade together with *Z. variegata* Warszewicz (CAPUTO *et al.* 1996; these authors report *Z. polymorpha* as *Zamia* sp. nov. and *Z. variegata* as *Z. picta*). *Zamia variegata* occurs from Chiapas, Mexico to Belize and Guatemala and is characterised by having distinct yellow spots on the leaflets. The species occupies primary and secondary forest and, in particular, disturbed and regenerated forest. Morphologically, it is quite variable within its range. This variability parallels that found in *Z. paucijuga* and *Z. polymorpha* in leaf and leaflet characters. *Zamia variegata* also exhibits variability in number and size of the achlorophyllous spots and the size and colour of the hypogeous stem. There appear to be both sun and shade forms which are related to the amount of habitat disturbance and hence exposure. Connected to this is cytological variation in chromosome number, with $2n=21$ and 22 (MORETTI *et al.* 1991) (Table 2). Thus, as in *Z. paucijuga* and *Z. polymorpha*, cytological variability is correlated with morphological and habitat variability.

Among the species listed in Table 2, *Z. fur-*

furacea, *Z. loddigesii* and *Z. sparteae* form a clade considered the most closely related to *Z. paucijuga* and *Z. polymorpha* (as *Zamia* sp. nov. in CAPUTO *et al.* 1996). In comparison to the latter two species each of the former three has a much narrower geographic range, little morphological variation in leaf and leaflet characters, and more specialised habitat preferences. All three have a chromosome number of $2n=18$ (Table 2), with no variation reported in spite of numerous observations. Each of these three species has distinctive morphological feature separating them, with no intermediates. For example, *Z. furfuracea* is the only species of *Zamia* that retains pubescence on its mature leaflets and has obovate, extremely coriaceous, sclerophyllous, blunt leaflets. It grows in coastal Veracruz, Mexico (Fig. 1) in sandy soils and often on cliffs above the sea. In contrast, *Z. sparteae* has glabrous, very narrow, oblanceolate, adaxially canaliculate, coriaceous but not sclerophyllous, attenuate leaflets. It is found in only two known populations in the area of the Isthmus of Tehuantepec, at the Oaxaca-Chiapas border in Mexico (Fig. 1). *Zamia loddigesii* has a somewhat greater variability in leaflet morphology and appears intermediate in general shape and size between *Z. furfuracea* and *Z. sparteae*. This lead some authors to suggest that *Z. loddigesii* represents a hybrid between *Z. sparteae* and *Z. furfuracea* (NORSTOG 1987, 1990; SCHUTZMAN 1987). Indeed, cytological data support this or at least do not militate against it, because the karyotypes of all three are identical (MORETTI 1990) as that of the hybrid synthesised by Norstog, reported here. However, leaflets of *Z. loddigesii* have neither the persistent pubescence at maturity, obovate shape, blunt apices, and sclerophyllous features found in *Z. furfuracea* nor the narrow, adaxially canaliculate, attenuate apices found in *Z. sparteae*. Moreover, when artificial hybrids between *Z. furfuracea* and *Z. sparteae* are produced, the F_1 plants are exclusively intermediate (NORSTOG 1990) and the F_2 plants exhibit continuous variation between the extremes of parental species (NORSTOG 1990), but the results of two-tailed t-tests on the F_2 plants support the contention that the two parental species are in

fact distinct (NORSTOG 1990) and the continuity of characters is not found in the field. The clade composed of these three species is an unresolved trichotomy (CAPUTO *et al.* 1996), with *Z. furfuracea* and *Z. spartea* each having autapomorphies, whereas *Z. loddigesii* has none observable. All of this does not mean that *Z. loddigesii* is not of hybrid origin as a result of past events. However, the present day distribution of all three (Fig. 1) and the fact that species of *Zamia* are insect pollinated with each *Zamia* having strictly species-specific pollinators (Dennis Wm. Stevenson, personal communication) indicate that such hybrid events no longer occur.

Analysis of the ecological situations in areas in which the five species grow may supply reasons for the presence and absence of chromosome variation. Stable habitats are characteristic of *Z. furfuracea*, *Z. loddigesii* and *Z. spartea*. Although each grows under extreme conditions, particularly *Z. furfuracea* in very sandy soils and often exposed to salt spray and *Z. spartea* in dry, chaparral vegetation, each also exhibits specialised adaptations for these habitats which themselves are stable. *Zamia loddigesii* grows in more mesic situations preferring open but stable areas of subcloud forest. The karyotype of these taxa is also stable and the species exhibit little morphological diversity within themselves. In contrast, *Z. paucijuga* and *Z. polymorpha* grow in unstable habitats subjected to both natural and anthropogenous disturbance. Both these species have high karyotypic variability, wide morphological variability, and wide habitat conditions. Thus, once more, karyotypic variability would seem correlated with broad habitat conditions.

In order to find further support for this hypothesis we may consider other less closely related species of *Zamia* from northern Central America and the West Indies. Part of these species are members of the same clade (CAPUTO *et al.* 1996), whereas others were not investigated by CAPUTO *et al.* (1996). The Central American species are *Z. cremnophila* Vovides *et al.*, *Z. fischeri* Miq., *Z. inermis* Vovides *et al.*, *Z. purpurea* Vovides *et al.*, *Z. soconuscensis* Schutzman *et al.*, *Z. splendens*

Schutzman, and *Z. vazquezii* D. Stevenson *et al.* (Table 2). Each of these species has specialised, stable habitats and little if any morphological variability. For example, *Z. cremnophila* grows only on limestone cliffs in Tabasco, Mexico and *Z. fischeri* grows only in cloud forests in San Luis Potosí, Mexico. Each of these species is $2n=16$, the only exception being *Z. vazquezii* with $2n=18$ (Table 2); none of them exhibits variability in their karyotype (CAPUTO *et al.* 1996; MARCHANT 1968; MORETTI 1990; MORETTI *et al.* 1991; SCHUTZMAN 1984; SCHUTZMAN *et al.* 1988; STEVENSON *et al.* 1995-1996b; VOVIDES 1983). Similarly, each of the West Indian species, *Z. amblyphyllidia* D. Stevenson, *Z. integrifolia* L. f. (from Florida, USA, but phytogeographically related to West Indian species), *Z. portoricensis* Urban, *Z. pumila* L., and *Z. pygmaea* (Table 2), grows in a specialised, stable habitat, exhibits little morphological variability, and has a nonvariant chromosome number of $2n=16$ (MARCHANT 1968; MORETTI 1990; MORETTI *et al.* 1991; NORSTOG 1980; STEVENSON 1987).

All of these examples support the hypothesis that stable intraspecific diploid numbers are associated with morphological specialisation and stable habitats, and that unstable intraspecific diploid numbers are related to intraspecific morphological diversity as well as to habitat diversity. Thus, not so much extreme habitats but rather habitat variability leads to intraspecific cytological and morphological diversity. In fact, species such as *Z. furfuracea* or *Z. cremnophila*, that live in extreme habitats, at the same time occupy stable habitats.

Karyological data here reported for the hybrid *Z. furfuracea* x *Z. spartea* may help clarify other aspects in the karyosystematics of *Z. paucijuga* and *Z. polymorpha*. The supposed hybrid origin of *Z. loddigesii* from *Z. furfuracea* and *Z. spartea* (NORSTOG 1987, 1990; SCHUTZMAN 1987), together with morphological and distributive relationships between *Z. loddigesii*, *Z. paucijuga* and *Z. polymorpha* (CAPUTO *et al.* 1996; NORSTOG 1980, 1987, 1990; SCHUTZMAN 1987; SCHUTZMAN *et al.* 1988; VOVIDES & OLIVARES 1996) led us to suppose that these five species may have a

common origin. Within this group, *Z. paucijuga* and *Z. polymorpha* would occupy a relatively derived position, due to the suggested apomorphic state of their high numbered karyotypes (CAPUTO *et al.* 1996; MORETTI 1990; MORETTI & SABATO 1984; MORETTI *et al.* 1991; VOVIDES & OLIVARES 1996; and present data). On these bases, we may imagine that the original cytotype giving rise to the cytotypic series in *Z. paucijuga* and *Z. polymorpha* should belong to an ancestor common to all five species and possibly with $2n=16$ or 18 , with $2n=18$ being the chromosome number found in *Z. furfuracea*, *Z. spartea*, and *Z. loddigesii* (Table 2). As a result, we may also hypothesise that the area, in which *Z. furfuracea*, *Z. spartea*, and *Z. loddigesii* actually occur (Fig. 1), represents a centre of diversifi-

cation for species of *Zamia*. From this area, ancestral species might have sprayed both towards the Atlantic coast, where *Z. loddigesii*, *Z. furfuracea* and *Z. polymorpha* today live, and towards the Pacific coast, where *Z. paucijuga* occurs (Fig. 1). In the diffusion of *Z. paucijuga*, a particular role could have been played by *Z. spartea*, since this species is today intermediate in distribution between *Z. paucijuga* and the other three species (Fig. 1). *Zamia spartea* is localised on the Isthmus of Tehuantepec, the only geographical bridge between the two oceanic coasts in North America. *Zamia polymorpha* and *Z. paucijuga*, in colonizing the coasts, would have adapted to new and variable habitats by means of karyotypic changes.

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