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Nuclear DNA variation in diploid and polyploid taxa of *Larrea* (Zygophyllaceae)

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A study of nuclear DNA content was made in telophase nuclei (2C) of the root apex of germinating seed in nine populations of the following species and cytotypes of *Larrea*: *L. nitida* (2x), *L. divaricata* (2x), *L. cuneifolia* (4x) and *L. tridentata* (2x, 4x, 6x). There were no significant differences in DNA content per basic monoploid genome among the diploid taxa nor between the latter and the tetraploid, among tetraploids or between tetraploids and the hexaploid. On the other hand, the difference between means was significant when all diploids were compared with the hexaploid cytotype. These results would indicate:

(1) Speciation at the diploid level in *Larrea* has not produced great differences in DNA content per basic genome. This is in contrast with the related genus *Bulnesia*.
(2) In *Larrea* there is a slight diminution in DNA content per basic genome when there is an increase in ploidy level.
(3) Species of *Larrea*, *Bulnesia* and *Pintoa* (Zygophyllaceae) that inhabit the most arid environments are the ones possessing the highest DNA content.
(4) This increase is due to an increment in ploidy level in *Larrea* and an augment of intrachromosomal DNA in *Bulnesia* and *Pintoa*.

INTRODUCTION

The genus *Larrea* has an interesting disjunct amphitropical distribution covering arid and semi-arid regions of Argentina, Chile, Bolivia, Perú, México and Southwestern United States. It is subdivided into two taxa: Sect. Larrea, comprising the South American diploid ($2n = 26$) multifoliolate species *L. nitida* and *L. ameghinoi* and Sect. Bifolium, which includes bifoliolate species such as the South American "jarillas" *L. divaricata*, diploid, and *L. cuneifolia*, tetraploid ($2n = 52$), and the North American "gobernadora" or "creosote bush" *L. tridentata*. This last taxon comprises a diploid cytotype ($2n = 26$), occurring in the Chihuahuan desert, a tetraploid ($2n = 52$) in the Sonoran desert, and an hexaploid ($2n = 78$) in the Mohave desert (Hunziker *et al.* 1977, 1978).

The three cytotypes have almost exclusively an allopatric distribution and there is a correlation between the increase in ploidy level and the increment in aridity, the Mohave desert being the most extreme. Tetraploid and hexaploid populations would have originated through interracial autopolyploidy (Hunziker *et al.*, 1972, 1978; Yang *et al.*, 1977).

Bennett (1976, 1987) has suggested that interspecific variation in DNA content has adaptive significance and is correlated with the environment and the geographical distribution. In the present investigation the 2C DNA content of *L. nitida*, *L. divaricata*, *L. cuneifolia* and the three cytotypes of *L. tridentata* is studied. An analysis is attempted of the variations that might have occurred during speciation and the possible correlation with adaptation to arid environments.

MATERIALS AND METHODS

The seed was usually collected from 25 randomly chosen individuals in each population. Representative herbarium specimens are deposited at the herbaria of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (BACFC), the Missouri Botanical Garden (MO) and the Instituto de Botánica Darwinion (SI).

Abbreviations correspond to J. H. Hunziker (JHH) and A. D. Burghardt (ADB).

The origin of the materials is as follows:

L. nitida. Argentina, Prov. Mendoza, Depto. Las Heras, Between Punta de Vacas and Arroyo El Tambillito, 2200 m elevation. JHH 9870.

L. divaricata. Argentina, Prov. Río Negro, Depto. San Antonio, Las Grutas, 20 m elevation. ADB 31. Argentina, Prov. La Pampa, Depto. Caleu Caleu, Anzoategui, 100 m elevation. ADB 5.

L. cuneifolia. Argentina, Prov. La Rioja, Depto. Capital, Near the airport and Río de la Rodadera, 500 m elevation. JHH 9723. Argentina, Prov. Río Negro, Depto. San Antonio, Route 251, 9 Km North of junction of routes 3 and 251. ADB 52.

L. tridentata. (2x). U.S.A., Arizona, Mountview., *ca.* 25 miles SE of Tucson, 3550 ft. Col. T. W. Yang.

L. tridentata. (4x). U.S.A. Arizona, Tucson, Bellevue, 2490 ft. Col. T. W. Yang.

L. tridentata. (6x). U.S.A., California, San Bernardino Co., Victorville, Vacant lot, 200-300 m from hotel. JHH 10025. U.S.A., Nevada, Nye Co., route 95, between Tonopah and Scotty's junction, 52 miles South of Tonopah. JHH 10026.

DNA content was measured in telophase nuclei (2C) at the root apex of germinating seed. For germination, seeds were placed in petri dishes on wet filter paper. Roots of 0·5–1 cm length were fixed in 3 absolute ethanol:1 acetic acid during 1–4 days. *Allium cepa* roots were used as a standard. After fixation, the roots were rinsed 30 minutes in distilled water. Hydrolysis was carried out with 5 N HCl at 20°C (Deitch *et al.*, 1968; Fox, 1969). Different times of hydrolysis were investigated and the optimum period determined was 50 minutes. After hydrolysis, the material was rinsed three times with distilled water for 10 or 15 minutes.

Staining was carried out with Feulgen stain at *p*H 2·2 for 2 hours (Teoh and Rees, 1976). The material was then rinsed three times in SO_2 water for 10 minutes each rinse, then rinsed again with distilled water (5 to 15 minutes) and, finally, squashed in 45 per cent acetic acid. The coverslip was removed after freezing with CO_2 and the material was dehydrated in absolute alcohol and mounted in euparal.

The amount of Feulgen staining per nucleus, expressed in arbitrary units, was measured at a wavelength of 570 mm using the scanning method in a Zeiss Cytoscan. The basic genome DNA content expressed in pg was calculated using *Allium cepa* as a standard (2C = 33·5 pg; Bennett and Smith, 1976). The differences in DNA content between populations and species were tested through an analysis of variance and comparisons between means using the Games and Howell method (Sokal and Rohlf, 1981) or Scheffé's method (Scheffé, 1959).

Chromosome studies were based on squashes from root tips pretreated with saturated aqueous solution of paradichlorobenzene and fixed in a 3 ethyl alcohol:1 acetic acid solution. After maceration in 5N HCl at 20°C; 2 per cent acetic haematoxylin was used for staining.

RESULTS

Chromosome numbers were determined for all strains. *L. divaricata* and *L. cuneifolia* were found to be diploid and tetraploid as expected and this confirmed several counts from different regions of their range made previously on these species (Hunziker *et al.*, 1978). The chromosome numbers obtained in *L. tridentata* agree with the innumerable cytological determinations made by Yang (1967, 1968, 1970) and confirms the diploid, tetraploid and hexaploid nature of the Chihuahuan, Sonoran and Mohave cytotypes, respectively (Yang *et al.*, 1977).

Table 1 Nuclear DNA content in different populations of *Larrea divaricata* (2x), *L. cuneifolia* (4x) and *L. tridentata* (6x)

Species	Population	DNA content (2C) (A.U.)	No. of nuclei	G & H(1)
L. divaricata	ADB n°31	1·416 ± 0·067	24	2·946 NS[a]
(2x)	ADB n°5	1·728 ± 0·082	17	
L. cuneifolia	ADB n°52	2·217 ± 0·115	14	1·171 NS[b]
(4x)	JHH n°9723	2·523 ± 0·227	16	
L. tridentata	JHH n°10025	3·042 ± 0·120	34	1·832 NS[b]
(6c)	JHH n°10026	3·541 ± 0·244	12	

Level of significance: $\alpha = 0{\cdot}01^{a}$; $\alpha = 0{\cdot}05^{b}$.
(1) Games and Howell method, program MCHETV, Sokal and Rohlf (1981).

Table 2 Nuclear DNA content in populations of species of *Larrea*

Species or cytotype	Ploidy level	2*n*	No. of nuclei	DNA content (2C) $\bar{X} \pm$ S.E. (A.U.)	DNA content (2C) pg.	DNA per basic genome A.U.	DNA per basic genome pg.
L. nitida	2x	26	60	1·557 ± 0·090	1·625	0·779	0·813
L. divaricata	2x	26	41	1·545 ± 0·056	1·614	0·773	0·807
L. tridentata	2x	26	38	1·439 ± 0·086	1·502	0·719	0·751
L. tridentata	4x	52	60	2·436 ± 0·072	2·543	0·609	0·636
L. cuneifolia	4x	52	30	2·380 ± 0·136	2·485	0·595	0·621
L. tridentata	6x	78	46	3·173 ± 0·113	3·313	0·529	0·552

DNA contents in arbitrary units and in picograms are summarized in tables 1 and 2. Figure 1 shows the frequency histograms of the species and cytotypes indicating the number of nuclei studied and their DNA content in arbitrary units. For each population 2 or 3 replicates were made which presented nonsignificant differences.

In *L. divaricata* (diploid), *L. cuneifolia* (tetraploid) and *L. tridentata* (hexaploid) two populations from different localities were studied (table 1. In the three species, using an approximate test of equality of means (Games and Howell method, Sokal and Rohlf, 1981) the differences between averages of different populations of each species were non significant. As a consequence data were pooled for each species in table 2.

An analysis of variance for the DNA content per basic genome of the species listed in table 2 indicates that there are significant differences among the taxa ($F = 9{\cdot}776$, $P < 10^{-7}$). Comparisons made through Scheffé's method indicated that there are no significant differences in DNA content per basic genome either among diploids or between the tetraploids *L. cuneifolia* and *L. tridentata.* There are no significant differences either when the following pairwise comparisons are made: (1) *L. tridentata* (diploid) with *L. tridentata* (tetraploid) and (2) *L. tridentata* (tetraploid) with *L. tridentata* (hexaploid). On the other hand, the difference between means was significant when each diploid was compared separately with the hexaploid cytotype of *L. tridentata.* These results would indicate that there is a slight diminution in DNA content per basic genome when there is an increase in ploidy level.

For evaluating the variation in DNA content, 2C and per basic genome, in relation to ploidy level, a regression variance analysis was made. In both cases linear regression was highly significant and the results that were obtained are represented in fig. 2. The 2C DNA content increases significantly with ploidy level ($y = 0{\cdot}64 + 0{\cdot}43x$; $F = 98{\cdot}62$; $P < 10^{-10}$) but the estimated regression line has a gentler slope than a calculated hypothetical line, which assumes that when the number of genomes increases, DNA is added as an exact multiple of the DNA content per basic genome (fig. 2(A)). This difference is correlated with the obtention of a negative regression when DNA content per basic genome is plotted against ploidy level ($y = 0{\cdot}81 - 0{\cdot}047x$; $F = 97{\cdot}76$; $P < 10^{-11}$; fig. 2(B)).

In fig. 3 the 2C DNA content is plotted against ploidy level for the species of *Larrea* studied here and for the related genera *Bulnesia* (Poggio and Hunziker, 1986) and *Pintoa* (Poggio and Naranjo, in press).

DISCUSSION

Within the genus *Larrea* the multifoliolate group (Sect. Larrea) is the most primitive and comprises the two diploid species *L. ameghinoi* and *L. nitida*, having 3–16 leaflets. The supposedly more advanced group Sect. Bifolium consists of bifoliolate (*L. divaricata* ($2n = 26$), *L. cuneifolia* ($2n = 52$) and *L. tridentata* ($2n = 26$, 52, 78)) which have their leaves reduced to only one pair of leaflets. The bifoliolate species seem to represent a reductional trend in response to aridity (Hunziker *et al.*, 1977).

The DNA 2C contents of *L. nitida* and *L. divaricata* (table 2), both diploid, but one from the primitive section and the other from the more advanced, respectively, do not differ significantly. *L. divaricata* has a much wider distribution (Hunziker *et al.*, 1977, figs. 2.3 and 2.1) and has a wider range of habitats. *L. nitida* is restricted to the coolest and more humid habitats of the whole range occupied by *L. divaricata.* DNA content in Patagonian *L. ameghinoi* was not studied because

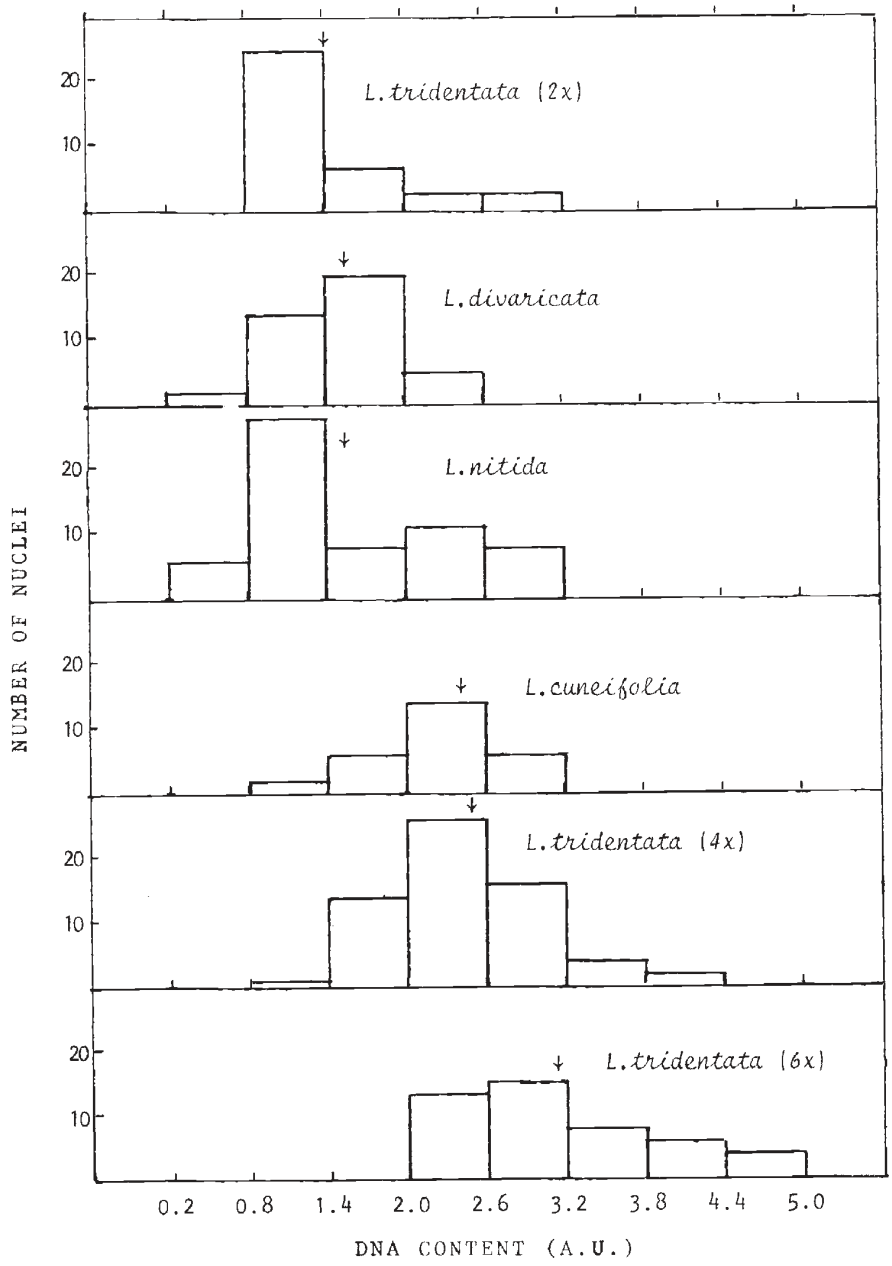

Figure 1 Frequency histograms: 2C DNA content in arbitrary units (A.U.) plotted against number of nuclei.

of lack of viable seed but it is likely that its content does not differ greatly from that found in *L. nitida* since their natural hybrid is completely fertile, shows normal meiosis with good pairing, without noticeable heteromorphic bivalents and both taxa could be considered partially sympatric semi-species (Hunziker *et al.*, 1978). The morphological and physiological variations producing adaptation to aridity and other rigorous conditions (low temperatures, wind, etc) in the diploid species of *Larrea* are apparently not correlated with great variation of their DNA content. We may conclude that, speciation at the diploid level in *Larrea* has not produced great differences in DNA content

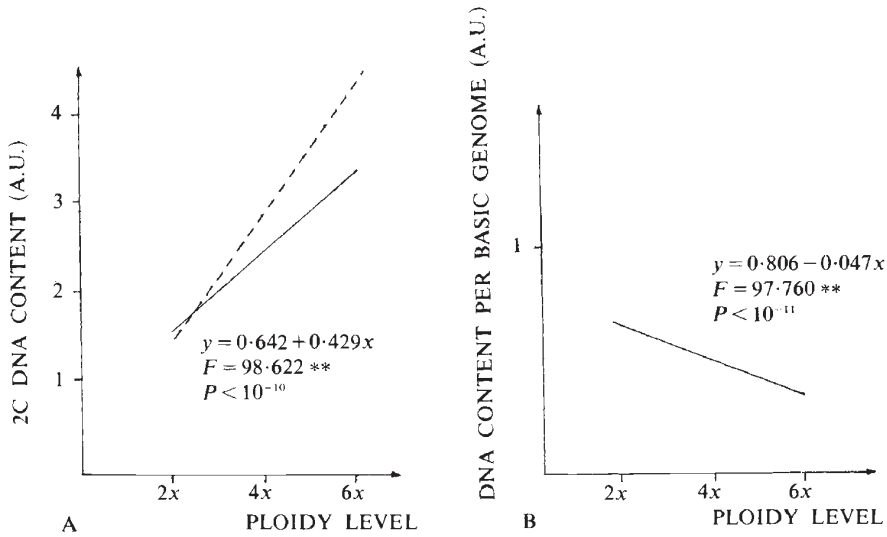

Figure 2 A = Regression 2C total DNA content on ploidy level in cytotypes of *Larrea*. Solid line = observed, broken = expected regression. B = DNA content per basic genome plotted against ploidy level (regression line).

per basic genome. The opposite has occurred in the closely related genus *Bulnesia* (Poggio and Hunziker, 1986; Poggio *et al.*, 1986).

On the other hand, when the DNA contents per basic genome of tetraploid *L. cuneifolia* and tetraploid and hexaploid cytotypes of *L. tridentata* are analyzed a slight tendency towards diminution is observed when there is an increase in polyploidy (table 2, fig. 2(B)).

Even when a stronger compaction of DNA in polyploid nuclei produces an underestimate of the measurements (Verma and Rees, 1974; Kenton, 1984*b*) it has been observed in several cases that polyploids have smaller chromosomes and lower DNA content than the expected (Darlington, 1965; Grant, 1976, 1987; Bennett, 1987; Martínez and Ginzo, 1985; Poggio and Hunziker, 1986; Poggio and Naranjo, in press). In *Larrea* due to the very small size of its chromosomes it is not known whether the diminution affects all chromosomes or only part of the complement. DNA content per basic genome in the diploid taxa of *Larrea* (0·75–0·81 pg, see table 2) is slightly higher than the content of the species that Palacios and Hunziker (1984) considered as primitive in the closely related genus *Bulnesia* (*B. schickendantzii* and *B. foliosa*, 0·58 pg and 0·50 pg, respectively; Poggio and Hunziker, 1986). In *Bulnesia* octoploid *B. bonariensis* has the smallest DNA content per basic genome and the smallest chromosomes (Poggio and Hunziker, 1986; Poggio *et al.*, 1986).

The DNA content of autotetraploid *L. tridentata* does not differ from that of *L. cuneifolia*. The latter species is an allopolyploid, one of its parental diploids being *L. divaricata* and the other a now extinct and unknown diploid (Hunziker *et al.*, 1978).

The similarity of DNA content in these auto and allotetraploid taxa would indicate either that DNA per genome has decreased in like manner at the tetraploid level or that some of the ancestral diploids had lower DNA content than present day diploids.

Two alternative explanations have been offered to explain why polyploid taxa may have less DNA per basic genome than the diploids. According to one, the polyploids could have been originated by diploids, perhaps extinct at present, that possessed lower DNA content.

These diploids, having the smallest chromosomes, would have been preadapted to give rise to polyploids (Darlington, 1956).

Another explanation is that saltatory changes occurred in the genome (DNA reduction) and this could have happened at the diploid or polyploid level (Grant, 1987).

The fact that interracial autotetraploids of *L. tridentata* have less DNA content per basic genome than the related diploids (*L. tridentata*, *L. divaricata*) suggests that the elimination of DNA has occurred in the polyploids and not in their ancestral diploids. It could be postulated that at polyploid level, the DNA elimination leads to a more adequate balance between total DNA content and a certain cellular parameter. Moreover, at the polyploid level, with genetic material dupli-

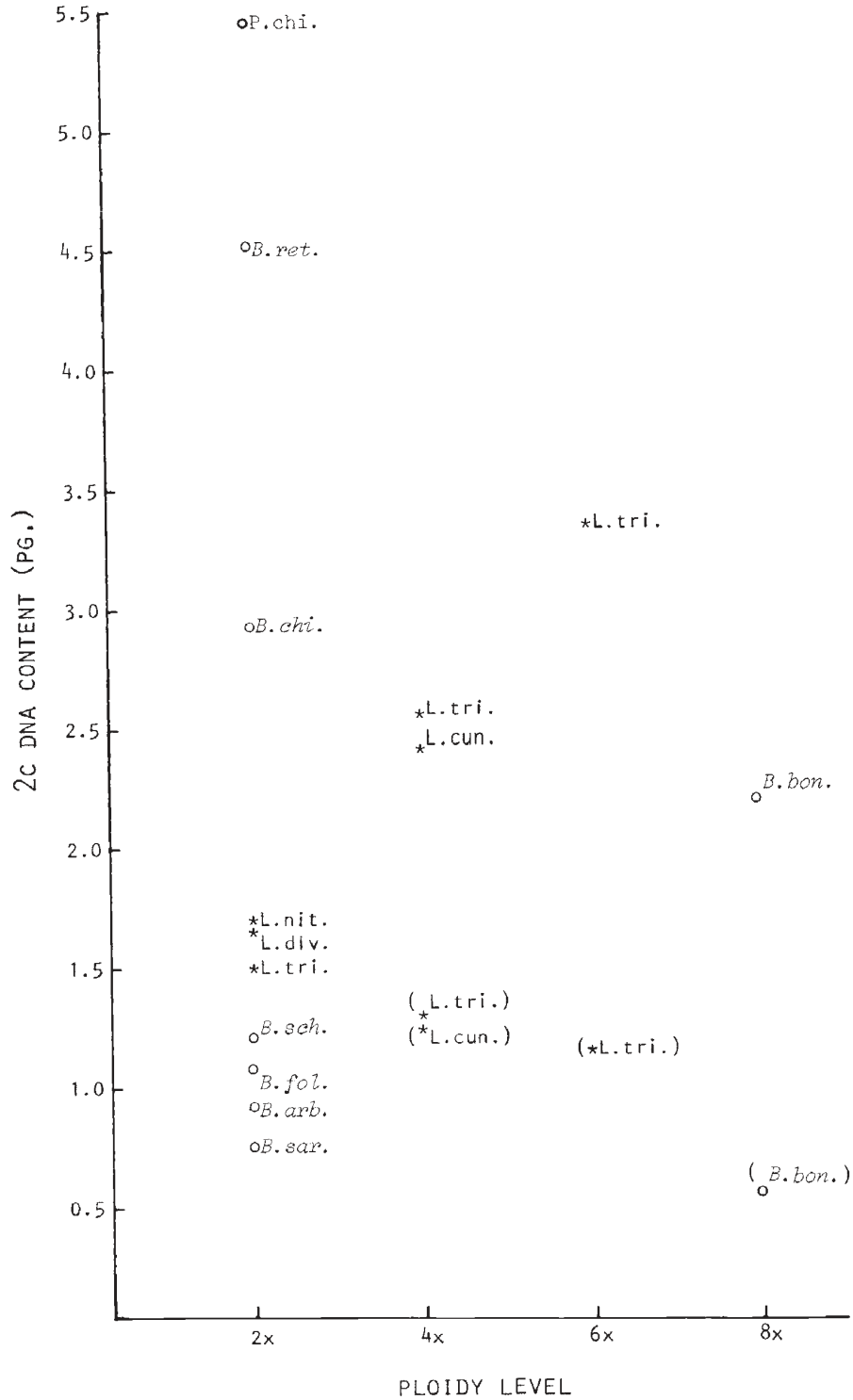

Figure 3 2C total DNA content plotted against ploidy level in cytotypes and species of *Bulnesia* (from Poggio and Hunziker, 1986), *Pintoa* (from Poggio and Naranjo, in press) and *Larrea*. Between parenthesis the DNA content per diploid genome. Abbreviations are as follows: *Bulnesia* = B. arb. = *B. arborea*; B. bon. = *B. bonariensis*; B. chi = *B. chilensis*; B. fol. = *B. foliosa*; B. ret. = *B. retama*; B. sar. = *B. sarmientoi*; B. sch. = *B. schickendantzii*. *Larrea* = L. cun. = *L. cuneifolia*; L. div. = *L. divaricata*; L. nit. = *L. nitida*; L. tri. = *L. tridentata*. *Pintoa* = P. chi. = *P. chilensis*.

cated, the partial elimination of DNA material is more easily tolerated.

In *L. tridentata* the ploidy level and therefore the total DNA content increases towards the west with a concomitant increase in aridity (Hunziker *et al.*, 1977, 1978). If total DNA content of Zygophyllaceae of the genera *Larrea*, *Bulnesia* and *Pintoa* is compared (fig. 3) it can be seen than those species that inhabit the most arid environments (*B. retama*, *B. chilensis*, *P. chilensis*, *L. tridentata* 6x) are the ones possessing highest DNA content. This increase is due to an increment in ploidy level in *Larrea* and an augment of intrachromosomal DNA in *Bulnesia* and *Pintoa* (Poggio and Hunziker, 1986; Poggio and Naranjo, in press).

It is possible that changes in DNA content can influence the ecological properties of species. Increased repetitive DNA may play an important role in gene regulation while gene duplication may increase ecological amplitude because of fixation of different alleles at loci having equal function or through the acquisition of new genic functions, (Levin and Funderberg, 1979). It is interesting to point out, however, that in both cases (increment in ploidy level and intrachromosomal DNA) nucleotypic parameters are altered whose expression is more a function of total DNA content than the informational content (Bennett, 1987). Moreover, if DNA variation is correlated with morphological or ecological diversity it would seen likely to be adaptive (Kenton, 1984*a*). The results obtained in *Bulnesia*, *Pintoa* and *Larrea* suggest that there is a correlation between environmental factors and total DNA content and that the latter may have adaptive significance.

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