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Abstract

Notolathyrus is a section of South American endemic species of the genus *Lathyrus*. The origin, phylogenetic relationship and delimitation of some species are still controversial. The present study provides an exhaustive analysis of the karyotypes of approximately half (10) of the species recognized for section *Notolathyrus* and four outgroups (sections *Lathyrus* and *Orobus*) by cytogenetic mapping of heterochromatic bands and 45S and 5S rDNA loci. The bulk of the parameters analyzed here generated markers to identify most of the chromosomes in the complements of the analyzed species. Chromosome banding showed interspecific variation in the amount and distribution of heterochromatin, and together with the distribution of rDNA loci, allowed the characterization of all the species studied here. Additionally, some of the chromosome parameters described (*st* chromosomes and the 45S rDNA loci) constitute the first diagnostic characters for the *Notolathyrus* section. Evolutionary, chromosome data revealed that the South American species are a homogeneous group supporting the monophyly of the section. Variation in the amount of heterochromatin was not directly related to the variation in DNA content of the *Notolathyrus* species. However, the correlation observed between the amount of heterochromatin and some geographical and bioclimatic variables suggest that the variation in the heterochromatic fraction should have an adaptive value.

Keywords (separated by '-') Heterochromatin - Karyotype - *Lathyrus* - rDNA loci

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REGULAR PAPER

Karyotype characterization and evolution in South American species of *Lathyrus* (*Notolathyrus*, Leguminosae) evidenced by heterochromatin and rDNA mapping

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Abstract *Notolathyrus* is a section of South American endemic species of the genus *Lathyrus*. The origin, phylogenetic relationship and delimitation of some species are still controversial. The present study provides an exhaustive analysis of the karyotypes of approximately half (10) of the species recognized for section *Notolathyrus* and four outgroups (sections *Lathyrus* and *Orobus*) by cytogenetic mapping of heterochromatic bands and 45S and 5S rDNA loci. The bulk of the parameters analyzed here generated markers to identify most of the chromosomes in the complements of the analyzed species. Chromosome banding showed interspecific variation in the amount and distribution of heterochromatin, and together with the distribution of rDNA loci, allowed the characterization of all the species studied here. Additionally, some of the chromosome parameters described (*st* chromosomes and the 45S rDNA loci) constitute the first diagnostic characters for the *Notolathyrus* section. Evolutionary, chromosome data revealed that the South American species are a homogeneous group supporting the monophyly of the section. Variation in the amount of heterochromatin was not directly related to the variation in DNA content of the *Notolathyrus* species. However, the correlation observed between the amount of heterochromatin and some geographical and bioclimatic variables suggest that the variation in the heterochromatic fraction should have an adaptive value.

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Keywords Heterochromatin · Karyotype · *Lathyrus* · rDNA loci

Introduction

The genus *Lathyrus* L. (Leguminosae, tribe Fabeae) includes around 150 species of annual and perennial herbs that were arranged into 13 sections according to morphological features, life cycle and geographic distribution (Kupicha 1983). This genus is distributed throughout the temperate regions of the Northern Hemisphere and extends into tropical East Africa and South America (Kupicha 1983). The main center of species diversity is located in the Mediterranean and Irano-Turanian regions, and two smaller ones are found in North and South America (Kupicha 1983).

There are about 23 species of *Lathyrus* endemic to South America (Burkart 1935, 1942; Neubert and Miotto 2001; Rossow 1982) which were placed within section *Notolathyrus* (Kupicha 1983). These species are mostly distributed in temperate-cold climates from the North of Colombia to South Argentina along the Andes Mountains, and from the Pacific to the Atlantic coast. *Lathyrus pusillus* Elliot, from North America, is also included in section *Notolathyrus*. According to the geographic distribution of the species, three major centers of diversity have been recognized for this section: the Paraná-Uruguay River basin, the Precambian mountains of the bioma Pampa, and the Patagonian forests (Burkart 1935; Seijo 2002).

Some of the recognized *Notolathyrus* species are widely distributed, while others are endemic to very small areas or known only from the type collection (*L. paraguariensis* Hassler). In some cases, specimens were not collected any more since several decades ago (e.g. *L. paraguariensis*, *L.*

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parodii Burkart, *L. hookerii* G. Don). Some of the species can be clearly distinguished using exomorphological characters, while others constitute morphological complexes and their taxonomic treatment is still a matter of debate (Neubert and Miotto 2001; Rossow 1982).

In spite that *Notolathyrus* is considered as a fairly homogeneous group, the section does not have any diagnostic character. Moreover, several South American species have one to several morphological features in common with species of other well defined sections of the genus distributed in the Northern Hemisphere (Kupicha 1983); for instance: uniyugate leaves, narrowly elliptic leaflets with parallel venation, double stigma and false "woolly" septa between seeds. The presence of some plesiomorphic characters and the lack of synapomorphies that can be used to define the section *Notolathyrus* have hampered the understanding of the origin of the South American species and their relationship with other members of the genus.

Burkart (1935) and Kupicha (1983) suggested that the South American species of *Lathyrus* dispersed into the region from North America via the Andes Mountains. The first molecular phylogenetic analysis of the genus supported this scenario suggesting that the *Notolathyrus* section should be considered as a derived group of the transberingian flora (Asmussen and Liston 1998). A more recent analysis of DNA sequences placed the *Notolathyrus* group outside the transberingian clade (which contains all the extant North American species sampled) and proposed long distance dispersal events from the Mediterranean nucleus of species to explain the origin of South American taxa (Kenicer et al. 2005). This position was supported in the recent phylogeny of the Fabeae tribe (Schaefer et al. 2012), in which it was suggested that *Notolathyrus* members split from a Mediterranean ancestral lineage about 8.6–6.1 Mya.

From a cytological point of view, most of the species of the genus are diploid with a basic chromosome number $x = 7$ (Goldblatt 1981; Krapovickas and Fuchs 1957; Senn 1938). Polyploids are rare exceptions; there is only one tetraploid species (*L. venosus* Müh., $2n = 4x = 28$), and triploid, tetraploid and hexaploid cytotypes were found only in very few species (Senn 1938; Gutiérrez et al. 1994; Kawaha et al. 1995; Chalup et al. 2012). The karyotype analyses by conventional staining of *Lathyrus* species from Europe and North America (Lavania and Sharma 1980; Yamamoto et al. 1984; Sahin et al. 2000), and more recently of the section *Notolathyrus* (Battistin and Fernández 1994; Klamt and Schiffino Wittman 2000; Seijo and Fernández 2003; Seijo and Solís Neffa 2006) revealed a large interspecific variation in chromosome size, although the chromosome morphology and the patterns of nucleolar organizer regions are conserved (Ali et al. 2000; Murray et al. 1992; Seijo and Fernández 2003). Variations in chromosome morphology allowed the identification of species groups associated mainly with the life cycle and to a lesser extent to the taxonomic sections (Seijo and Fernández 2003; Yamamoto et al. 1984). However, the variation in chromosome features detected by classical techniques was neither enough to characterize most of the taxa at the species level nor to establish the evolutionary trends of chromosome change.

Heterochromatin detection together with physical mapping of DNA sequences by fluorescent in situ hybridization (FISH) have proved to be useful techniques to develop chromosome markers to delimit species (Moscone et al. 1999; Raina et al. 2001; Robledo et al. 2009), to investigate the species relationships (Robledo and Seijo 2010; Thomas et al. 1997) and to analyze the patterns of karyotype evolution (Fonsêca and Pedrosa-Harand 2013; Maluszynska and Heslop-Harrison 1993; Moscone et al. 2007; Wolny and Hasterok 2009) in many plant groups with poor differentiation in chromosome morphology. In *Lathyrus*, heterochromatin detection by C-banding was carried out in a few species of the old world (Lavania and Sharma 1980; Narayan 1982; Narayan and McIntre 1989), but mainly focused on investigating the causes and consequences of genome size variation within the genus. Fluorescent banding was also applied to small group of species, most of which were the same taxa analyzed by C-banding assays (Ünal et al. 1995). Both types of approaches used to detect heterochromatin provided a set of new chromosome markers and were useful to improve the karyotype characterization of the genus.

Similarly, the localization of the 45S and 5S ribosomal RNA genes (rDNA) by FISH has been applied to seven *Lathyrus* species of the Old World (that belong to sections *Aphaca*, *Clymenum* and *Lathyrus*) (Ali et al. 2000) and to only one South American species, *L. nervosus* (Chalup et al. 2012). In spite of the small representation of the species analyzed, the number and distribution of the ribosomal loci showed interspecific variation and demonstrated that they can provide useful cytogenetic markers to identify homologous chromosomes and to improve the karyotype characterization of the species.

DNA content variation of 1.7-fold was registered for *Notolathyrus* species and, notably, four groups of species with different DNA content were determined (Chalup et al. 2014). The 2C values were correlated with the total chromosome length obtained by Feulgen's staining (Seijo and Fernández 2003) and as the karyotype formula remained almost constant it was proposed that DNA changes occurred proportionately in all the chromosome arms. In spite of the variation detected and the relationships established in that report, there is no information about the genome fraction associated to the DNA content variation in the South American species. In this sense, a comparative analysis of the heterochromatin/euchromatin fractions in relation to the DNA content may provide useful

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information to understand the mechanisms of chromosome evolution of this section.

In the present study, we physically mapped the 45S and the 5S rDNA loci by FISH and the heterochromatic bands by using CMA and DAPI fluorochromes in ten species of section *Notolathyrus* and in four species of the North Hemisphere. Additionally, some of the karyotype parameters studied here were analyzed in relation to geographic and bioclimatic variables. These analyses were conducted aiming to analyze the goodness of the chromosome markers in delimiting the species of section *Notolathyrus*, to investigate the existence of any diagnostic chromosome markers for this section, to gain insights into the ecological significance of the chromosome variation observed, and to analyze the patterns of chromosome change during the diversification of the South American taxa.

Materials and methods

Plant materials

The plant materials studied here and their provenances are presented in Table 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). In order to compare the results obtained for South American species, four species of sections *Lathyrus* and *Orobus* from the North Hemisphere were also included in the study.

Chromosome preparations

Chromosome preparations were made from root tips obtained from germinated seeds on moist filter paper in petri dishes. Roots were pre-treated with 2 mM 8-hydroxyquinoline for 4 h at room temperature, then fixed in absolute ethyl alcohol: glacial acetic acid (3:1), and kept in the same solution at −20 °C until use. Root tips were incubated in 1 % (w/v) *Trichoderma viridae* cellulase plus 10 % (w/v) *Aspergillus niger* pectinase dissolved in 40 % (v/v) glycerol in 0.01 M citrate buffer (pH 4.8) at 37 °C for 20–45 min, and then squashed in 45 % (v/v) aqueous acetic acid (Schwarzacher et al. 1980). Coverslips were removed with carbon dioxide, and the slides were aged for 1–2 days at room temperature, and then kept at −20° C until use.

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

Species	Collector[b], accession number and provenance
Section Notolathyrus	
L. cabrerianus Burkart	S.1604. Argentina, Neuquén. Los Lagos. Correntoso lake. (ASN-CTES-F-GH- K-MBM-NY-SI-TEX)
L. crassipes[a] Hook. & Arn.	S. 2349. Argentina, Corrientes. Empedrado. El Sombrero. (CTES-G-U)
L. hasslerianus Burkart	S. 2000. Argentina, Misiones. San Pedro. Aº Tambero. (CTES-MBM-NY-SI)
L. macrostachys Vogel	S. 1258. Argentina, Corrientes. Santo Tomé. Virasoro. (BAB-CTES-MA-SPF-UPCB)
L. magellanicus Lam. var. *magellanicus*	S. 1182. Argentina. Río Negro. Bariloche. Gutiérrez lake. (CTES-F-GH-MEXU-MICH-NY)
L. multiceps Lam.	S. 1195. Argentina, Neuquén. Los Lagos. Villarino lake. (CTES-GH-MBM)
L. nitens Vogel	S., SN., Pe., So. 2685. Uruguay, Tacuarembó. Road to Gruta de los Cuervos. (CTES-MEXU-MO-SI-U)
L. paranensis Burkart	S., SN. 3954. Uruguay. Rivera. Cañada de Santa Bárbara. (ASU-CTES-FUEL-IBGE-MBM)
L. pubescens Hook. & Arn.	S., SN., P., So. 2491. Uruguay. Rivera. Bajada de Pena. (CTES-CESJ-MEXU-MO-SI-SPF-EX)
L. tomentosus Lam.	S. 1207. Argentina, Buenos Aires. Tornquist. E. Tornquist Prov. Park. (CTES-GH-MBM-MEXU)
Section Lathyrus	
L. latifolius L.	S. 1738. Adventitious in Argentina, Mendoza. San Rafael. (CTES-K-MBM-NY-SI)
L. odoratus[a] L.	S. 2008. Cultivated at Corrientes, Argentina. Origin: Japón. (CTES)
L. sylvestris L.	S. s/n. Cultivated at Corrientes, Argentina. Origin: Matsuyama, Japón. (CTES)
Section Orobus	
L. japonicus Willd.	S. 2353. Cultivated at Corrientes, Argentina. Origin: Japan Shikoku. Ehime. Matsuyama. Kashima. (CTES)

Herbaria are cited according to Holmgren et al. (1990)

[a] Annual species

[b] Collectors: P, C. Peichoto; S, G. Seijo; SN, V. Solís Neffa; So, M. Sosa

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Chromosome banding and in situ hybridization

Slides were stained with chromomycin A_3 (CMA) for 90 min, then with 4, 6-diamidino-2-phenylindole (DAPI) for 30 min and mounted in Mc Ilvaine's (pH 7.0) buffer:glycerol v/v 1:1 (Schweizer 1976). Fluorescent in situ hybridization (FISH) using 45S and 5S probes (rDNA) of *Arachis hypogaea* (Robledo and Seijo 2008) was carried out according to Moscone et al. (1996 and Chalup et al. 2012). Chromosomes were analyzed and photographed with a Leica DMRX epifluorescence microscope (Leica, Heerbrugg, Switzerland). Digital images were combined using IM 1000 Leica software and then imported into Photoshop, version 7.0 (Adobe, San Jose, California, USA) to process color balance, brightness, and contrast uniformly across the image.

Karyotypes

All measurements were made on metaphase plates stained with DAPI. To describe the chromosome morphology at least ten metaphases were measured for each species (3–6 individuals), using the free version of the MicroMeasure 3.3 software (Reeves 2001). The karyotype formula was described using the centromeric index (ci = short arm × 100/total length) following the Levan et al. (1964) nomenclature. Accordingly, chromosomes were classified into three categories: metacentric (*m*)i = 50–37.5, submetacentric (*sm*) i = 37.5–25 and subtelocentric (*st*) i = 25–12.5. Chromosome lengths and centromeric indices from homologous chromosomes were combined to mean values for each metaphase. The average chromosome length (CL) and the total chromosome length (TCL) of each species were established by averaging the lengths of all chromosomes and by adding the average lengths of chromosomes of the complement, respectively. Karyotype asymmetry was determined using the intrachromosomal asymmetry index (A_1) = [S(*b*/*B*)/*n*]; and interchromosomal asymmetry index (A_2) = *s*/*x* (Romero-Zarco 1986), where *b* and *B* are the mean length of short and long arms of each pair of homologues, respectively, *n* is the number of homologues, *s* is the standard deviation, and *x* the mean chromosome length.

Mean karyotype values for each species were represented as haploid complements in the idiograms. Chromosomes were ordered primarily by morphology and then by decreasing size. Chromosome bands and rDNA loci were mapped using the index di = d × 100/a (d = distance of band center from the centromere, a = length of the corresponding chromosome arm) according to Greilhuber and Speta (1976). Total $DAPI^+$ heterochromatin was determined by adding the average lengths of the heterochromatic bands detected in each chromosome and was expressed as a percentage of the TCL.

Statistical procedures

All statistical analyses were done using the InfoStat software version 2013 (Di Rienzo et al. 2013). Interspecific variation of TCL, heterochromatin length and euchromatin length were performed by the ANOVA test, with application of Tukey's test. The relationship of some karyotype parameters with the DNA content for *Notolathyrus* species was also investigated by the ANOVA test. The DNA content data obtained by flow cytometry was recovered from Chalup et al. (2014) and Nandini and Murray (1997). Some of the karyotype parameters studied here were also analyzed in relation to geographic (latitude, longitude and altitude) and bioclimatic variables 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) and the Pearson correlation coefficient was used to test the relationships.

Results

The general karyotype features are listed in Table 2, and the patterns of heterochromatic bands and of rDNA loci mapped for the 14 *Lathyrus* species analyzed are listed in Table 3. Representative somatic metaphases stained with fluorochromes are shown in Figs. 1, 2 and 3 and, after FISH treatment, in Figs. 4 and 5. Consensus idiograms containing all the markers detected for each species are illustrated in Fig. 6.

General karyotype features

All the species studied here were diploids with $2n = 2x = 14$ (Figs. 1, 2, 3, 4 and 5). However, five different karyotype formulae were observed among them (Table 2). The karyotypes of the South American species (section *Notolathyrus*) were characterized by the presence of one *st*, a large proportion of *sm*, and from zero to two *m* chromosome pairs. By contrast, the North Hemisphere (sections *Orobus* and *Lathyrus*) species presented karyotypes composed of three to four *m* pairs and without *st* chromosomes. South American species characteristically possessed a secondary constriction that defines a large satellite on the long arm of pair #7 (Figs. 1, 4 and 6). The perennial species of the Northern Hemisphere had only one pair of satellites in the short arms of pairs #1, #2 or #4, while *L. odoratus* (annual) presented microsatellites in two pairs (#4 and #5) of chromosomes (Figs. 3, 5 and 6).

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Table 2 Chromosome features of *Lathyrus* species

Species	KF	TCL ± SE	CL ± SE	Range CL	CI	A_1	A_2	H
Section Notolathyrus								
L. cabrerianus	12 *sm* + 2 *st*	$54.22^{a,b}$ ± 1.98	7.74 ± 0.35	8.99–6.47	30.29	$0.56^{a,b}$	0.13	S
L. crassipes[a]	4 *m* + 8 *sm* + 2 *st*	44.89^{d} ± 1.76	6.41 ± 0.25	7.39–5.42	33.06	0.48^{c}	0.09	S
L. hasslerianus	2 *m* + 10 *sm* + 2 *st*	$50.56^{b,c}$ ± 1.40	7.22 ± 0.19	8.81–6.40	31.49	0.52^{b}	0.12	S
L. macrostachys	12 *sm* + 2 *st*	$49.61^{a,b}$ ± 3.05	7.09 ± 0.66	7.95–6.04	30.86	0.53^{b}	0.12	S
L. magellanicus var. magellanicus	2 *m* +10 *sm* +2 *st*	54.03^{a} ± 1.95	7.71 ± 0.39	8.99–6.98	31.84	0.52^{b}	0.09	S
L. multiceps	12 *sm* + 2 *st*	$48.36^{c,d}$ ± 0.40	6.91 ± 0.13	8.12–5.54	29.89	$0.57^{a,b}$	0.11	S
L. nitens	2 *m* + 10 *sm* + 2 *st*	55.36^{a} ± 2.02	7.91 ± 0.28	8.96–6.84	31.55	0.53^{b}	0.09	S
L. paranensis	2 *m* + 10 *sm* + 2 *st*	$48.47^{c,d}$ ± 0.71	6.92 ± 0.10	7.85–6.03	31.88	0.52^{b}	0.10	S
L. pubescens	12 *sm* + 2 *st*	51.95^{a} ± 2.01	7.42 ± 0.29	8.79–6.02	31.78	0.47^{c}	0.13	S
L. tomentosus	12 *sm* + 2 *st*	57.12^{a} ± 0.47	8.16 ± 0.16	10.01–6.44	30.14	0.57^{a}	0.15	S
ANOVA		F = 18,17				F = 18,35		
		$P \leq 0.0001$				$P \leq 0.0001$		
Section Lathyrus								
L. latifolius	6 *m* + 8 *sm*	69.71^{b} ± 3.59	9.56 ± 0.10	13.77–9.73	37.47	0.40	0.13	N
L. odoratus[a]	6 *m* + 8 *sm*	49.83^{a} ± 1.92	7.12 ± 0.58	8.90–5.71	37.90	0.39	0.14	N
L. sylvestris	8 *m* + 6 *sm*	62.72^{b} ± 5.70	8.96 ± 0.67	10.73–7.67	41.12	0.33	0.15	N
ANOVA		F = 489.68				F = 3.49		
		$P \leq 0.0001$				$P \leq 0.099$		
Section Orobus								
L. japonicus	8 *m* + 6 *sm*	42.20 ± 1.68	6.03 ± 0.24	8.16–4.58	39.96	0.35	0.19	N

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

KF Karyotype formula, *TCL* Total length of the haploid complement, *CL* Mean chromosome length, *Range CL* Range of chromosome length, *CI* Mean centromeric index, A_1 Intrachromosome asymmetry index, A_2 Interchromosome asymmetry index, *H* Hemisphere, *SE* Standard error, *m* Metacentric, *sm* Submetacentric, *st* Subteleocentric, *S* South, *N* North

[a] Annuals species

Total chromosome length (TCL) was statistically different among the species of each of the sections analysed (Table 2). Moreover, the TCL observed for South American species were different from those observed in *Lathyrus* and *Orobus* sections (F = 18.06, $p = 0.0001$). Within each section, annual species had shorter (F = 5.67, $p = 0.0221$) chromosome complements than perennial ones (Table 2). The centromeric indices of *Notolathyrus* species were smaller than those observed in species of the other sections (Table 2). The asymmetry index A_1 of *Notolathyrus* species were slightly higher (F = 53.55, $p = 0.0001$) than in species of the other sections analyzed (Table 2; Fig. 7). The asymmetry index A_2 (Table 2) was relatively small in all the species and not statistically different (F = 0.40, $p = 0.6714$).

Heterochromatin

In all the species, $CMA^+/DAPI^0$ heterochromatin (Fig. 1) was observed exclusively at the secondary constrictions of the chromosomes where the 45S rDNA genes mapped (Fig. 6, see below). In contrast, $CMA^0/DAPI^+$ heterochromatin (hereafter designated as $DAPI^+$) was arranged in different patterns, which varied in the number, size and location of the bands among the taxa. All the species showed $DAPI^+$ bands at the centromeres, but the number of chromosome pairs that borne these bands ranged from three to seven (Table 3; Figs. 2, 3 and 6). The size of the bands varied from dotted like (mostly in *Notolathyrus* species, Figs. 2 and 6) to large solid blocks (in section *Lathyrus*, Figs. 3, 6).

In addition to the centromeric bands, proximal, interstitial and distal bands were rarely observed in few species of *Notolathyrus* but were more frequent in the perennial species of the *Lathyrus* section (Table 3; Figs. 2, 3). The heterochromatin amount per karyotype, measured as a percentage of the TCL, varied largely among the species analyzed (Table 3). The total amount of $DAPI^+$ heterochromatin varied in more than five times within section *Notolathyrus* and around two within section *Lathyrus* (Table 3). Considering all the species here analyzed, the amount of heterochromatin observed in *L. japonicus* (section *Orobus*) was in the range

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Table 3 Karyotypic features and genome size (2C) of *Lathyrus* species obtained by DAPI banding and fluorescence in situ hybridization (FISH) of 5S and 45S rDNA

Species	DAPI$^+$ HT	Centromeric bands	Proximal bands	Interstitial bands	Distal bands	Total bands number	5S DNAr	45S DNAr	2C (pg)[B]
Section Notolathyrus									
L. cabrerianus	4.52^{a,b} (8.47 %)	7	0	0	0	7	1 [2*p* (P)]	1 [7*q* (I)]	21.32 ± 0.08$^{(1)}$
L. crassipes[A]	1.21^{d} (2.69 %)	3 (1, 5, 7)	0	0	0	3	1 [3*p* (I)]	1 [7*q* (I)]	12.50 ± 0.15$^{(1)}$
L. hasslerianus	1.40^{d} (2.77 %)	6 (2, 3, 4, 5, 6, 7)	1 (1*p*)	0	0	7	1 [1*p* (P)]	1 [7*q* (D)]	21.13 ± 0.16$^{(1)}$
L. macrostachys	2.23^{c,d} (4.49 %)	5 (1, 2, 4, 5, 7)	0	0	0	5	1 [2*p* (I)]	1 [7*q* (I)]	17.31 ± 0.41$^{(1)}$
L. magellanicus var. magellanicus	5.71^{a} (10.56 %)	7	0	0	0	7	1 [4*p* (P)]	1 [7*q* (D)]	18.38 ± 0.06$^{(1)}$
L. multiceps	7.15^{a} (14.79 %)	7	1 (1*q*)	3 (1, 3, 6*q*)	0	11	1 [4*p* (P)]	1 [7*q* (I)]	20.67 ± 0.09$^{(1)}$
L. nitens	2.80^{c} (5.73 %)	7	0	0	0	7	1 [2*p* (P)]	1 [7*q* (D)]	18.01 ± 0.22$^{(1)}$
L. paranensis	4.38^{a,b} (9.05 %)	7	1 (6*q*)	2 (2, 7*q*)	0	10	1 [3*p* (P)]	1 [7*q* (D)]	15.70 ± 0.60$^{(1)}$
L. pubescens	3.87^{b,c} (7.45 %)	7	0	0	0	7	1 [3*p* (I)]	1 [7*q* (D)]	17.28 ± 0.29$^{(1)}$
L. tomentosus	6.50^{c} (11.40 %)	7	2 (2*p*; 5*q*)	4 (1, 3, 6, 7*q*)	2 (4*q*; 6*p*)	15	1 [4*p* (P)]	1 [7*q* (D)]	17.34 ± 0.35$^{(1)}$
ANOVA	F = 38.89								
	$P \leq 0.0001$								
Section Lathyrus									
L. latifolius	19.57^{b} (20.91 %)	7	0	1 (6*q*)	11 (1, 2, 3, 4, 6,7*p*; 1,2,3,5,7-*q*)	19	1 [4*p* (P)]	1 [4*p* (D)]	23.3$^{(2)}$
L. odoratus[A]	5.04 ^{a} (10.13 %)	7	0	1 (4*p*)	0	8	–	2 [4, 5*p* (D)]	14.3$^{(2)}$
L. sylvestris	10.31^{c} (16.43 %)	5 (2, 4, 5, 6, 7)	0	3 (1, 6*p*; 3*q*)	6 (1, 2, 3, 4*p*; 4, 6*q*)	14	1 [2*p* (P)]	1 [2*p* (I)]	23.3$^{(2)}$
ANOVA	F = 60.14								
	$P = 0.0001$								
Section Orobus									
L. japonicus	3.50 (8.30 %)	7	0	0	0	7	2 [1*p* (D); 2*p* (P)]	1 [1*q* (I)]	No data

Different lowercase letters indicate significant differences in the mean values of DAPI$^+$ heterochromatin ($P < 0.05$) obtained by the Tukey's test

DAPI $^+$ HT Total amount of DAPI$^+$ heterochromatin expressed as absolute length in μm and the numbers in parentheses indicate the percentage of heterochromatin in relation to the total length of the haploid complemen, *Centromeric*, *Proximal* and *Interstitial bands* are indicated as the total number per haploid complement, and their location is indicated in parentheses, *5S rDNA and 45S rDNA* indicate the number of rDNA loci per haploid complement, the chromosome pair is indicated in square brackets and their position in parentheses. *P* Proximal, *I* Interstitial, *D* Distal, *p* Short arm, *q* Long arm

[A] Distinguish annuals species

[B] DNA content values were recovered from the literature: [(1)] Chalup et al. (2014); [(2)] Nandini and Murray 1997

of values registered for *Notolathyrus* species, while those of the species of section *Lathyrus* were the highest (F = 4.02, $p = 0.05$). Considering the species of *Notolathyrus*, the total amount of heterochromatin was not correlated to the length of the karyotypes (r = 0.50, $p = 0.17$).

Concerning the life cycles of the species (in *Notolathyrus* and *Lathyrus* sections), the annuals had lower (not statistically different) heterochromatin content than the perennials (except for *L. hasslerianus*, which had the lowest heterochromatin of the perennial species here studied) (Table 3). The heterochromatin was largely restricted to the centromeres in the annuals, while bands located in other chromosome positions aside of the centromeric regions were more frequently observed in the perennials (Table 3).

TCL and heterochromatin vs DNA content and geo-climatic variables

Neither the TCL, (r = 0.33, $p = 0.34$) nor the total DAPI$^+$ heterochromatin were correlated (r = 0.33, $p = 0.34$) to the DNA contents published for *Notolathyrus* species (compiled in Table 3). Similarly, TCL was not correlated with any geographic or bioclimatic variables (data not shown). However, the total amount of DAPI$^+$ heterochromatin measured in *Notolathyrus* species was directly correlated with the three geographical variables analyzed (Table 4) but inversely and significantly correlated (r values between −0.68 and −0.89) with 11 of the 19 bioclimatic variables considered here (Table 4).

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Ribosomal genes

The patterns of rDNA loci distribution revealed by FISH were fairly conserved among species within the same section but differed among species from different sections. A single pair of 45S rDNA loci and a single pair of 5S rDNA loci were observed in all *Notolathyrus* species (Table 3; Figs. 3, 5). Among these species, the 45S rDNA loci mapped always in interstitial or distal position on the long arms of the *st* chromosomes (pair #7). The 5S rDNA loci were more variable and mapped in proximal or interstitial position of the short arms of different chromosome pairs (#1–#4) of the species complements.

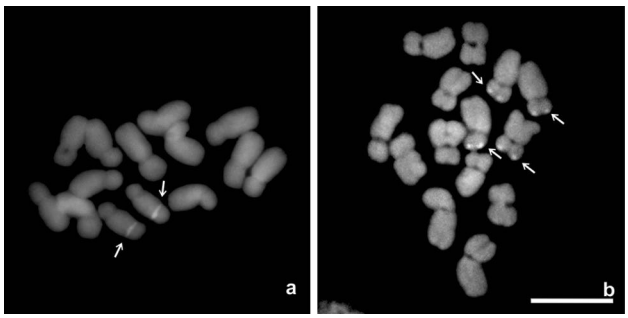

Fig. 1 CMA banding patterns in different species of *Lathyrus*. **a** *L. pubescens* (section *Notolathyrus*), **b** *L. odoratus* (section *Lathyrus*)

Within section *Lathyrus*, the two perennial species have a single pair of 45S rDNA loci and a single pair of 5S rDNA loci (Table 3; Figs. 5a, c, 6). Both markers mapped on the short arms of pair #2 (*L. sylvestris*) or pair #4 (*L. latifolius*) in adjacent positions. In *L. odoratus*, the 45S rDNA loci were localized in distal position of pairs #4 and #5 and the 5S rDNA loci were not detected (Figs. 5b, 6). *Lathyrus japonicus* of section *Orobus* presented one pair of 45S rDNA loci in the long arms (pair #1) and two pairs of 5S rDNA loci in the short arms (pairs #1 and #2) (Table 3; Figs. 5d, 6).

Discussion

Large chromosomes and a wide variation in the length of their karyotypes are unique features of *Lathyrus* species (and allied genera of the Fabeae tribe) among legume species. Although the genus was one of the biological models used to investigate the C-paradox in plant species (Narayan 1991), molecular cytogenetic works in *Lathyrus* species are very scarce, and little is known about the chromatin

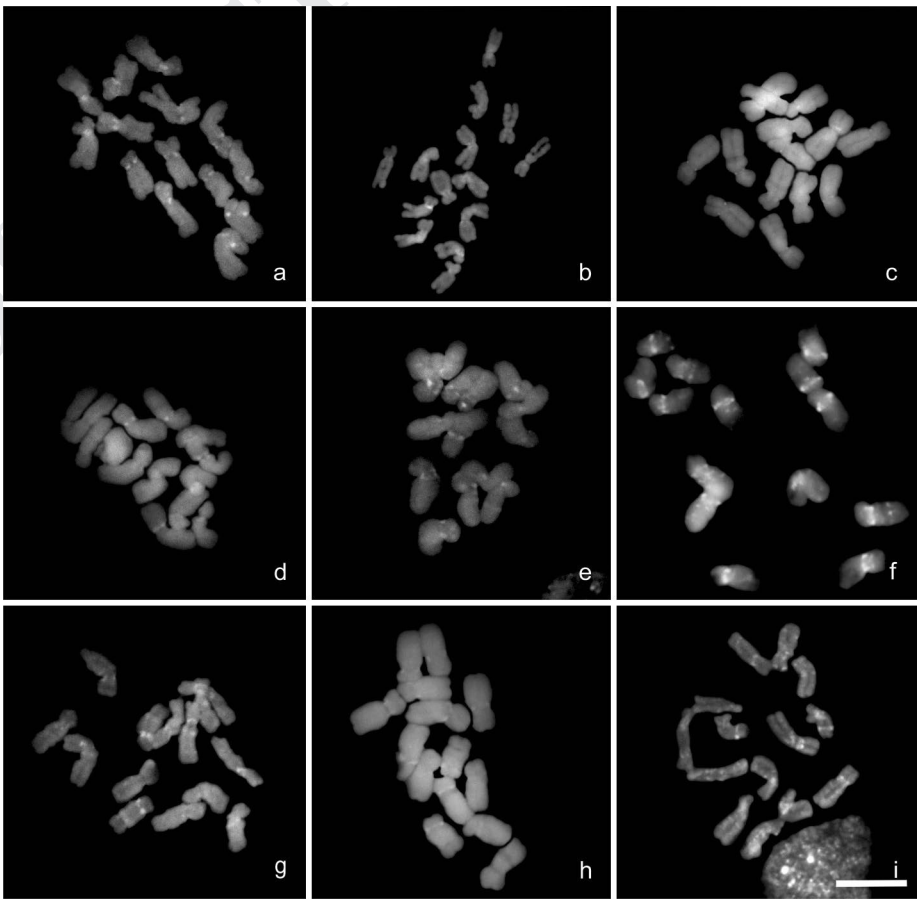

Fig. 2 DAPI banding patterns in different species of section *Notolathyrus*. **a** *L. cabrerianus*, **b** *L. crassipes*, **c** *L. hassleri-anus*, **d** *L. macrostachys*, **e** *L. magellanicus* var. *magellanicus*, **f** *L. multiceps*, **g** *L. paranensis*, **h** *L. pubescens*, **i** *L. tomentosus*. *Scale bar* 10 μm

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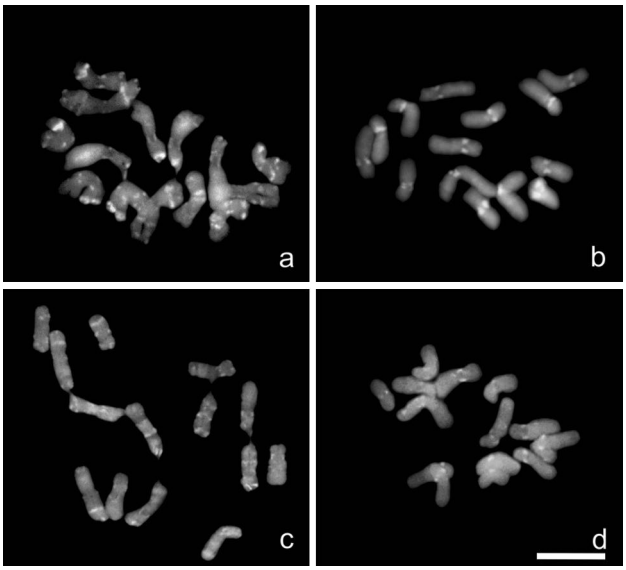

Fig. 3 DAPI Banding patterns species of sections *Lathyrus* and *Orobus*. **a–c** section *Lathyrus* **d** section *Orobus*. **a** *L. latifolius*, **b** *L. odoratus*, **c** *L. sylvestris* and **d** *L. japonicus*. *Scale bar* 10 µm

organization and patterns of chromosome evolution, especially in New World species. The present work constitutes the first comprehensive cytogenetic analysis using banding techniques and physical mapping of ribosomal DNA species of the South America endemic *Notolathyrus* section. It is also the first report in which chromosome parameters of wild *Lathyrus* species were analyzed in relation to geographic and bioclimatic variables and discussed in a taxonomic and phylogenetic context.

General karyotype features

Among South American species, the karyotype formulae established for *L. cabrerianus*, *L. crassipes*, *L. hasslerianus* and *L. magellanicus* var. *magellanicus* were coincident with those reported before for these species using Feulgen staining (Seijo and Fernández 2003). For the other species studied, the karyotype formulae were slightly different from those reported previously (Battistin and Fernández 1994; Klamt and Schifino Witman 2000; Seijo and Fernández 2003). The differences observed might be due

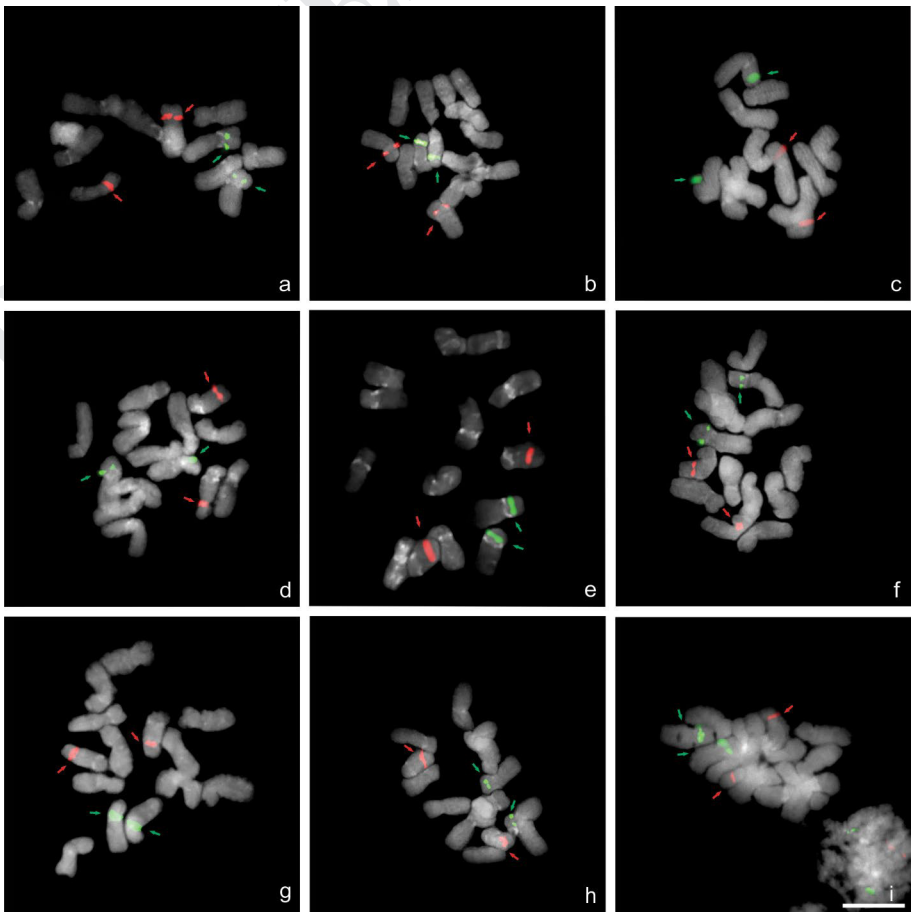

Fig. 4 Somatic metaphases and interphase nuclei of *Notolathyrus* section after double fluorescent in situ hybridization (FISH). Green fluorescein isothiocyanate (FITC) signals correspond to the 5S rDNA loci, and red tetramethyl-rhodamine isothiocyanate (TRITC) signals to the 45S rDNA loci. **a** *L. cabrerianus*, **b** *L. crassipes*, **c** *L. macrostachys*, **d** *L. magellanicus*, **e** *L. multiceps*, **f** *L. nitens*, **g** *L. paranensis*, **h** *L. pubescens*, and **i**. *L. tomentosus. Scale bar* 10 µm

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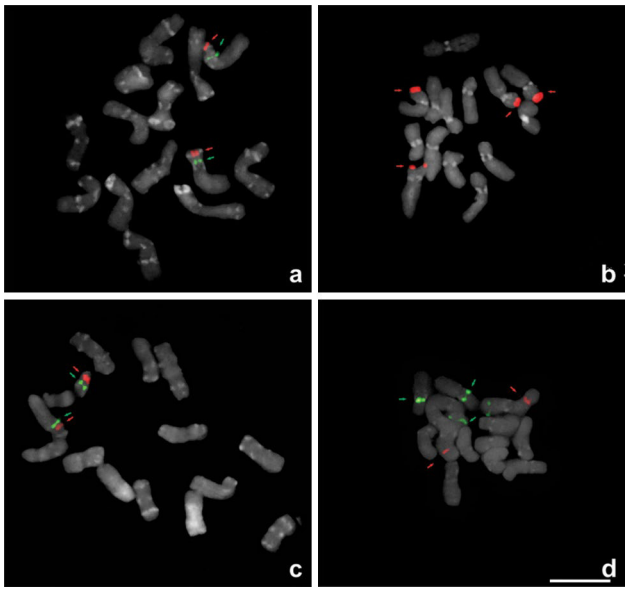

Fig. 5 Somatic metaphases and interphase nuclei of *Lathyrus* species of *Lathyrus* and *Orobus* sections after double fluorescent in situ hybridization (FISH). Green fluorescein isothiocyanate (FITC) signals correspond to the 5S rDNA loci, and red tetramethyl-rhodamine isothiocyanate (TRITC) signals to the 45S rDNA loci. **a–c** *Lathyrus* section, **d** *Orobus* section. **a** *L. latifolius*, **b** *L. odoratus*, **c** *L. sylvestris*, **d** *L. japonicus*. *Scale bar* 10 µm

to small changes in chromosome morphology caused by fluorochrome staining, although minor intraespecific variation in the morphology of the chromosomes due to structural changes cannot be ruled out. The karyotype obtained for the rare *L. nitens* is the first cytogenetic information provided for the species. The karyotype formulae observed for Northern Hemisphere species were, in general, concordant with the available data published, although minor differences are registered in the reports published by different authors (Ali et al. 2000; Badr 2007; Narayan 1982; Ünal et al. 1995). The analysis of chromosome morphology revealed that the *Notolathyrus* species constitute a homogeneous group, different from any of the North Hemisphere species so far studied cytogenetically.

A comparative analysis of the karyotypes among the species of section *Notolathyrus* demonstrated that there is a difference of 12.23 µm in TCL between the longest and the shortest complements. This variation represents about 27 % of the TCL (of the shortest complement) and it is equivalent to almost two middle size chromosomes of the haploid complement. Although the South American species analyzed constitute less than 10 % of the species recognized for the genus, the range of variation in TCL observed represent around half of the total variation reported for the genus (Arzani 2006; Seijo and Fernández 2003; Yamamoto et al. 1984). These results evidenced that the variation in chromosome size was one of the major changes that has occurred during the divergence and evolution of the chromosome complements of *Notolathyrus*. This finding is in agreement with the general trend of chromosome change proposed for the genus (Narayan and Rees 1976). Concerning the karyotype length in relation to the life cycle of the species of sections *Notolathyrus* and *Lathyrus*, the shortest complements observed in annual species suggest that a reduction of the genome size may have occurred during the transition from perennial to annual life cycle considering the traditional evolutionary pathway (Strassburger 1984). In the recent phylogeny published for Fabeae (Schaefer et al. 2012) annual life form was reconstructed as ancestral in the tribe and the perennial life form evolved at least 20 times independently followed by several reversals to annual life form. Since the two annual species analyzed correspond to reversals of the perennial state this phylogenetic analysis supports a reduction of genome size in the transition from perennial to annual life cycle.

The asymmetry A_1 and A_2 indices evidenced that the South American taxa have karyotypes with a higher degree of asymmetry than those observed in all the North Hemisphere species. However, since the phylogenetic position of *Notolathyrus* is still under discussion (Asmussen and Liston 1998; Kenicer et al. 2005; Kupicha 1983; Schaefer et al. 2012), it is difficult to determine if a more asymmetric karyotype correspond to a derived or ancestral character within the genus.

Chromosome banding

The analysis of metaphases with base-specific fluorochromes revealed more pronounced karyological differentiation among the *Lathyrus* species than that revealed with classical techniques, including variation in the number and location of heterochromatic bands. The comparative analysis of the DAPI and CMA heterochromatin is the first done for any species of *Lathyrus*.

The analysis of the metaphases with CMA revealed that the GC rich heterochromatic bands were conserved and restricted to the secondary constrictions of the SAT chromosomes in all the species. In plant chromosomes, the chromatin associated to the nucleolus organizer regions is usually CMA^+ and GC rich (Deumling and Greilhuber 1982; Schweizer 1976). The NOR associated heterochromatin represents a special kind composed of rDNA that may exist in distinct chromatin conformations determined by specific epigenetic codes, such as cytosine methylation and post-translational changes in histones (Neves et al. 2005).

The patterns of $DAPI^+$ heterochromatic bands were different for the 14 species analyzed. The variation in the amount of heterochromatin and in the distribution and intensity of the bands were the characters with highest

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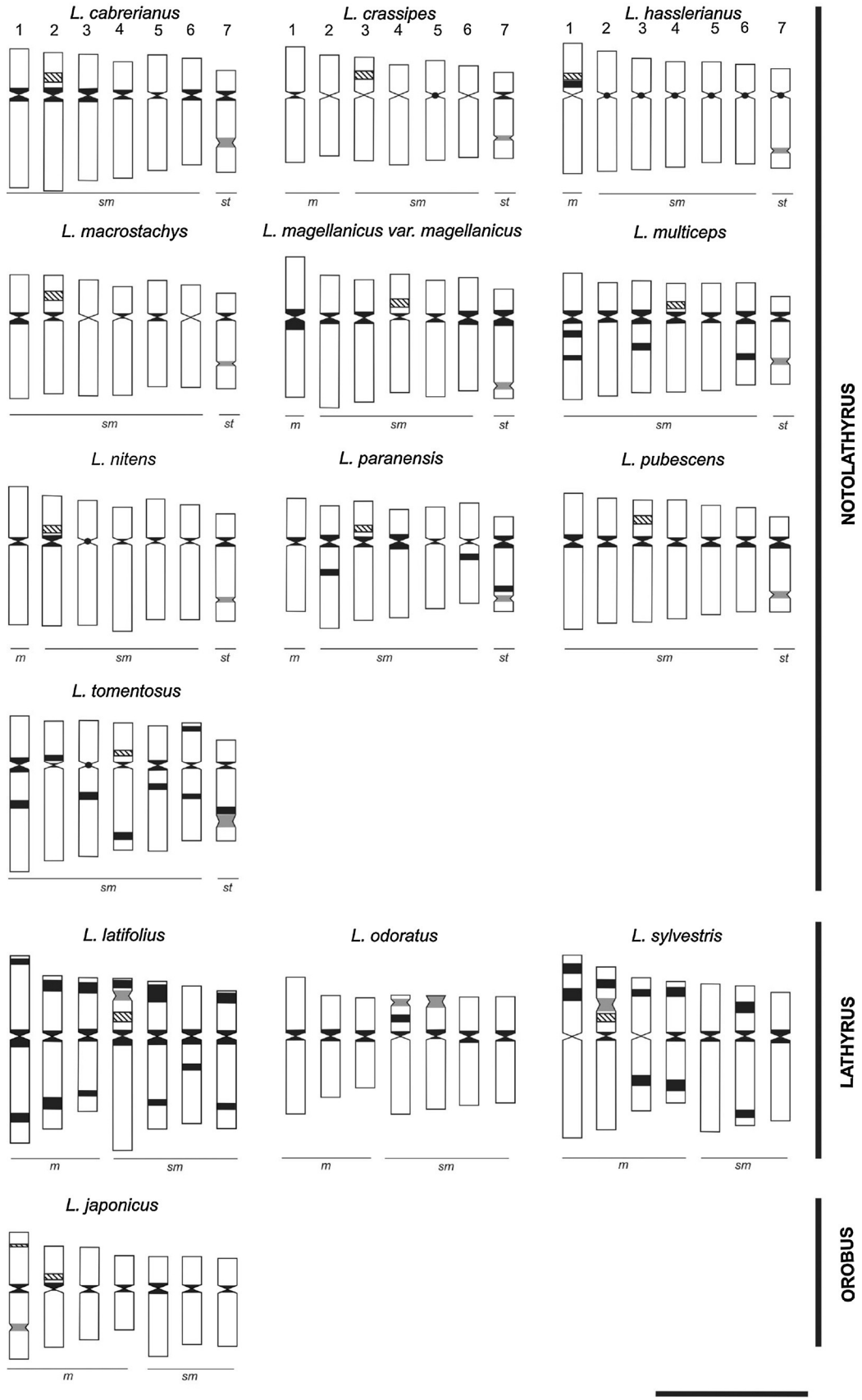

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◀ **Fig. 6** Idiograms of the *Lathyrus* species analyzed. $CMA^0/DAPI^+$ heterochromatic bands (*black*), 5S rDNA (*diagonal stripes*) and 45S rDNA colocalized with the $CMA^+/DAPI^0$ heterochromatic bands (*grey*) are shown. The chromosomes were ordered primarily by morphology and secondarily by decreasing size. *Dots* in the centromeres of some chromosomes indicate small $CMA^0/DAPI^+$ bands, not in scale. *Scale bar* 10 μm

taxonomic value at the species level. From a cytogenetic point of view, the patterns of DAPI banding provided new chromosome markers that facilitated the precise identification of most (except in *L. crassipes* and *L. hasslerianus*) of the homologous pairs within each karyotype.

The prevalence of $DAPI^+$ centromeric bands observed in all the species analysed here was consistent with the patterns reported previously for a small sample of *Lathyrus* species using C- and Q-banding (Lavania and Sharma 1980; Ünal et al. 1995; Verma 1978) and after FISH (Ali et al. 2000; Murray et al. 1992). Notoriously, and in spite that the total amount of heterochromatin was not statistically correlated with the length of the complements in *Notolathyrus* species, the shortest karyotypes tended to have the lowest number of centromeric bands. Consistently, the interstitial bands were only observed in perennial species with long karyotypes. The patterns of heterochromatin distribution observed were in accordance with those observed in some groups of plants, in which the largest karyotypes tend to present interstitial and distal heterochromatic blocks more frequently than the shortest ones (Guerra 2000).

The difference in the total amount of $DAPI^+$ heterochromatin observed between annual and perennial species of section *Notolathyrus* was mainly due to the lower number and smaller size of the centromeric bands. By contrast, in section *Lathyrus*, the main difference in heterochromatin content between annual and perennial species was not due to the size and number of centromeric bands but mainly to the lack of interstitial and distal heterochromatin in the annual *L. odoratus*. These facts evidenced that the reduction of the heterochromatic content in the annuals of different sections affected different chromosome regions and suggest the existence of particular chromosome constrains for the evolution of the karyotypes within each group of species.

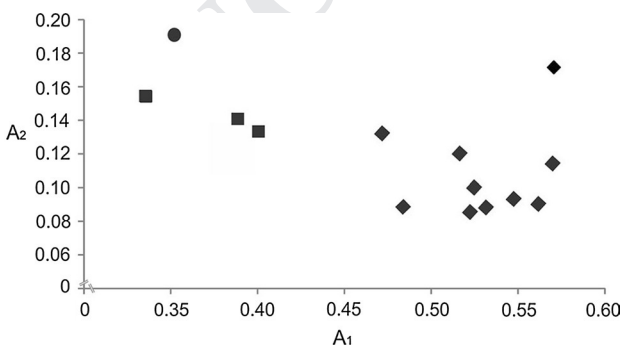

Fig. 7 Scatter diagram of the Romero-Zarco asymmetry indices. Species are clustered by sections Values of A_1 and A_2 are summarized in Table 2. The *symbols* show the different sections, *circle Orobus*, *squares Lathyrus* and *diamonds Notolathyrus*

Concerning the composition of the heterochromatin in *Lathyrus*, all the bands so far revealed (except the NOR associated heterochromatin) in Old World species were AT rich (Ünal et al. 1995; Ali et al. 2000, this report). Similarly, our results indicated that the heterochromatin (except the NOR associated heterochromatin) in *Notolathyrus* species is AT rich. In spite of the similarity in base composition of the heterochromatin detected in phylogenetically distant species, the satellites sequences associated with that heterochomatin are highly species specific or even chromosome specific (Ceccarelli et al. 2010). Therefore, the variation in the heterochromatic banding pattern observed along with the chromosome or species specificity of satellites sequences reported for *Lathyrus* suggest that this genomic fraction may have been one of the most dynamic in the karyotype evolution in the genus.

Changes in total amount of heterochromatin were directly related to variations in genome size in different plant groups (Bennett et al. 1977; Mercado-Ruaro and Delgado Salinas 1998). However, in others, the amount of heterochromatin varies regardless the amount of euchromatin or the nuclear DNA content (Guerra 2000). The latter seems to be the case of *Notolathyrus* since the total amount of heterochromatin per complement was neither correlated (statistically) with the total chromosome length nor with the available genome sizes of the species (Chalup et al. 2014). Therefore, the variation in genome size observed in *Notolathyrus*, cannot be fully explained by amplification/reduction of the satellites sequences of the heterochromatic fraction, and suggest that the repetitive component of the euchromatin, probably transposable elements, may have also been important drivers of the changes in genome size, as demonstrated in some other plant genus, e.g.: *Arachis* (Bertioli et al. 2013), *Beta* (Kubis et al. 1998).

Some reports have evidenced variation in the heterochromatin content within and among species correlated to latitude and other geographic and climatic parameters (Furuta and Nishikawa 1991). Within South American *Lathyrus* species, the correlations observed with geographic variables evidenced that the species living further North, East and at lower altitudes tend to have lower contents of heterochromatin than those that live further South, West and at higher altitudes. The analysis of the total amount of $DAPI^+$ heterochromatin in relation to the climatic variables revealed that the species with lower heterochromatic

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Table 4 Values of the geographic and bioclimatic variables registered for the samples of the *Notolathyrus* species analyzed

Species	Latitude	Longitude	Altitude	AMT	MMTR	I	TS	MTWM	MTCM	TAR	MTWeQ	MTDQ	MTWaQ	MTCQ	AP	PWM	PDM	PS	PWeQ	PDQ	PWaQ	PCQ
L. cabreri-anus	40°44′11″	71°40′20″	806	7.60	11.40	51.02	408.50	20.70	−1.50	22.20	3.20	12.80	12.80	2.80	1490	271	46	54.10	651.00	181.00	181.00	636.00
L. cras-sipes	27°40′09″	58°46′24″	61	21.60	11.20	49.40	426.70	33.40	10.80	22.60	24.50	16.40	26.80	16.40	1233	156	44	39.40	425.00	145.00	391.00	145.00
L. hassle-rianus	26°45′58″	54°10′56″	392	18.80	13.10	57.40	355.60	30.10	7.20	22.90	15.90	15.50	23.00	14.40	1766	177	114	12.70	471.00	371.00	462.00	438.00
L. macros-tachys	28°04′20″	56°02′06″	121	21.00	12.00	52.10	400.70	33.00	9.90	23.10	20.80	16.10	25.90	16.10	1610	167	90	16.50	462.00	326.00	390.00	326.00
L. magel-lanicus	41°11′46″	71°23′41″	938	6.30	11.80	52.90	414.10	19.70	−2.70	22.40	1.40	11.60	11.60	1.40	1119	206	29	59.30	19.80	116.00	116.00	515.00
L. multi-ceps	40°28′25″	71°30′32″	944	5.1	11.80	51.90	434.80	18.40	−4.40	22.80	−0.20	10.50	10.50	0.20	895	159	24	59.00	409.00	97.00	97.00	409.00
L. nitens	31°37′12″	56°02′30″	239	17.90	12.10	47.80	478.90	31.70	6.30	25.40	21.10	12.30	24.00	12.30	1302	130	89	14.40	363.00	287.00	309.00	287.00
L. paran-ensis	31°33′22″	55°30′23″	170	18.00	12.10	48.50	461.00	31.70	6.70	25.00	21.10	12.70	23.90	12.70	1293	134	82	14.30	366.00	287.00	306.00	287.00
L. pube-scens	31°08′33″	55°54′54″	262	17.70	12.20	49.00	463.80	31.20	6.30	24.90	20.90	12.30	23.60	12.30	1413	146	93	14.80	392.00	308.00	344.00	308.00
L. tomen-tosus	38°04′24″	61°58′33″	481	13.20	13.00	46.40	549.60	29.30	1.30	28.00	19.70	6.70	20.10	6.70	743	101	27	37.00	245.00	97.00	229.00	97.00
r	0.887	0.699	0.735	−0.826	0.043	−0.279	0.488	−0.708	−0.867	0.273	−0.623	−0.867	−0.766	−0.863	−0.771	−0.035	−0.703	0.617	−0.399	−0.680	−0.892	0.140
p	0.00062	0.02	0.02	0.0032	0.90	0.43	0.15	0.02	0.0011	0.44	0.0011	0.01	0.0012	0.01	0.01	0.92	0.02	0.06	0.25	0.03	0.0005	0.70

The Pearson correlation coefficient (r) indicates the correlation values of heterochromatin with each of the variables analyzed

AMT annual mean temperature, *MMTR* mean monthly temperature range, *I* isothermality (2/7) (×100), *TS* temperature seasonality (STD × 100), *MTWM* max temperature of warmest month, *MTCM* min temperature of coldest month, *TAR* temperature annual range (5–6), *MTWeQ* mean temperature of wettest quarter, *MTDQ* mean temperature of driest quarter, *MTWaQ* mean temperature of warmest quarter, *MTCQ* mean temperature of coldest quarter, *AP* annual precipitation, *PWM* precipitation of wettest month, *PDM* precipitation of driest month, *PS* precipitation seasonality (CV), *PWeQ* precipitation of wettest quarter, *PDQ* precipitation of driest quarter, *PWaQ* precipitation of warmest quarter, *PCQ* precipitation of coldest quarter

content tend to be distributed in the regions with higher annual mean temperature and precipitation. This is consistent with the correlations found for the geographic variables since the two variables, high temperatures and high precipitation, are found in the lowlands of the Northeast of the distribution area of *Notolathyrus*. Other climatic variables correlated with the heterochromatic amount follow a similar trend to the two formerly discussed.

Our analyses revealed that the species living in the Patagonian forest (*L. magellanicus* var*, magellanicus*, *L. multiceps* and *L. cabrerianus*) have the highest values of heterochromatin, those that live in the pre-Cambrian ranges of the Pampean region have high (*L. tomentosus* and *L. paranensis*) to medium values of heterochromatin (*L. pubescens* and *L. nitens*); while those that live in subtropical areas, usually collected in flooded plains or open patches of the Parananense forest, have the lowest values of heterochromatin (*L. macrostachys*, *L. hasslerianus* and *L. crassipes*). These data suggested that the species that live in the subtropical lowlands tend to have lower amounts of heterochromatin than those that live in more temperate and higher altitudes and latitudes, and suggest that the variation of the heterochromatin content may have an adaptive role for the *Notolathyrus* species.

Ribosomal DNA mapping

The fluorescent in situ hybridization on metaphases using the 45S and 5S rDNA as probes provided useful diagnostic markers for identifying one or two pairs of chromosomes unequivocally. Moreover, a characteristic pattern of the 45S and 5S rDNA loci was detected for each section analysed.

The conserved position of the 45S rDNA loci on the long arms of the *st* pair of chromosomes seems to be exclusive of the South American species since it was not detected in any *Lathyrus* species from the Northern Hemisphere studied so far (Ali et al. 2000; Murray et al. 1992; Ceccarelli et al. 2010; this report). In this sense, this cytogenetic marker would be the first diagnostic character for the South American species. The detection of the 5S rDNA loci in only one pair of chromosomes different from that of the 45S rDNA loci also constitute a diagnostic marker for the section *Notolathyrus*. At the interspecific level, the variable location of the 5S rDNA loci was useful for identifying some of the species on this section.

Among the species of section *Lathyrus*, the finding of 45S rDNA loci in the terminal position of the short arms of two pairs of chromosomes in the annual *L. odoratus* is coincident with the number and position of loci identified by Murray et al. (1992) for this species. The number of loci is in agreement with the pattern observed in *L. sativus*, but differs significantly from other annual species of the section *Lathyrus* (Ali et al. 2000; Murray et al. 1992). One possible explanation to our inability to detect the 5S rDNA loci in *L. odoratus* is that the size of these gene clusters were too small to be detected by the FISH procedure; however, this hypothesis needs to be confirmed in future experiments. The location of the 45S and 5S rDNA loci in the same chromosome arm as observed in the perennial species of section *Lathyrus* is coincident with previous results (Ceccarelli et al. 2010) and it is a distinctive characteristic of them. The more variable localization of 45 S rDNA loci among the annual species compared to the perennial ones of *Lathyrus* section may evidence the occurrence of a more dynamic re-patterning of this gene clusters in the former group of species. The presence of two pairs of 5S rDNA located in different chromosome pairs, one of them in the same chromosome than the 45S rDNA loci (but in different arms), was exclusive for *L. japonicus*.

Our FISH analysis, together with the available molecular cytogenetic data (Ali et al. 2000; Chalup et al. 2012; Murray et al. 1992), showed the existence of a limited polymorphism in the number of 45S rDNA loci among the *Lathyrus* species. However, the homogeneity registered in the number of 5S rDNA for the *Notolathyrus* species is in disagreement with the relatively high polymorphism (from one to four pairs of loci) observed in other sections of *Lathyrus* (Ali et al. 2000). In general, the patterns of rDNA loci observed and those reported before suggest that the 45S rDNA loci may have more genomic or karyotypic constrains for changes in number and position than the 5S rDNA loci in the *Notolathyrus* section and probably in the genus.

Taxonomic and phylogenetic considerations

The analysis of the chromosome data showed that within the *Notolathyrus* section, the species have significant differences in TCL, A_1 asymmetry index and in the amount of heterochromatin. However, the observed variation in these parameters is not in accordance with the variation in exomorphological characters among the species. Considering only *Notolathyrus*, it was reiteratively exposed that their species does not have any morphological diagnostic character (Burkart 1942; Kupicha 1983; Seijo and Fernández 2003). However the chromosome data obtained here revealed that the South American species are a homogeneous group characterized by karyotypes composed mainly of *sm* and one *st* chromosomes, and by a large satellite in the long arm of the smallest chromosome pair (#7). The detection of 45S rDNA loci in only one pair of chromosomes different from those that born 5S rDNA loci also constitutes a diagnostic marker for the section *Notolathyrus*. All these characteristics evidenced that South American species are a compact group of taxa and clearly differentiate *Notolathyrus* species from any other of the North Hemisphere

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species so far studied cytogenetically. Moreover, the pattern of DAPI$^+$ heterochromatin together with the variable location of the 5S rDNA loci was useful for identifying most of the species studied here.

The taxonomic position of South American species is controversial and the validity of the section *Notolathyrus* was argued (Asmussen and Liston 1998). *Notolathyrus* was established mainly using geographical criteria by Kupicha (1983), who advanced the hypothesis that South American species derived from North American taxa. The phylogenetic hypothesis derived from RFLP of cpDNA (Asmussen and Liston 1998) suggested that section *Orobus* is monophyletic only when the section *Notolathyrus* is included in it and based on the taxa investigated in that study, the authors suggested that the two sections should be combined. By contrast, in a later analysis of DNA sequences, South American species formed a well-supported (100 % bootstrap support) monophyletic clade and it was proposed that the group should be reinstated as a different section (Kenicer et al. 2005). Moreover, *Notolathyrus* and *Pratensis* were the only sections (of the 13 recognized for the genus based on morphological characters) recovered as monophyletic in the phylogenetic analysis of the tribe Fabeae (Schaefer et al. 2012). In complete agreement, the chromosome data here obtained, evidenced that the *Notolathyrus* species constitute a single evolutionary linage within the genus and support their treatment as a different section, in accordance with the Kupicha (1983) criteria.

Several biogeographic scenarios have been proposed to explain the origin of *Notolathyrus* species. The phylogenetic hypothesis derived from RFLP of cpDNA (Asmussen and Liston 1998) supported the origin of South American species from North American taxa involving southern ward dispersion through the Panama isthmus and the Andes Mountains after 1.4 Mya (Bässler 1966; Burkart 1935; Kupicha 1974; 1983). Nevertheless, the comparison of the karyotypes of the *Notolathyrus* species with those available for the *Orobus* section (here *L. japonicus*, Lavania and Sharma 1980; Yamamoto et al. 1984) do not support that hypothesis, since the species differ in the karyotype formula, in the banding pattern and in the distribution of rDNA loci. Karyotype data are more concordant with the treatment of the section *Notolathyrus* as monophyletic group different from the transberingean species of section *Orobus* as proposed by Kenicer et al. (2005). However, a direct link to the species of other sections was not evidenced in this study.

The increase of the karyotype asymmetry in the *Notolathyrus* species, in comparison with other taxa of the genus *Lathyrus*, suggests that they should be a derived group. Although the karyotype data are still scarce in the genus, the chromosome morphology and structure of the species of section *Notolathyrus* are more similar to those of the species in the east Mediterranean region, mainly to those of section *Lathyrus*. Similar information was recovered from the phylogenetic analysis done based on the chloroplast and ITS sequences (Kenicer et al. 2005). The last phylogeny that included many species of the *Lathyrus* section and other species of the genera belonging to the Fabeae tribe using molecular clock and ancestral range analyses reconstructed several long distance dispersion events from the Mediterranean center of origin to the New World (Schaefer et al. 2012). One of these events involved the origin of South American species, dated in 8.6–6.1 Mya. The calculated time divergence is compatible with the fact that the *Notolathyrus* species are highly diverse and adapted to very different environmental conditions, in spite being a homogeneous group from the karyotype point of view.

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