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Relationships among Genome Size, Environmental Conditions and Geographical Distribution in Natural Populations of NW Patagonian Species of *Berberis* L. (Berberidaceae)

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Variation in genome size of 24 populations belonging to 11 NW Patagonian species of *Berberis* was analysed as a function of the environment and geographical location. The variation showed three levels of discontinuity, two of which corresponded to diploid species ($2n = 28$) while the third corresponded to polyploid species ($2n = 56$). Diploids with DNA content ranging from 1·463 pg to 1·857 pg included *Berberis cabrerae*, *B. chillanensis*, *B. montana*, *B. serrato-dentata* and *B. bidentata*. Diploids with DNA content ranging from 2·875 pg to 3·806 pg included *B. linearifolia*, *B. darwinii*, *B. parodii* and *B. empetrifolia*. The genome size of the polyploid species *B. buxifolia* and *B. heterophylla* ranged from 5·809 pg to 6·844 pg. Principal component analysis (PCA) was applied to represent the variability of environmental conditions. The eigenvectors of the principal component axes showed that PC1 discriminates the populations according to rainfall, types of vegetation and geomorphology; altitude and latitude, on the other hand, contribute to PC2 and PC3, respectively. From these results it is concluded: (1) that diploids with lower DNA content grow in high-elevation sites having greater rainfall but lower water availability; (2) diploids with higher DNA content are associated with half-elevation forests where the vegetative period is longer, the water availability is greater and the temperatures are higher; and (3) the distribution pattern of polyploids is considerably wider than that of diploids, which are geographically and ecologically restricted to forest areas. These results suggest that the C-value plays an important role in the ability of the species to adapt to different growing conditions.

Key words: *Berberis* L., barberry, calafate, michay, genome size, DNA content, environmental correlation, Patagonia.

INTRODUCTION

Many examples of wide intra- and interspecific variation in nuclear DNA content affecting genome size can be found in the literature (Bennett and Smith, 1976, 1991; Price, 1988; Poggio and Naranjo, 1990; Cavallini and Natali, 1991; Bennett and Leitch, 1995, 1997). The influence exerted by genome size on several characteristics has been revealed by numerous studies showing that interspecific and intraspecific variation in DNA C-value is often strongly correlated with many phenotypic features of cells and organisms, such as chromosome volume or length, minimum duration of the cell cycle, minimum generation time, as well as with ecological behaviour, phenological factors, optimum environment and range of cultivation of crop and non-crop species (for a review, see Bennett and Leitch, 1995). For instance, it has been demonstrated that important ecological characteristics of plant species in natural habitats vary with genome size, e.g. timing of spring growth, cell size and rate of leaf expansion in early season growth (Grime *et al.*, 1985), frost resistance (Macgillivray and Grime, 1995), xeric conditions (Poggio *et al.*, 1989) and altitude (Poggio *et al.*, 1998).

The term 'nucleotype' (Bennett, 1971, 1972, 1987) defines those conditions of the nuclear DNA affecting the phenotype independently of its encoded informational content.

Taking into account the above-mentioned relationships, Bennett (1976, 1987) suggested that variation in DNA content has adaptative significance and is correlated with the environment and the geographical distribution of species. This has been confirmed, for example, for Argentinian populations of maize cultivated at different altitudes and latitudes, each population being characterized by an optimum nucleotype. In these populations, the frequency of accessory chromosomes (B) showed a positive correlation with altitude of cultivation, whereas the A-DNA content and mean number of DAPI+ heterochromatic bands were negatively correlated with both altitude and the mean number of B chromosomes per plant (Poggio *et al.*, 1998; Rosato *et al.*, 1998). These examples show that there is no simple explanation for the variation in genome size; several reports in the literature, however, give support to the hypothesis that in higher plants genome size should be predictable, adaptive and of evolutionary significance.

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The Andino-Patagonian region was subjected to several great climatic changes, such as glaciations and fluctuations of sea level, during the Pliocene and Pleistocene (Romero, 1986). Moreover, natural disturbances (landslides, volcanic, glacial and tectonic activities, fires, introduction of exotic herbivores) have resulted in varied environmental conditions that favour diversification and speciation. This is an ideal location in which to develop a genetic and evolutionary study in relation to climatic and geographical factors.

The genus *Berberis* L. is well represented in the South American Andes and its species have a wide geographical distribution in Argentinian Patagonia (Orsi, 1974). They are evergreen and semi-evergreen shrubs which grow under a wide range of ecological conditions. The purpose of the present work was to analyse variation in DNA content as a function of the environmental conditions and geographical location of 33 natural populations belonging to 11 NW Patagonian species.

MATERIALS AND METHODS

Plant material

Individuals belonging to 33 natural populations from 11 species were collected at 24 sites in NW Argentinian Patagonia (Fig. 1). The sites are representative of their distribution area and correspond to different environmental conditions (Table 1). Seeds and young buds were collected in each locality by MCJB. Representative accessions were deposited in the herbarium of the Instituto de Botánica Darwinion (SI), Argentina.

Determination of DNA content

DNA content was measured in telophase nuclei ($2n$) of root tips from germinating seeds and in immature anther walls. They were fixed in 3 : 1 absolute alcohol : glacial acetic acid. As the germination rate was very low and DNA measurements in roots and anther walls yielded no significant differences, DNA content was measured in the latter. *Amaranthus cruentus* L. 'Don Giem' (2C = 1·26 pg) was used as a standard to calculate genome size in picograms because of the low C-values of *Berberis*. It was calibrated according to Bennett and Smith (1976) using *Allium cepa* 'Ailsa Craig' (2C = 33·55 pg) (Greizerstein, 1995). Root tips of both the standard and experimental material were fixed simultaneously as described above. At least three to five individuals from each locality were used and 20 to 40 nuclei in each sample analysed.

The staining method was performed as described in Tito *et al.* (1991) with minor modifications. After fixation, roots and anthers were rinsed for 30 min in distilled water. Hydrolysis was carried out in 5 N HCl at 20°C, the optimum period being 40 min. The material was rinsed three times in distilled water for 15 min and stained for 2 h in Schiff's reagent at pH 2·2 (Teoh and Rees, 1976). Material was then rinsed three times in SO_2 water for 10 min each, kept in distilled water, and squashed in 45 % acetic acid. The coverslips were removed after freezing with CO_2 and the material was dehydrated in absolute alcohol, mounted in Euparal and maintained in the dark until measurements were made. The amount of Feulgen staining per nucleus, expressed in arbitrary units, was measured at a wavelength of 570 nm using the scanning method with a

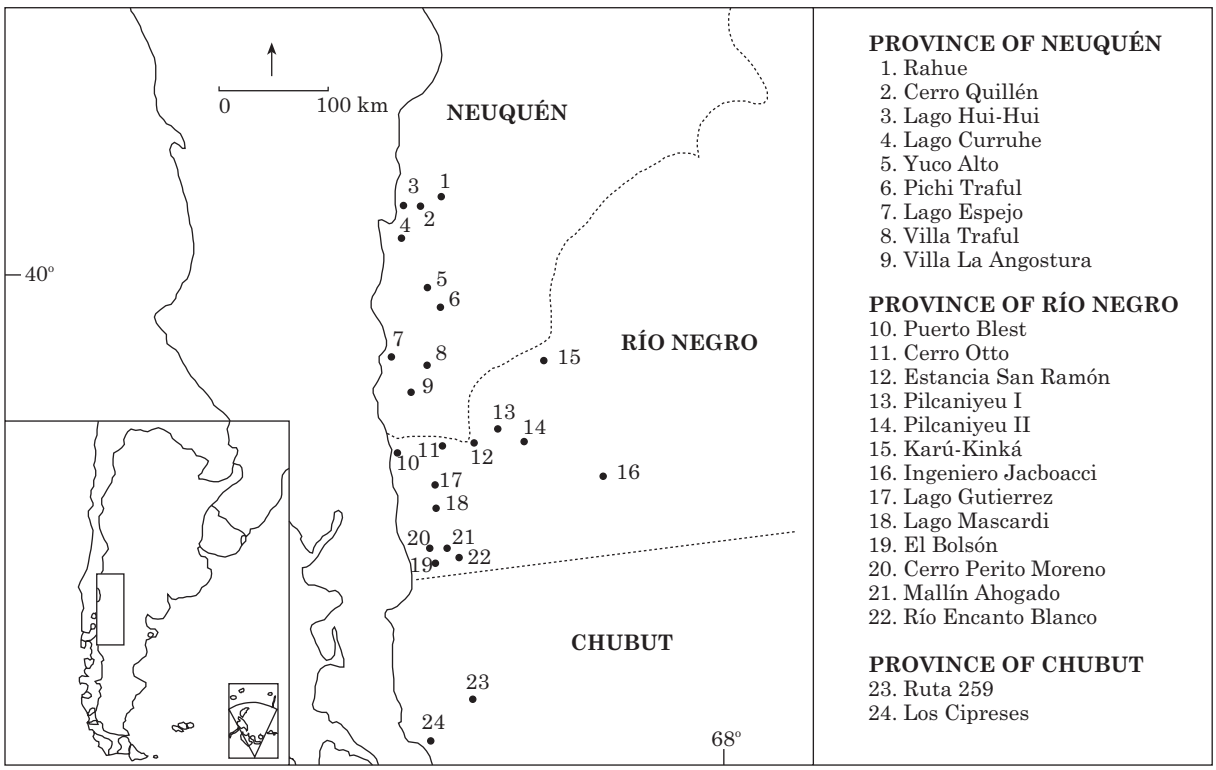

FIG. 1. Geographic distribution of the populations studied.

TABLE 1. *Populations of* Berberis *studied and environmental parameters*

Species	Population	Latitude south	Longitude west	Altitude (above sea level)	Average annual rainfall (mm)	Type of vegetation	Geomorphological region
B. cabrerae	Cerro Quillén (2)	39°22′	71°41′	1000	2000	*N. nervosa* and *N. oblicua* forest	Mountains, cordilleran valley
B. montana	Lago Hui Hui (3)	39°15′	71°15′	1250	2500	*N. nervosa* and *N. oblicua* forest	Mountains, cordilleran valley
	Puerto Blest (10)	41°01′	71°49′	1000	3000	*N. dombeyi* forest	Mountains, cordilleran valley
B. chillanensis	Cerro Quillén (2)	39°22′	71°41′	1000	2000	*N. nervosa* and *N. oblicua* forest	Mountains, cordilleran valley
B. serrato dentata	Cerro P. Moreno (20)	41°58′	71°31′	2000	1500	*N. pumilio* forest	Mountains, cordilleran valley
	Cerro Otto (11)	41°08′	71°17′	1300	1300	*N. pumilio* forest	Mountains, cordilleran valley
B. bidentata	Lago Gutierrez (17)	41°18′	71°20′	850	1600	*N. dombeyi* forest	Mountains, cordilleran valley
	Lago Mascardi (18)	41°21′	71°31′	850	1600	*N. dombeyi* forest	Mountains, cordilleran valley
	Villa Traful (8)	40°41′	71°12′	800	1500	*N. dombeyi* forest	Mountains, cordilleran valley
	Villa La Angostura (9)	40°47′	71°40′	845	1200	*N. dombeyi* forest	Mountains, cordilleran valley
B. linearifolia	Villa Traful (8)	40°41′	71°12′	850	1500	*N. dombeyi* forest	Mountains, cordilleran valley
	Villa La Angostura (9)	40°47′	71°40′	850	1500	*N. dombeyi* forest	Mountains, cordilleran valley
B. darwinii	Lago Mascardi (18)	41°21′	71°31′	950	1500	*N. dombeyi* forest	Mountains, cordilleran valley
	Villa Traful (8)	40°41′	71°12′	800	1500	*N. dombeyi* forest	Mountains, cordilleran valley
	Mallín Ahogado (21)	41°58′	71°31′	600	1000	*N. dombeyi* forest	Mountains, cordilleran valley
B. parodii	Lago Espejo (7)	40°47′	71°40′	970	1800	*N. antarctica* and *L. hirsuta* forest	Mountains, cordilleran valley
	Lago Curruhe (4)	39°50′	71°22′	900	1500	*N. antarctica* and *L. hirsuta* forest	Mountains, cordilleran valley
	Cerro Quillén (2)	39°22′	71°41′	1000	2000	*N. antarctica* and *L. hirsuta* forest	Mountains, cordilleran valley
	Pichi Traful (6)	40°45′	71°46′	800	1200	*N. antarctica* and *L. hirsuta* forest	Mountains, cordilleran valley
B. empetrifolia	Lago Mascardi (18)	41°21′	71°31′	950	1500	Heaths	Mountains, cordilleran valley
	Río Blanco (22)	41°58′	71°31′	1000	1500	Heaths	Mountains, cordilleran valley
	Rahue (1)	39°22′	70°56′	1000	1000	Heaths	Rocky soils, volcanic stones
	Yuco Alto (5)	40°08′	71°30′	1100	1700	Heaths	Mountains, cordilleran valley
B. buxifolia	Ea. San Ramón (12)	41°04′	71°00′	960	585	Grassland Patagonian steppe	Rocky soils, volcanic stones
	Ruta 259 (23)	42°31	71°27′	500	600	Grassland Patagonian steppe	Rocky soils, volcanic stones
	Lago Cipreses (24)	43°11′	71°40′	400	1500	*Austrocedrus chilensis* forest	Mountains, cordilleran valley
	Cerro Otto (11)	41°08′	71°17′	1100	1300	*A. chilensis* forest	Mountains, cordilleran valley
	El Bolsón (19)	41°58′	71°31′	297	800	*A. chilensis* forest	Mountains, cordilleran valley
	Mallín Ahogado (21)	41°58′	71°31′	465	800	*A. chilensis* forest	Mountains, cordilleran valley
B. heterophylla	Karú Kinká (15)	40°35′	70°38′	1000	200	Grassland shrubby Patagonian steppe	Volcanic stones
	Pilcaniyeu I (13)	41°05′	70°03′	1200	270	Grassland shrubby Patagonian steppe	Volcanic stones
	Pilcaniyeu II (14)	41°05′	70°03′	780	270	Grassland shrubby Patagonian steppe	Volcanic stones
	Ing. Jacobacci (16)	41°18′	69°36′	1000	158	Grassland shrubby Patagonian steppe	Volcanic stones

Numbers in parentheses refer to populations of NW Argentinian Patagonia indicated in Fig. 1.

Zeiss Universal Microspectrophotometer (UMSP 30), and finally expressed in picograms by reference to the standard.

Statistical analysis

DNA content. Differences in DNA content between populations and species were tested using analysis of variance (ANOVA) and comparisons between means were made using Scheffe's methods (Sokal and Rohlf, 1995). This allowed detection of populations with different DNA contents.

Environmental analysis. Geographical location, habitat conditions (annual average rainfall in mm) and typical vegetation (Marcolín *et al*., 1987) were recorded for each of the collection sites. The annual average rainfall was recorded by Cordow *et al*. (1993). A matrix of basic data was built. The operational taxonomic units are the populations collected defined by the name of the species to which they belong, and the variables are the environmental traits (Table 1).

Simple correlation between DNA content and environmental factors (latitude, longitude, rainfall and type of vegetation) was calculated using STATISTICA for Windows 4.5 f (Stat Soft, Inc. 1993). Pearson's coefficient for the correlation product-moment was used as well as a *t*-test for this coefficient applying $n = 2$ degrees of freedom (Sokal and Rohlf, 1995).

Principal component analysis (PCA) was used to study the variability of environmental factors under which individuals of different populations grow. The principal axis, which corresponds to the largest eigenvalue, is the dimension that accounts for the greatest amount of variance in the sample. The second principal axis accounts for the second largest amount of variance, and so on (Sneath and Sokal, 1973).

PCA was performed on standardized data and on a similarity matrix based on Pearson product–moment correlation coefficient (Sneath and Sokal, 1973; James and McCulloch, 1990). Numerical analysis was made using NTSYS-pc 2.0f (Rohlf, 1998).

RESULTS

Table 1 shows the environmental factors prevailing in the locations where the populations were collected.

Table 2 summarizes the DNA content (2C) in picograms and chromosome numbers of the 33 populations belonging

TABLE 2. *Chromosome numbers, nuclear DNA content of different populations of* Berberis *spp., and nuclear DNA content of species*

Species	Population	Name	2*n*	Population DNA (2C) pg $\bar{x} \pm$ s.e.	Species DNA (2C) pg $\bar{x} \pm$ s.e.
B. cabrerae	Cerro Quillén	caCQ	28	1·636 ± 0·107 a	1·653 ± 0·112 A
B. montana	Lago Hui Hui	moHH	28	1·683 ± 0·078 a	1·696 ± 0·093 A
	Puerto Blest	moPB	28	1·708 ± 0·178 a	
B. chillanensis	Cerro Quillén	chCQ	28	1·711 ± 0·120 a	1·711 ± 0·120 A
B. serrato dentata	Cerro P. Moreno	sdPM	28	1·857 ± 0·194 a	1·842 ± 0·198 A
	Cerro Otto	sdCO	28	1·831 ± 0·202 a	
B. bidentata	Lago Gutierrez	biLG	28	1·463 ± 0·138 a	1·495 ± 0·153 A
	Lago Mascardi	biLM	28	1·495 ± 0·201 a	
	Villa Traful	biVT	28	1·548 ± 0·080 a	
	La Angostura	biVA	28	1·473 ± 0·191 a	
B. linearifolia	Villa Traful	liVT	28	3·243 ± 0·439 b	3·401 ± 0·394 B
	La Angostura	liVA	28	3·570 ± 0·348 b	
B. darwinii	Lago Mascardi	daLM	28	3·222 ± 0·414 b	3·097 ± 0·816 B
	Villa Traful	daVT	28	2·875 ± 0·461 b	
	Mallín Ahogado	daMA	28	3·145 ± 1·575 b	
B. parodii	Lago Espejo	paLE	28	3·379 ± 0·419 b	3·129 ± 0·338 B
	Curruhe	paCU	28	3·145 ± 0·394 b	
	Cerro Quillén	paCQ	28	3·040 ± 0·310 b	
	Pichi Traful	paPT	28	3·125 ± 0·200 b	
B. empetrifolia	Lago Mascardi	emLM	28	3·349 ± 0·498 b	3·629 ± 0·577 B
	Río Blanco	emRE	28	3·721 ± 0·541 b	
	Rahue	emRA	28	3·689 ± 0·439 b	
	Yuco Alto	emYA	28	3·806 ± 0·832 b	
B. buxifolia	San Ramón	buSR	56	6·712 ± 1·463 c	6·055 ± 0·602 C
	Ruta 259	bu259	56	5·809 ± 0·579 c	
	Lago Cipreses	buLC	56	5·940 ± 0·450 c	
	Cerro Otto	buCO	56	6·480 ± 0·288 c	
	El Bolsón	buEB	56	5·830 ± 0·137 c	
	Mallín Ahogado	buMA	56	6·005 ± 0·515 c	
B. heterophylla	Karú Kinká	heKK	56	6·844 ± 1·013 c	6·222 ± 0·659 C
	Pilcaniyeu I	heP1	56	6·741 ± 0·020 c	
	Pilcaniyeu II	heP2	56	5·827 ± 0·152 c	
	Ing. Jacobacci	heIJ	56	6·695 ± 0·742 c	

According to Sheffe's methods: Means followed by the same lowercase letter indicate no significant differences among populations. Means followed by the same capital letter indicate no significant differences according to Scheffe's methods ($P < 0·05$).

to 11 species *of Berberis*. Analysis of variance for the DNA content indicated that the differences between mean DNA content of different populations of each species were non-significant; consequently our measurements were pooled to indicate the average DNA content of the species. The same capital letter indicates no significant differences among species according to Scheffe's methods ($P < 0{\cdot}05$) (Sokal and Rohlf, 1995).

Analysis of interspecific variation of DNA content showed three levels of genome size discontinuity (Table 2, Fig. 2): two levels correspond to diploids and the third one to polyploids.

(1) Diploids with DNA content ranging from 1·463 pg (*B. bidentata* Lechl.) to 1·857 pg (*B. serrato–dentata* Lechl.). This group includes, in addition, *B. cabrerae* Job, *B. chillanensis* (C. K. Sheid.) Sragne., and *B. montana* Gay.
(2) Diploids with DNA content ranging from 2·875 pg (*B. darwinii* Hook.) to 3·806 pg (*B. empetrifolia* Lam.). This group includes, in addition, *B. linearifolia* Phil. and *B. parodii* Job.
(3) Polyploids with DNA content ranging from 5·809 pg (*B. buxifolia* Lam.) to 6·844 pg (*B. heterophylla* Juss.).

When individuals belonging to the populations of diploid and polyploid species were considered, a significant correlation was found between DNA content and all the variables analysed (environmental conditions and geographical location). Correlations with rainfall, longitude and altitude were negative, but those with types of vegetation and latitude were positive (Table 3).

Principal component analysis (PCA) was used to describe the variability of environmental conditions. This analysis indicated that the first three principal components (PC) explain 82·56 % of the total variability. The first principal component (PC1) represents 49·62 % of the total variability, the second (PC2) 21·47 %, and the third (PC3) 11·47 % (Table 4). Analysis of these three principal components can often permit an environmental interpretation for each component axis. The eigenvectors of the principal component axes (Table 5) show that in PC1, rainfall (−0·9135), types of vegetation (0·8145) and geomorphology of the region (0·7647) discriminate between the populations, while altitude (−0·7530) and latitude (0·5497) contribute to PC2 and PC3, respectively (Fig. 3A and B). The first principal component coefficients point to an association between mean annual rainfall, type of vegetation and geomorphology of the region. Rainfall in the Andino-Patagonian region has a profound effect upon the altitudinal distribution (the most important factor in PC2) of plant communities due to the fact that the north-south orientation of the Andes constitutes a high barrier for the humid winds from the Pacific Ocean, thereby determining a marked environmental gradient. Latitude is the most important factor in the third principal component. It is associated with the mean annual temperatures and the decreasing length of the growing season along the north-south gradient.

Figure 3A, a two-dimensional graph of PC1 and PC2, explains 71·08 % of the total variability. Populations of

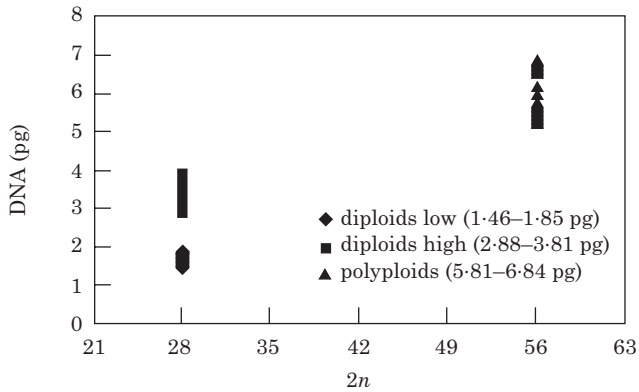

FIG. 2. Relationship between DNA content and ploidy levels.

polyploid species (*B. buxifolia* and *B. heterophylla*) are grouped together (Sokal and Rohlf, 1995) in the positive part of axis 1. *B. heterophylla* has a high positive value along PC1 because it grows in grassland of the shrubby Patagonian steppe with volcanic stones, where rainfall is in the range 150–270 mm. *B. buxifolia* shows a wide distribution along the positive axis 1 of the graph because it is a component of the grasslands of the Patagonian steppe (sandy soils and volcanic stones) and also of the forests of

TABLE 3. *Correlation coefficient and* t*-test between nuclear DNA content and environmental traits*

Correlation	*r*
DNA–rainfall	−0·7186**
DNA–longitude	−0·5370**
DNA–altitude	−0·3198*
DNA–latitude	0·4590**
DNA–type of vegetation	0·9213**
DNA–geomorphological region	0·5974**

$^{**}P < 0.01$; $^{*}P < 0.05$.

TABLE 4. *Eigenvalue, percentage of total variation and cumulative percentage of total contribution*

Eigenvalue	Percent total	Percent cumulative
2·977	49·615	49·615
1·288	21·466	71·082
0·688	11·467	82·549
0·484	8·070	90·619
0·356	5·934	96·554
0·207	3·446	100·000

TABLE 5. *Eigenvectors of the principal components axes*

	PC1	PC2	PC3
Latitude	0·5448	−0·5584	*−0·5497*
Longitude	−0·7160	−0·5355	−0·1100
Altitude	−0·2910	*−0·7530*	−0·5175
Rainfall	*−0·9135*	−0·0895	−0·0630
Type of vegetation	*0·8145*	−0·1909	−0·1336
Geomorphological region	*0·7647*	0·2793	0·1358

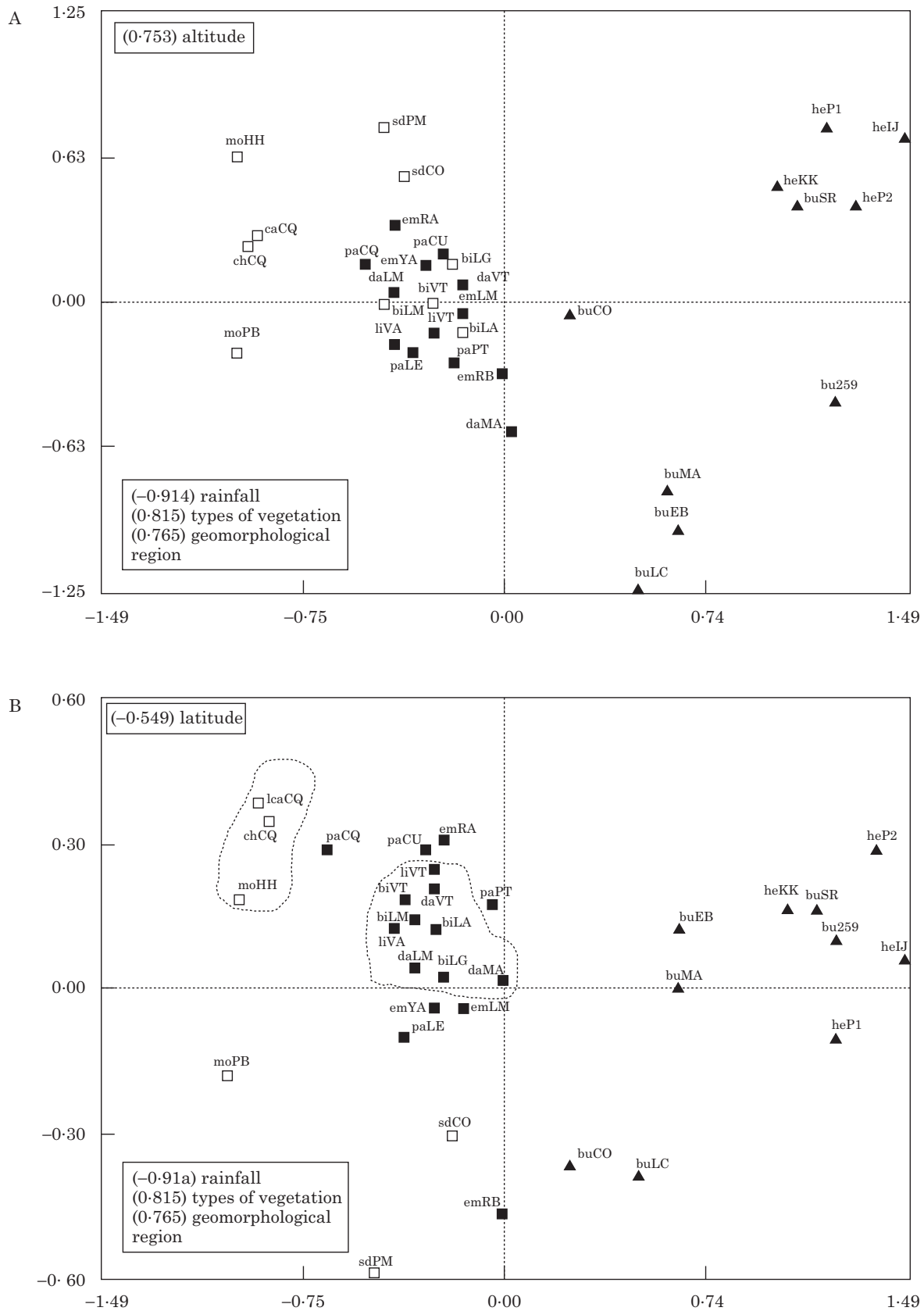

FIG. 3. A, Principal component analysis. Two dimensional graph of PC1 and PC2. Total variability: 71·08 %. B, Two dimensional graph of PC1 and PC3. Total variability: 61·08 %. □, Diploids low (1·46–1·85 pg); ■, diploids high (2·88–3·81 pg); ▲, polyploids (5·81–6·84 pg).

Austrocedrus chilensis (D. Don) Flor. & Boutl. (mountains and valleys), with total rainfall between 585 and 1500 mm.

On the other hand, all the diploid populations form a group in the negative end of axis 1. These species grow in the *Nothofagus* Blume forest of mountains and valleys, where the precipitation range is 1200–3000 mm. Diploid species of lower DNA content (1·46–1·85 pg) are located in the most extreme position on axis 1, except *B. serrato-dentata* and *B. bidentata* which appear together with the species of greater genome size (2·88–3·88 pg). For the second component, altitude has the higher coefficient. Thus *B. serrato-dentata* appears away from the rest of the group because this species inhabits forests (of *Nothofagus pumilio* (Poepp. Et Endl.) Krasser) at a higher altitude in the sampled area. The second component also separates the two polyploid species, *B. heterophylla* and *B. buxifolia*, the first being located in the positive end and the second in the negative end, with the exception of population buSR, collected from the Patagonian steppe.

Figure 3B shows the two-dimensional graph of PC1 *vs.* PC3 which explains 61·08 % of the total variability. The third axis shows the clustering of different populations as a function of latitude. *B. darwinii*, *B. linearifolia* and *B. bidentata* are grouped together in the top left quadrant near the origin of coordinates. On the other hand, *B. empetrifolia*, *B. buxifolia* and *B. parodii* are dispersed along the axis.

Figure 4 shows a three-dimensional graph which combines DNA content, types of vegetation and altitude. These environmental characteristics were selected for the reason that they have the highest correlation coefficient. The graph shows that the species can be assembled in three discontinuous classes. These classes are composed of the same species that form the groups plotted in Fig. 2.

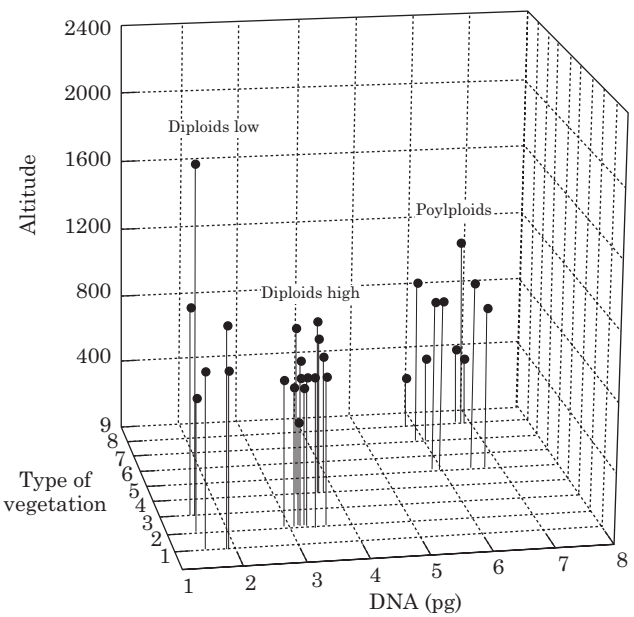

FIG. 4. Three dimensional graph showing DNA content, types of vegetation and altitude.

DISCUSSION

Intra- and interspecific variation in DNA content

The genus *Berberis* shows two ploidy levels, namely diploids with $2n = 28$ and tetraploids with $2n = 56$ chromosomes (Bottini *et al.*, 1997, 1999). The total DNA content does not show intraspecific variation, in contrast with the wide interspecific variation existing throughout the genus (Table 2, Fig. 2), which, however, is not continuous.

At the diploid level, the species have been grouped in two classes, one of them including those with lower DNA content ($\bar{x} = 1{\cdot}679$ pg) and the other including those with higher DNA content ($\bar{x} = 3{\cdot}314$ pg). The polyploids have the highest DNA content ($\bar{x} = 6{\cdot}139$ pg).

Discontinuous patterns of DNA variation have also been reported previously for the genera *Clarkia* Pursh and *Nicotiana* L. (Narayan, 1998), *Vicia* L. (Martin and Shanks, 1966), and *Microseris* L. (Price and Bachmann, 1975).

Differences in DNA content among diploid species

Diploid species can be classified in two groups according to their DNA content. The first group is formed by *B. cabrerae*, *B. chillanensis*, *B. montana* and *B. serrato-dentata*, species characterized by their lower genome size, specialized morphological characteristics and restricted geographic range. These species grow in deciduous forest of *Nothofagus nervosa* (Phil.) Dim. et Mil., *Nothofagus oblicua* (Mirb.) Blume and *N. pumilio*, where the annual average rainfall is high, especially in winter, but water availability, however, is low during that season because the soils are frozen. In spring the weather begins to get warmer and favourable environmental conditions reappear; the short flowering period starts at the end of winter and concludes in early spring. It is worth mentioning that Grime and Mowforth (1982) indicated that the most actively growing species in early spring are associated with a progressive reduction in genome size.

The group formed by *B. cabrerae*, *B. chillanensis* and *B. montana* (Fig. 3B) has no sharp morphological discontinuities and intermediate forms from one species to another were detected. Moreover, it can be seen in Figs 2–4 that these species are grouped together in the extreme left of axis 1, which is an indication that they have similar water requirements.

Although *B. bidentata* belongs to the group of lower genome size, it grows in different ecological and geographical conditions since it was found in habitats disturbed by human activities. This fact is very common in hybrid plants since they can only establish themselves in disturbed soils if they are adaptively superior to the parent species (Grant, 1981). Based on morphological studies, *B. bidentata* appears to have originated by hybridization of two diploid species, the putative parental species being *B. darwinii* and *B. linearifolia* (Orsi, 1974; Bottini *et al.*, 1998). The lower C-value in this species with respect to its putative parents might be due to its hybrid condition.

The other diploid group with higher DNA content includes *B. empetrifolia*, *B. darwinii*, *B. linearifolia* and *B. parodii*. The latter species grow in forests of evergreen

N. dombeyi, *Lomatia hirsuta* and deciduous *N. antarctica* (800–1000 m above sea) where the climatic conditions are more stable than in forests at higher elevations. The water availability is higher than in the first group (*B. cabrerae*, *B. chillanensis* and *B. montana*) and the flowering period begins later. *B. empetrifolia* constitutes a particular case within this group. This species grows in the xeric conditions of the trans-Andean latitudinal gradient where the soil is typically granitic with metamorphic rock and/or sand. The water availability is low because the infiltration rate is high. Its genome size is similar to other species of the group but its large altitudinal and latitudinal range is explained by its ecological characteristics.

Bennett (1976) has shown that cultivation of species with high DNA amounts per diploid genome tends to be localized in temperate latitudes. The cline for DNA amount and latitude is a natural phenomenon (Bennett, 1987). In our work, DNA content is strongly associated with the type of vegetation and the mean annual rainfall, with the association with latitude being less significant (Table 5). Water availability during growing and developmental stages is a constraining factor which severely affects the distribution of *Berberis* ssp with different DNA contents in the Andino-Patagonian region.

DNA content in polyploid species

Polyploids of *Berberis* have the highest genome size. Generally polyploid Patagonian *Berberis* inhabit environments with low rainfall. *B. heterophylla* is widely distributed in the Patagonian steppe (xeric environments) where the mean annual rainfall is less than 200 mm (Orsi, 1974; Veblen *et al*., 1995). *B. buxifolia* has a more extensive distribution range, growing in *Austrocedrus* forest, mixed forest of *Austrocedrus* and *Nothofagus*, and the forest-steppe ecotone (585–1200 mm).

It is possible that changes in DNA content can influence the ecological properties of species. The increase in repetitive DNA may play an important role in gene regulation, while gene duplication may enlarge the ecological amplitude through the acquisition of new genic functions (Levin and Funderberg, 1979). Increased ecological diversity and the opening of new habitats for colonization of xeric areas, as a result of climate changes, provide an opportunity for polyploids to exhibit their adaptive abilities and to contribute to the fight against deserts in Patagonia (Bottini *et al*., 1999). It is interesting to point out that the correlation with climate, especially the association of polyploids with dry conditions (*B. heterophylla* and several populations of *B. buxifolia*) are in accordance with Grime and Mowforth's theory (1982) in which phenology (the seasonal timing of shoot expansion) is the key phenomenon.

The results show that *Berberis* is a good model in which to study relationships between genome size and ecological and geographic diversity, since there is a strong correlation between environmental factors and total DNA content. This suggests that C-values play an important role in the ability of species to adapt to different growing conditions.

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