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DNA content in South American endemic species of *Lathyrus*

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Abstract The genome size was surveyed in 13 Notolathyrus species endemic to South America by flow cytometry and analyzed in an evolutionary and biogeographic context. A DNA content variation of 1.7-fold was registered, and four groups of species with different DNA content were determined. Although, the 2C values were correlated with the total chromosome length and intrachromosomal asymmetry index (A_1), the karyotype formula remained almost constant. The conservation of the karyotype formula is in agreement with proportional changes of DNA in the chromosome arms. Species with annual life cycle and shorter generation time had the lowest DNA content and the data suggest that changes in DNA content involved reductions of genome size in the perennial to annual transitions. The variation of 2C values was correlated with precipitation of the coldest quarter and, to some extent, with altitude. Additional correlations with other variables were observed when the species were analyzed separately according to the biogeographic regions. In general, the species with higher DNA content were found in more stable environments. The bulk of evidence suggests that changes on genome size would have been one of the most important mechanisms that drove or accompanied the diversification of Notolathyrus species.

Keywords Notolathyrus · DNA content · Flow cytometry · Karyotype evolution

Introduction

The genus *Lathyrus* L. (Fabaceae, Fabeae, n. v. sweet peas) includes ca. 150 species classified into 13 sections according to their morphological features, life cycles and geographic distribution (Kupicha 1983). These species are mostly distributed throughout the temperate regions of the Northern Hemisphere, extending out towards the subtropical Africa and South America (Kupicha 1983).

Nearly 23 species of *Lathyrus* are endemic to South America. These species are of particular taxonomic and evolutionary interest since they present a suit of traits that individually define different taxonomic sections of the Northern Hemisphere. Therefore, all South American species (about 23) were included in section Notolathyrus based on a geographical criterion exclusively (Kupicha 1983). Notolathyrus species are also ecologically diverse, inhabiting the patagonian forest, interandean valleys, mountain grasslands, temperate savannas and grasslands, temporarily flooded swamps and open patches of the subtropical paranaense forest (Seijo 2002). Around half of the species are relatively frequent whereas the remaining species are rare or very rare.

Cytogenetically, the Notolathyrus species ($x = 7$) studied so far are diploids (Battistin and Fernández 1994; Chalup et al. 2012; Klamt and Schifino-Wittmann 2000; Krapovickas and Fuchs 1957; Seijo and Fernández 2003; Seijo and Solís Neffa 2006) with similar karyotype

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formula. However, these species have significant variation (almost twofold) in the total chromosome length (TCL) (Klamt and Schifino-Wittmann 2000; Seijo and Fernández 2003). According to those data, it was suggested that the variation in the genome size would have played an important role in the diversification of Notolathyrus (Seijo and Fernández 2003), although the 2C values of these species remain unknown.

In spite of the fact that the 2C values of Notolathyrus species have still to be determined, the species of *Lathyrus* were amongst the earliest subjects of genome size measurement in plants (Rees and Hazarika 1969). The analyses of the DNA content in a small set of species belonging to six different sections from the Northern Hemisphere showed a fourfold variation in 2C nuclear DNA amount (Ali et al. 2000; Narayan 1982; Rees and Hazarika 1969). The distribution of DNA contents suggested that the variation in genome size is not gradual and that there are different steady states of 2C values within the genus (Narayan 1982). On the contrary, a more recent study including only species of section Lathyrus suggested that the DNA content variation is continuous (Nandini and Murray 1997).

Considering the impact that the changes in the genome size would have on the karyotypes, it is still a subject of debate how the variation in DNA amounts is distributed among the chromosomes of a given complement. Several authors, considering non random changes, proposed that changes in genome size would be equally distributed among all chromosomes of the complements, while others pointed out that those changes would be proportionally distributed. Equal distributions of DNA among the karyotypes were proposed for the Northern Hemisphere species of *Lathyrus* (Narayan and Durrant 1983; Rees and Narayan 1988), while proportional changes along the chromosome complement were suggested for *Vicia* (Naranjo et al. 1998) and other angiosperms (Brandham and Doherty 1998; Moscone et al. 2003; Seal 1983; Seal and Rees 1982). However, none of these two patterns of DNA distribution among the chromosomes of a complement could be determined in other plant groups (Poggio and Naranjo 1990).

According to the nucleotype hypothesis (Bennett 1987), the DNA content variation is a crucial factor that may be linked with many aspects in the biology of the species. It has been correlated with several environmental traits, affecting the plant life-histories (Bennett 1972; Nandini and Murray 1997; Price and Bachmann 1975), phenology (Baranyi and Greilhuber 1999; Grime and Mowforth 1982) and plant species distribution (Bennett 1976; Bottini et al. 2000; Knight and Ackerly 2002; Levin and Funderburg 1979; Mac Gillivray and Grime 1995; Ohri and Khoshoo 1986; Poggio et al. 1989, 1998; Wakamiya et al. 1993). Moreover, correlations between DNA variation and geographic or climatic parameters were suggested for many plant species (Bennett 1995; Caceres et al. 1998; Rayburn and Auger 1990; Schmuths et al. 2004; Temsch and Greilhuber 2000, 2001). Considering *Lathyrus* species, the DNA content variation observed in a few species of the Northern Hemisphere was associated to the life cycles, breeding systems (Nandini and Murray 1997; Rees and Hazarika 1969; Rees and Narayan 1988) and to the frequency of chiasmata in meiotic cells (Narayan and McIntre 1989). Additionally, genome size variation in the cultivated *L. sativus* L. was significant and positively correlated with longitude (Ghasem et al. 2011), although, no ecological interpretation was provided for these data. However, to the best of our knowledge, the variation in genome size in wild species of *Lathyrus* was not analyzed in the context of geographical or ecological ranges yet.

In this study we analyzed the nuclear DNA content of 13 species from section Notolathyrus with the objectives of (1) estimating, for the first time, the genome size of the South American species of *Lathyrus*, (2) analyzing the patterns of genome size variation and the direction of those changes (3) investigating the impact of the genome size variation on the karyotypes (4) assessing the significance of genome size variation in a biological and biogeographical context.

Materials and methods

Plant materials

The species here studied and their provenances are presented in Table 1 and geographically localized in Fig. 1. Vouchers specimens are deposited at the Herbarium of the Instituto de Botánica del Nordeste (CTES) and duplicates were distributed to herbaria of different institutions of the world (Table 1).

Nuclear DNA measurements

DNA content was estimated by flow cytometry using fresh or silica gel dried young leaves. For each species, the mean of 2C value was obtained from measurements of three individuals with three replicates per individual. Nuclei of *Pisum sativum* var. ĹCtirad (2C = 9.09 pg) and *Secale cereale* var. ĹDaňouské (2C = 18 pg) (Doležel et al. 2007) were used as internal standards. To release nuclei from the cells, 0.5 cm^2 of leaf tissue of *Lathyrus* was chopped together with 0.5 cm^2 of leaf tissue of the internal standard in 0.5 ml buffer (High resolution DNA kit, Partec GmbH, Münster, Germany). Subsequently, 5 U ml^{-1} of RNAse were added and incubated for 2–5 min at room temperature. The suspension was filtered through a 30 µm nylon

Table 1 List of the *Lathyrus* species studied including collector, accession number, provenance and herbaria in which the vouchers are deposited

Species	Collector, accession number and provenance
Section Notolathyrus	
L. cabrerianus Burkart	S. 1604. Argentina, Neuquén. Dpt. Los Lagos. Correntoso lake. (ASN-CTES-F-GH-K-MBM-NY-SI-TEX)
*L. crassipes** Hook. et Arn.	S. 2349. Argentina, Corrientes. Dpt. Empedrado. El Sombrero. (CTES-G-U)
L. hasslerianus Burkart	S. 2000. Argentina, Misiones. Dpt. San Pedro. Tambero river. (CTES-MBM-NY-SI)
L. macropus Gillies ex. Hook. *et* Arn.	S. 1730. Argentina. Mendoza. Malargüe. Los Molles. Salado river. (CTES-MO)
L. macrostachys Vogel	S. 1258. Argentina, Corrientes. Dpt. Santo Tomé. Virasoro. (BAB-CTES-MA-SPF-UPCB)
L. magellanicus Lam. var. *magellanicus*	S.1199. Argentina, Neuquén. Dpt. Los Lagos. Villarino lake. (CTES-GH-MBM)
L. multiceps Lam.	S. 1201. Argentina, Neuquén. Lacar. San Martín de los Andes. Chapelco. (BAB-CEN-CTES)
L. nervosus Lam.	S. 1188. Argentina, Río Negro. Los Lagos. Villa La Angostura. Nahuel Huapi lake. (CTES-GH -LIL-NY)
L. nitens Vogel	S. SN. 3966. Uruguay, Tacuarembó. Road to Gruta de los Cuervos. (CTES-UTEP)
L. paranensis Burkart	S., SN. 3954. Uruguay, Rivera. Cañada de Santa Bárbara. (ASU-CTES FUEL-IBGE-MBM-)
L. pubescens Hook. et Arn.	S., SN., P., So. 2491. Uruguay, Rivera. Bajada de Pena. (CESJ-CTES- MEXU-MO-SI- SPF-TEX-)
L. subulatus Lam.	S., SN. 3962. Uruguay, Tacuarembó. Road to Gruta de los Cuervos. (CTES)
L. tomentosus Lam.	S. 1207. Argentina, Buenos Aires. Tornquist. E. Tornquist Prov. Park. (CTES-GH-MBM-MEXU)

Annuals species are referenced by an asterisks (*)

Dpt., department. Collectors: P, C. Peichoto; S, G. Seijo; SN, V. Solís Neffa; So, M. Sosa

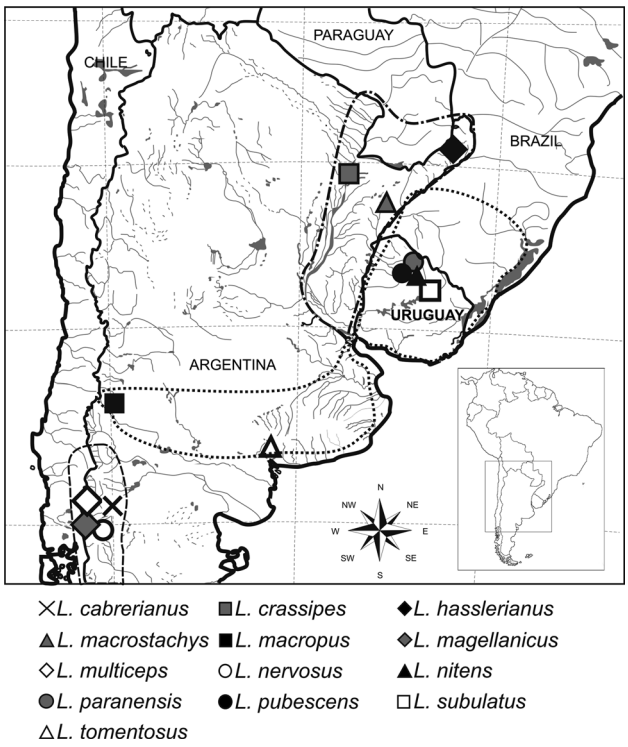

Fig. 1 Geographic distribution of the samples of the Notolathyrus species analyzed in this study. For location details see Table 1. The *dash-doted line* denotes the subtropical–paranaense region, the *doted line* the template-pampean region and the *dashed line* the cold–patagonian region

mesh. After this period, 1.5 ml of staining solution containing 1 μg μl^{-1} propidium iodide was added. Within 1 h of staining, measurements were performed with a Partec CA–II flow cytometer. About 10,000 nuclei were measured for each sample. The data analysis was performed using the Partec PA II FloMax software (Partec GmbH, Münster, Germany). All fluorescence values were converted to fluorescence ratios, relative to the closest reference standard. The absolute value of DNA content of each sample was calculated by the formula: DNA content of the sample = ($\overline{X}$ peak of sample × G1 DNA content (2C) of the standard)/$\overline{X}$ G1 peak of the standard (Doležel and Bartos 2005).

Data analysis

The mean, standard deviation and the coefficient of variation of 2C value were calculated for each species from three different individuals. Differences in DNA content between species were tested by one–way analysis of variance (ANOVA) at a significance level of 5 % ($\alpha = 0.05$). The Tukey 5 % post hoc test was used to test differences between each pair of species. DNA content variation in Notolathyrus section was compared to the available data for the genus (Table S1) (Ali et al. 2000; Ghasem et al. 2011; Nandini and Murray 1997).

The pattern of genome size variation was analyzed by plotting in an increasing order the DNA values of each Notolathyrus species studied. Groups of species with similar DNA content were established on the basis of the gaps observed in the distribution of the dataset. The mean, standard deviation, and range of variation of DNA values were calculated for each group. Tukey's test 5 % was carried out to test differences between each pair of groups.

To analyze whether the patterns of DNA variation observed in Notolathyrus species fit with those published previously for the Northern Hemisphere species, the mean and range of variation of DNA values of the groups detected in Notolathyrus were further compared with those established by Narayan (1982).

To infer the effect of DNA content variation on the TCL, the ratio between maximum and minimum values of DNA content and TCL were firstly compared among the Notolathyrus species and then considering the values of DNA content (Table S1) and TCL (Table S2) so far published in the genus.

The impact of the DNA variation on the karyotype morphology and on the patterns of chromosome change in Notolathyrus species was evaluated by comparing the values of DNA content here obtained with the karyotype parameters (mean values for each species of TCL; intrachromosomal asymmetry index, A_1; and interchromosomal asymmetry index, A_2) and karyotype formula previously published by our Laboratory group (Seijo and Fernández 2003). The karyotype values of Notolathyrus used to make the correlation analyses are the means published from at least ten drawn metaphases from two to four populations (including three to eight individuals) per species. The exceptions in which the published data were obtained from only one population were *L. hasslerianus*, *L. macropus* and *L. tomentosus*, which are rare species: the former one is known from only the type collection.

The expected changes in the values of karyotype parameters (TCL; ratio between length of the shortest chromosome and the longest one of each complement, R; length difference between the longest and the shortest chromosome of the complement, L-S; A_1 and A_2 asymmetry indices) and on each chromosome (C1 and C2) of the complement [chromosome length (CL), centromeric index (CI), and relative length of each chromosome in relation to the total length of the complement (RL)] were calculated for 30, 50 and 100 % increases of DNA content under models that considered equal and proportional changes of DNA amounts among all the chromosome arms of a theoretical complement composed by two chromosomes (a metacentric and a submetacentric ones). The models assumed that an increase of DNA content produce an equivalent increase in the chromosomes length.

The biological significance of the DNA content variation was evaluated in relation to the life cycle, the minimum generation time and the distribution range of the studied species. These data (not shown) were recovered from Burkart (1935, 1942), Seijo (2002), from personal observations of the authors in wild and cultivated populations, and from herbarium material. The DNA content of the species here studied was also analyzed in relation to geographic (latitude, longitude and altitude) and 19 bioclimatic variables (Table S3) to gain insights into the ecological significance of the variation observed. Data for bioclimatic variables were extracted from the WorldClim database (Hijmans et al. 2005). To standardize the analysis we selected only one population per species. We adopted this criteria since for some species we only have one sample and because extensive sampling has revealed striking stability in plant genome sizes, specially for chromosome stable groups (Bennett et al. 2000; Bennett and Leitch 2005; Kron et al. 2007; Greilhuber 1998, 2005; Price and Johnston 1996), like Notolathyrus (within which aneuploid or polyploid plants were not reported).

The Pearson correlation coefficient was used to test whether mean genome sizes were related to karyotypic, geographic and bioclimatic variables. Correlations between DNA content with geographic and bioclimatic variables were done, firstly, considering all Notolathyrus species and, then, considering groups of species growing in different biogeographic regions (subtropical–paranaense, temperate–pampean; and cold–patagonian) as depicted in Fig. 1.

All statistical analyses were performed using the InfoStat software version 2013 (Di Rienzo et al. 2013).

Results

The DNA amounts determined for 13 species of Notolathyrus section are shown in Table 2. The flow cytometric measurements of all the *Lathyrus* species and the standards resulted in well defined and sharp peaks with species coefficient of variation lower than 5 % (Table 2), supporting the reliability of the flow cytometric assessments.

The 2C nuclear DNA amounts of the species here analyzed varied from 12.50 pg (*L. crassipes*) to 21.32 pg (*L. cabrerianus*), representing about twofold (1.70) of variation in genome size (Table 2). The distribution of DNA content showed four discrete groups of species (designated A, B, C and D in Fig. 2) with different genome size. The amount of variation in DNA content within each group were 0 (only one species included), 0.89, 1.10 and 0.65 pg, separated by three gaps of 2.31, 1.58 and 2.29 pg, respectively (Fig. 2). Statistical analysis revealed significant differences between species ($F = 441$; $P < 0.0001$, Table 2) and between groups of species with different DNA content ($F = 382$; $P < 0.0001$).

Three out of the four groups detected in Notolathyrus could be assigned to the groups established for the Northern Hemisphere species (Fig. 2). The A group (12.50 pg) laid within the range of the third group (12.15–14.93 pg) of Narayan (1982). Similarly, the C group (17.19–18.38 pg) could be assigned to fourth group, although with a major range of variation (16.78–18.38 pg),

Table 2 2C DNA content of the *Lathyrus* species analyzed

Lathyrus	Biogeographic region[A]	2C (pg)[B]	CV[C]	Group[D]		Range[E]	TCL ± SE (μm)[F]	A_1^G	A_2^H	KF[I]
				Narayan	Present study					
Section Notolathyrus										
L. cabrerianus	C–P	21.32 ± 0.08[h]	0.40	5	D	R	45.75 ± 0.78	0.57	0.10	12 sm + 2 st
*L. crassipes**	ST–P	12.50 ± 0.15[a]	1.23	3	A	W	31.96 ± 1.20	0.43	0.08	4 m + 10 sm
L. hasslerianus	ST–P	21.13 ± 0.16[h]	0.83	5	D	R	45.57 ± 1.25	0.51	0.07	2 m + 12 sm
L. macropus	T–P	15.63 ± 0.31[c]	1.01	4	C	W	41.13 ± 1.11	0.52	0.10	2 m + 10 sm + 2 st
L. macrostachys	ST–P	17.31 ± 0.41[d]	0.41	4	B	M	44.97 ± 1.33	0.51	0.12	4 m + 10 sm
L. magellanicus var. *magellanicus*	C–P	18.38 ± 0.06[f]	0.32	4	C	W	43.31 ± 1.04	0.53	0.11	2 m +10 sm +2 st
L. multiceps	C–P	20.67 ± 0.09[g]	0.23	5	D	M	41.15 ± 2.56	0.55	0.09	2 m +10 sm +2 st
L. nervosus	C–P	17.19 ± 0.33[d]	1.04	4	C	W	41.02 ± 0.89	0.54	0.09	2 m +10 sm +2 st
L. nitens	T–P	18.01 ± 0.22[ef]	0.73	4	C	M	–	–	–	–
L. paranensis	T–P	15.70 ± 0.60[c]	0.35	4	B	W	–	–	–	–
L. pubescens	T–P	17.28 ± 0.29[d]	1.32	4	C	W	44.22 ± 0.80	0.50	0.11	2 m +10 sm +2 st
L. subulatus	T–P	14.81 ± 0.37[b]	0.45	4	B	W	–	–	–	–
L. tomentosus	T–P	17.34 ± 0.35[de]	0.65	4	C	M	45.90 ± 1.42	0.52	0.14	4 m + 8 sm + 2 st
ANOVA		$F = 565.67$ $P = <0.0001$								

Annuals species are referenced by an asterisks (*)

The karyotype parameters were taken from Seijo and Fernández (2003)

[A] Biogeographic region indicates the region in which the species is mostly distributed. *C–P* cold Patagonia, *T–M* template–pampean, *ST–P* subtropical paranaense regions

[B] 2C represents the DNA content in pg. For ANOVA results, different lower-case letters indicate significant differences among population for mean values of each parameter at 5 % level using Tukey's test

[C] *CV* coefficient of variation

[D] Group refers to that of DNA content published by Narayan (1982) for North Hemisphere species of *Lathyrus*, and those established in the present study

[E] Range indicates the extension of the geographic distribution of each species. *M* medium, *R* restricted, *W* wide areas of distribution

[F] *TCL* total chromosome length

[G] A_1 intrachromosomal asymmetry index

[H] A_2 interchromosomal asymmetry index

[I] *KF* karyotype formula. *m* metacentric chromosome, *sm* submetacentric chromosome, *st* subtelocentric chromosome

and the D group (20.67–21.32 pg) to the fifth group (19.52–22.08 pg) identified by Narayan. The B group (14.81–15.70 pg) of the Notolathyrus species could not be assigned to any of the Narayan's groups and mostly laid in the gap between the third and the fourth groups.

The 2C values of Notolathyrus species were positively and significantly correlated with TCL ($r = 0.70$; $P = 0.02$, Fig. 3a). However, the ratio between the highest and the lowest 2C DNA values (1.70) was higher than the ratio between the longest and the shortest TCL (1.51) within the Notolathyrus. Similarly, the ratio for DNA content was approximately of fourfold (Table S1), while the ratio between the longest and the shortest complements was 2.42 (Table S2), when all the known data for the genus *Lathyrus* were analyzed.

The DNA variation was also directly correlated ($r = 0.79$; $P = 0.01$) with the A_1 intrachromosomal asymmetry index but not with the A_2 interchromosomal index (Table 2; Fig. 3b). In spite of the tendency to increase the A_1 index as the genome size increase, the karyotype formula of the species remained largely constant (Table 2). The only variation observed was the

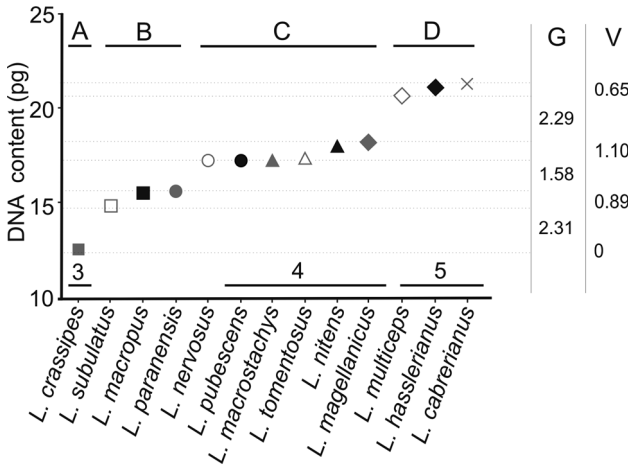

Fig. 2 DNA amount of the species of the Notolathyrus analyzed in this study. DNA contents were plotted in an increasing order. Data are the means of three independent measurements. *Upper horizontal lines* represent the groups of species (*A*, *B*, *C* and *D*) of Notolathyrus with statistical different DNA content. *Lower horizontal lines* represent the groups (*3*, *4* and *5*) established by Narayan (1982). *Numbers in the columns on the right* show the gaps in the distribution of DNA amount (pg) between adjacent groups (*G*) and the amounts of DNA content variation within each group of species (*V*). Species are represented by the *same symbols* used in Fig. 1

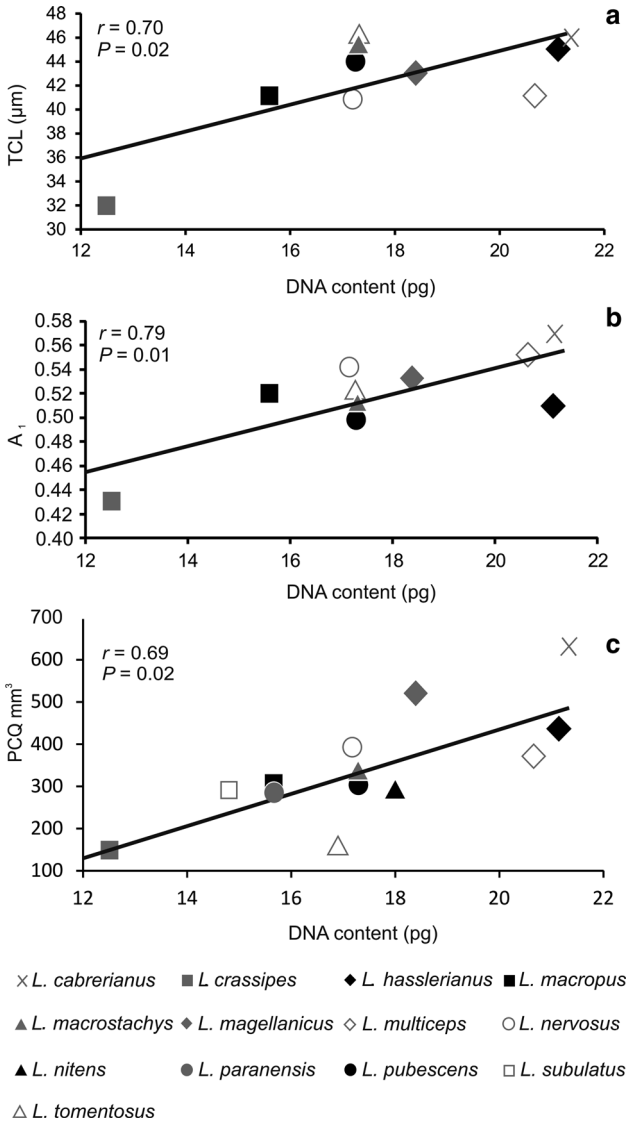

Fig. 3 Dispersion diagrams representing the relationships between 2C values with **a** total chromosome length (TCL), **b** intrachromosomal asymmetry index (A_1), and **c** precipitation in the coldest quarter (PCQ). *Values in the plots* show the coefficients of correlation between each pair of variables. A *trend line* was represented in each graph. Species are represented by the *same symbols* used in Fig. 1

replacement of one or two metacentric pairs by submetacentric ones (but with CI close to 37.50; Seijo and Fernández 2003) in the species with the largest genomes (Table 2).

In Fig. 4, we depicted the expected changes in different karyotype parameters considering proportional and equal distributions of DNA change among all chromosome arms of a theoretical karyotype composed by one metacentric and one submetacentric chromosomes. According to Fig. 4, proportional increments of the chromosome arms length up to 100 % by means of proportional distribution of DNA content did neither change the other chromosome parameters (CI and RL of the chromosomes remain unchanged) nor the karyotype parameters (chromosome ratio, A_1 and A_2 asymmetry indices) analyzed. Such proportional changes of DNA content led to the conservation of the karyotype formula. The alternative model that considered an equal distribution of DNA content variation among all chromosome arms irrespective of their length resulted in an increasing of CI and a decreasing of the A_1 and A_2 asymmetry indices, but in an increase of the relative length of the shortest chromosome. Such changes led to the modification of the karyotype formula, being more symmetric as the DNA content increase. The conservation of the karyotype formula among Notolathyrus species would fit well with the proportional changes of DNA content. However, the direct correlation of DNA content with the A_1 and the absence of a significant relationship with the A_2 asymmetry indices did not completely fit any of the models considered in Fig. 4.

The analysis of the relationships of DNA content with the life cycle of the Notolathyrus species showed that the annual *L. crassipes* had a significant lower DNA content (12.50 pg) than any of the perennial species (Table 2). Among the latter species, the 2C values ranged 15.70–21.32 pg (1.37-fold), with a mean value of 18.69 pg. The largest gap in DNA content was recorded in the transition between the only annual *L. crassipes* and the perennial species (Fig. 2). This trend was also detected

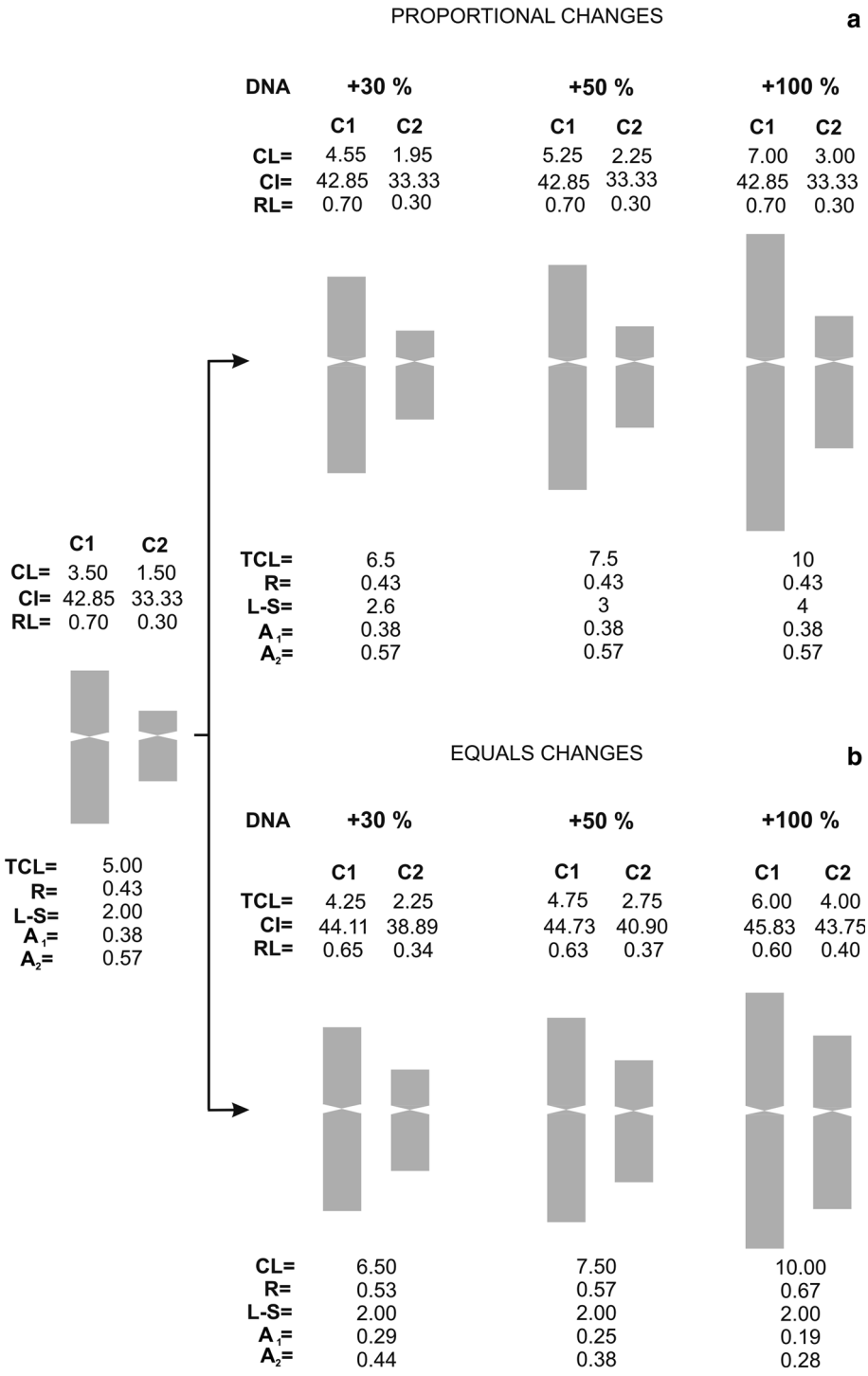

Fig. 4 Representation of **a** proportional and **b** equals changes of DNA in all chromosomes of the complement and their effects on chromosome and karyotype parameters. The theoretical complement is constituted by two chromosomes. Individual chromosome parameters (*CL*, *CI* and *RL*) are indicated above each chromosome (*C1* and *C2*). Complement parameters (*TCL*, *R*, *L-S*, A_1 and A_2) are indicated below each complement. Changes in DNA amount are indicated by the *plus symbol* followed by the percentage (30, 50, 100 %) of increase. *C1* chromosome 1, *C2* chromosome 2, *CL* chromosome length, *CI* centromeric index, *RL* relative length, *TCL* total chromosome length, *R* short/long chromosome ratio, *L-S* difference between largest chromosome and shortest chromosome of the complement, A_1 intrachromosomal asymmetry index, A_2 interchromosomal asymmetry index

among the species of section Lathyrus considering the values of DNA amounts recovered from the literature (Table S1). Among the perennial species of Notolathyrus, those with lower DNA content had shorter minimum generation time than species with larger genome size (data not shown).

The comparison of the DNA contents obtained here with the geographic ranges of the Notolathyrus species revealed that those with largest DNA content (*L. cabrerianus* and *L. hasslerianus*) were restricted to small areas while the species with intermediate (*L. magellanicus* and *L. nervosus*) to lower 2C values (*L. crassipes* and *L. subulatus*) were geographically extended (Table 2). The species with the largest genomes were found in patagonian and paranaense rain forests with more stable environments, while the species with medium to small genome sizes were found

in mountain grasslands, open savannas or temporarily flooded places that are more harsh and unstable.

The analysis of genome size variation observed in section Notolathyrus related to geographical and bioclimatic variables (Table S3), showed that DNA content tend to increase with altitude (with some exceptions, like *L. macropus*), relation that was more evident among species distributed below the 810 m above the sea level (a.s.l.) and that was directly correlated with the precipitations of the coldest quarter ($r = 0.69$; $P = 0.02$, Fig. 3c). The analysis of DNA content variation within the groups of species that belong to different biogeographic regions revealed additional relationships (not significant) with geographic and bioclimatic variables, particularly for the subtropical–paranaense region. In this region, the DNA content was positively and highly related with longitude ($r = 1$), altitude ($r = 0.91$), precipitation variables ($r = 1$–0.82), except precipitation seasonability ($r = -0.95$) variables, mean monthly temperature range ($r = 0.99$) and isothermality ($r = 0.97$), while was negatively related with all other temperature variables ($r = -0.89$ to 0.99), except temperature range ($r = 0.65$). However, DNA content was not correlated with any of the tested variables in the temperate–pampean and cold–patagonian regions (Table S3).

Discussion

This work constitutes the first analysis of genome size for *Lathyrus* species of the Notolathyrus section. It is also the first report in which DNA content variation of wild species of the genus *Lathyrus* was analyzed in relation to different karyotype features as well as to geographic and bioclimatic variables. To our knowledge, it is one of the first studies of this nature done in a closely related group of wild plants species of South America.

DNA content variation: continuous vs discontinuous

The range of 2C values (2C = 12.50–21.23 pg) measured for 13 species of section Notolathyrus covered 39 % of the total variation of DNA amount published for the genus *Lathyrus* (2C = 6.9–29.2 pg per 2C, Table S1). The distribution of the DNA amounts of Notolathyrus species in discrete groups separated by large gaps evidenced that the variation in DNA content in section Notolathyrus is not gradual. Notably, the magnitudes of the gaps observed in the distribution of DNA contents were higher (1.58–2.31 pg) between groups than the amount of variation in DNA content within each group (lower than 1.10 pg). These findings are in agreement with the discrete distribution of DNA content observed in the *Lathyrus* species of the Northern Hemisphere (Narayan 1982) and also with the patterns of variation reported in well studied groups of plants such as *Microseris* (Price and Bachmann 1975), *Nicotiana*, *Clarkia* and *Allium* (Narayan 1998), in which DNA content variation rather falls into discontinuous groups.

The facts that the magnitude of the gaps observed between each group of Notolathyrus species were similar to those recorded for the Northern Hemisphere species (Narayan 1982) and that the ranges of DNA content observed in three out of the four groups established for South American species fitted well with the Northern Hemisphere groups of species support the existence of different steady states of genome size within the genus (Narayan 1982).

DNA content variation and chromosome morphology: equal vs proportional changes of DNA

The results obtained in this study showed that the variation in genome size was directly related to the variation in the length of the chromosome complements of Notolathyrus species. The disparity observed between TCL and genome size ratios suggests that the variation in DNA content is influencing not only the chromosome length but also the chromosome volume (Bennett 1982a, b; Naranjo et al. 1998). Changes in the chromosome volume may also involved changes in the organization of the chromatin loops around the scaffold of the chromosomes affecting homology of chromosomes among related species. In this way, changes in genome size may lead or contribute to species differentiation among related groups of taxa.

The large variation in DNA content observed here in Notolathyrus without significant changes of the karyotype formula (Seijo and Fernández 2003) evidenced those changes in DNA amounts are no randomly distributed along the chromosome complements.

Comparisons of the observed chromosome and karyotype parameters of Notolathyrus species with those expected according to the models considered in Fig. 4, suggested that the conservation of the karyotype formula would support the proportional changes of DNA content. The fact that the changes observed in the A_1 and the A_2 asymmetry indices did not completely fit the models considered in Fig. 4 suggests that other factors like small structural chromosome rearrangements and/or higher order constrains for chromosome change like those proposed by Schweizer and Loidl (1987) are contributing in the modulation of the karyotypes of the Notolathyrus species.

Therefore, the nonrandom changes in DNA content along the chromosome complement that led to the conservation of the karyotype formula among South American taxa, in spite of 1.7-fold of genome size variation, may have been controlled by different intrinsic constrains of the

karyotype. An increasing amount of molecular and chromosome data evidences that changes in chromosomal size (by variation in DNA content) are constrained by the own and neighbor chromosomes structures, the organization in euchromatic and hetrochromatic blocks, the chromosome order and the organization of chromatin in functional nuclear domains as proposed in the models of Schweizer and Loidl (1987) and in that of the natural karyotype proposed by Bennett (1982b).

Genome size, life cycle and minimum generation time

DNA variation has different impacts on the morphology and physiology of the organisms (Bennett 1987). The lower DNA amounts observed in annual compared to perennial species of the section Notolathyrus is in agreement with the observations in the old world *Lathyrus* species (Nandini and Murray 1997; Rees and Hazarika 1969), and in other leguminous (Choi 1971; Naranjo et al. 1998) and non leguminous plants (Bancheva and Greilhuber 2006; Sims and Price 1985). For example, this trend was observed in the New World species of *Hordeum* (Jakob et al. 2004), in the Australian native species of *Sorghum* (Price et al. 2005), in the genus *Veronica* (Albach and Grilhuber 2004) and in many other herbaceous angiosperms (Bennett 1972). In the context of the nucleotype hypothesis, the lower DNA content was associated with a shortening of the cell cycle with the accompanying reduction not only of the life cycle but also with a reduction of the minimum generation time (Bennett 1972, 1987). In accordance, the lowest DNA content observed in Notolathyrus species is not only associated with the annual life cycle, but also with a shortening of the whole vegetative and reproductive periods. For example, in the paranaense group of species, the annual *L. crassipes* complete its life cycle in 3–4 months (August–October/November), while among the perennial species *L. macrostachys* complete the annual cycle (from sprouting to senescence of the aerial organs) in 4–5 months (August–November/December) and *L. hasslerianus* 5–6 months (August–December/January) (Burkart 1935; Seijo 2002). Therefore, the reduction of DNA content in the annuals and perennial species with a shorter minimum generation time within Notolathyrus section may be adapted and follows the predictions established by the nucleotype hypothesis (Bennett 1987).

DNA change vs geographical and bioclimatic variables

The phenotypic impacts of genome size variation have been initially demonstrated at the cellular level (Bennett 1972) and readily extended to the organism level (Bennett 1987). Whole-organism developmental rates, proposed to be affected by DNA content, may constrain the ecological lifestyle of the organism and the geographic and ecological preferences (Bennett 1987; Gregory 2001, 2005b; Knight et al. 2005; Price 1988). In this sense, the inverse association found in our results between DNA content and the geographic range of the species of Notolathyrus is in agreement with the "large genome constraint" hypothesis (Knight et al. 2005) that considers that the species with low DNA amounts tend to be widespread, whereas those with the highest C values are progressively excluded from increasingly harsh environments.

Considering the ecological requirements of Notolathyrus species, all of them vegetates and reproduces in cold–temperate environments. They are hydrophylic and most of them are heliophytic, living associated to open water streams. In general, these optimal conditions for Notolathyrus species are found in hills with open grasslands at medium altitudes (100–800 m.a.s.l. for perennials), where the humid wins condense and form small to medium size water courses. In the subtropical–paranaense and in the temperate–pampean regions the optimal conditions (middle temperatures, moisture and sun exposure) occurred in shorter periods as the altitude decrease. Encompassing the extension of the period with optimal conditions, the life cycle (in annual) or generation time (in perennials) of the species is shorter in lowlands. According to the nucleotype hypothesis (Bennett 1987), shortening of the generation time or life cycle would required a reduction of the DNA content. Consistently, our results showed that the species distributed at the lowest altitudes have lower DNA content and shorter generation time. Similarly, the species that live in the highest places (e.g. *L. macropus*) did not show the highest values of DNA content. The reduced genome size at high altitudes is expected because those localities are seasonally covered by snow and the optimal conditions to vegetate are shorter than at middle altitudes (up to 800 m.a.s.l.).

The direct association of DNA content with precipitations in the coldest quarter observed in Notolathyrus is directly related to the life story of these plant species. All Notolathyrus species initiates their vegetative cycle after the coldest quarter, therefore, precipitations during these period may be beneficial for the development of *Lathyrus* species, particularly in the initial phases, by regulating the temperature fluctuation and providing a source of humidity. The finding of species with higher DNA content in areas with higher precipitations during the coldest quarter supports that more benign environments may be more propitious for the development of species with larger genomes.

The analysis of the DNA content of the species within the three major biogeographic areas considered for this study, revealed other correlations that were not evident considering the section Notolathyrus as a whole, particularly in the subtropical paranaense region. The negative

association of higher DNA content with longitude in the subtropical–paranaense region is strongly related to the distribution of rainfall. In this region the rainfall decreases westward, and the vegetation turns from subtropical forest (the place of *L. hasslerianus*) to savannas (*L. macrostachys*) and seasonally dried grassland (*L. crassipes*). Since the Notolathyrus species are among the earliest to vegetate after winter in this region, the direct association of DNA content with larger amounts of precipitation in the coldest and driest quarter of the year suggests that the increase of rainfall provide more benign environment for the species with larger genomes. Similarly, the direct relationship of DNA content with altitude observed for Notolathyrus species in this region may be attributed to the fact that in the highest localities the period with optimal temperature in spring-summer is longer than in the lowlands, providing more stable environments for the development of species with larger genomes, like *L. hasslerianus*.

Direction of genomic change: amplifications vs reductions

In relation to the direction of DNA content change, most legumes are characterized by having small to medium size genomes (Doyle and Luckow 2003). However, species of the genera included in the Fabeae tribe (*Lathyrus*, *Lens*, *Pisum*, *Vicia* and *Vavilovia*) show a sharp increase in the diploid genome size that probably occurred during the differentiation of the tribe (Poggio et al. 2008). In the Notolathyrus section, and in the whole genus *Lathyrus*, the DNA contents of most perennial species are above 15 pg while those of the annuals are below 14 pg (Table S1). Assuming that annual species of *Lathyrus* are derived from the perennial ones, as it is largely accepted for angiosperms (Stebbins 1957), a significant reduction in the DNA amount should have occurred during the transition from perennial to annual life cycle in Notolathyrus section. Moreover, since the perennial–annual transition was recorded in, at least, three different clades of the *Lathyrus* phylogenetic tree (Kenicer et al. 2005), the reduction in DNA content may have independently occurred several times in the evolutionary history of the genus.

Although Bennetzen and Kellogg (1997) proposed that the plants may have a 'one-way ticket to genome obesity' as a consequence of retroelements accumulation and polyploidy, decreases in genome size accompanying angiosperm phylogeny have also been recorded (Soltis et al. 2003). Evolutionary decreases in genome size, similar to those observed in Notolathyrus, have been detected in the evolution of cotton relatives (Wendel et al. 2002), in the species of the genus *Sorghum* (Price et al. 2005) and in Brassicaceae (Johnston et al. 2005). It has been more recently recognized that genome shrinkage is a powerful and common process and can counteract the many mechanisms for genome growth (Petrov 2002). Among the mechanisms of genome size shrinkage, illegitimate recombination was recognized as the major factor responsible for most DNA removal in those plant and animal species that have been investigated to date (Bennetzen et al. 2005; Devos et al. 2002; Ma et al. 2004; Petrov and Hartl 1997; Wicker et al. 2003).

The mutational mechanisms that are proposed for genome size reduction may operate on (at least) three very different scales (Gregory 2005a). The first one is based on a predominance of deletions over insertions on scales <400 bp and constitutes the basis of the 'mutational equilibrium model' (Petrov 2002). However, this deletional mechanism was considered as extremely weak to play a major role in the large-scale genome size evolution (Bennetzen and Kellogg 1997; Gregory 2004). The second scale of deletional mechanisms involves recombination between homologous copies of the long terminal repeats characteristic of LTR retrotransposons (Kalendar et al. 2000). Recombination between these elements leaves behind only a 'solo LTR' (Devos et al. 2002) and is considered a more powerful mechanism than the first for large genome size reduction. The third scale, involves 'illegitimate recombination' between LTRs of non-homologous elements either on the same or different chromosomes that may lead to extensive DNA loss. This is the only one of the three scales of deletions capable of producing extensive genomic shrinkage over reasonable timescales (Bennetzen 2002; Bennetzen et al. 2005). In Notolathyrus species, although random occurrence of deletions at the three scales may have occurred, the conservation of the karyotype formula suggest that the extension of these processes may have been constrained by the chromosome or chromatin organization in the nuclei as proposed by Bennett (1982b) and (Schweizer and Loidl 1987).

Conclusions

The variation registered among the species evidenced that changes in DNA content were one of the most important mechanisms that accompanied or determined the diversification of the South American species of *Lathyrus*. These changes in genome size may have been constrained to produce proportional accumulation/reduction of DNA in the chromosome arms leading to the conservation of the karyotype formula. The association of genome size with the life cycle of the species and the correlations with some geographic and bioclimatic variables strongly suggest that variation in DNA amount has an adaptive significance in Notolathyrus.

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