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Karyotype Analysis and Chromosome Evolution in Species of *Lathyrus* (Fabaceae)

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Abstract: The karyotypes of 21 accessions of *Lathyrus* L. belonging to four sections were investigated. Although all the species have a chromosome number of 2n = 14, they could be differentiated by their karyotype formula and quantitative parameters of the karyotypes. Phenetic distance showed that in spite of the differences observed among entitied, they can be grouped in clusters that coincide with the taxonomic sections established by Kupicha and with the life cycle of the species. The section *Clymenum* can be distinguished by the presence of a subtelocentric pair. From an evolutionary point of view, variation in genome size, however, is congruent with morphological variation as well as with the life cycle.

Key words: Karyotype, *Lathyrus*, Fabaceae, chromosome evolution

INTRODUCTION

Lathyrus is distributed throughout the temperate regions of the Northern Hemisphere with 52 species in Europe, 30 species in North America, 78 species in Asia and 24 species extending into tropical East Africa and 24 species into temperate South America (Kupicha, 1983; Allkin *et al*., 1985; Goyder, 1986). The genus *Lathyrus* (Fabaceae, Papilionoidae, Vicieae) consists of 13 sections and comprises about 150-160 species of annual or perennial herbs with erect or, more usually, climbing and sprawling habit, which are predominantly self-pollinating (Kupicha, 1983). The main center of diversity is the eastern Mediterranean region, with two smaller centers in North and South America (Kupicha, 1983; Allkin *et al*., 1985; Simola, 1986). *Lathyrus* species occur in a diversity of habitats, including open woods, forest margins, meadows, pastures, fields, slopes, marshes, seashores, sand dunes and roadsides. *Lathyrus* exhibits a typical bee-pollinated papilionoid flower, which may be yellow, orange, red, purple, violet, bluish, or white (Conny *et al*., 1998). There are many species cultivated for forage and human food: *L. sativus* (grass pea), *L. hirsutus* (rough pea), *L. cicera* (flatpodded vetchling), *L. odoratus* (sweet pea), *L. ochrus (ochrus)*, *L. sylvestris* (flat pea).

Cytological investigations have shown that all species were diploid with 2n = 14 chromosomes, the basic chromosome number of x = 7 is constant throughout the genus and that most of the species are diploid, with polyploids as rare exceptions (Senn, 1938; Yamamoto *et al*., 1984; Broich, 1989; Battistin and Fernández, 1994; Klamt and Schifino-Wittmann, 2000; Seijo and Fernandez, 2001). Despite this stability in chromosome number, large variations in chromosome size have played an important role in the evolution of *Lathyrus* species, which are associated with a fourfold variation in 2C nuclear DNA amount (Narayan and Durrant, 1983). The species show marked similarity in their chromosome shapes and karyotype arrangements within complements. The amounts of nuclear DNA within this genus are discontinuously distributed. Many karyotypic studies have been performed on Old World members of *Lathyrus* (Lavania and Sharma, 1980; Yamamoto *et al*., 1984; Sahin *et al*., 2000), but there is a paucity of data for American species, with the karyotypes of only five South American entities described so far (Battistin and Fernández, 1994; Klamt and Schifino-Wittmann, 2000). From the available information, a number of conflicting observations have arisen. Some authors claim that, in addition to the numerical constancy, *Lathyrus* species display morphological uniformity of chromosomes and homogeneous karyotype arrangement (Lavania and Sharma, 1980; Narayan and Durrant, 1983; Klamt and Schifino-Wittmann, 2000). However, others have found enough interspecific karyotype differences to allow species characterization (Yamamoto *et al*., 1984; Murray *et al*., 1992a; Battistin and Fernández, 1994). Such discrepancy was also observed at the infraspecific level, mainly in the widely studied *L. odoratus* L. and *L. sativus* (Bhattacharjee, 1954; Sharma and Datta, 1959; Verma and Ohri, 1979; Murray *et al*., 1992b). From a karyosystematic point of view, Yamamoto *et al*. (1984) have noted that Old World species could be grouped according to their karyotype morphology and that some of them were coincident with the taxonomic sections proposed by Davis (1970). However, these authors did not propose any relationship, either among groups or considering the world infrageneric classification proposed by Kupicha (1983), Przyblska *et al*. (1999), Seijo and Fernández (2003) and Wong *et al*. (2006).

Genetic relationships within species of the genus *Lathyrus* were reported by Datta (1955) and Chaudhuri (1966). In addition to this numerical chromosome constancy species display uniformity in chromosome morphology (all chromosomes are metacentric or submetacentric). Evolution, nevertheless, has resulted in a large increase in chromosome size (Narayan and McIntyre, 1989). In general, the total length of somatic chromosome set was longer in perennial species than in annual ones as reported by Rees and Hazarika (1969). Species of the section *Clymenus*, *Nissolia* and *Aphaca* have chromosome complements larger than those in the section *Cicercula*. The main karyotypic difference between species of the genus *Lathyrus* involves the shape of satellite chromosome (Yamamoto *et al*., 1984). The divergence and evolution within this genus is accompanied by a 3-fold increase in chromosome size which is directly correlated with 4-fold increase in their nuclear DNA amounts. Comparisons of total DNA amounts showed discontinuity in the variation among species of this genus (Narayan, 1982).

It has been reported that infarageneric *Lathyrus* classification has varied markedly during its history and classifications based on morphology are problematic due to homoplasy and lack of diagnostic characters (Conny *et al*., 1998). Thus, the present investigation is concerned with karyotype analysis and idiogram of the genus *Lathyrus* to clarify the taxonomic relationships among species of this genus and to examine the pattern of chromosome variation in relation to the taxonomic position and the life cycle of the taxa.

MATERIALS AND METHODS

In the present work, cytological study was carried out on 21 accessions samples obtained as a donation from Western regional plant introduction station 59 Johnson Hall Pullman (PI), Washington 99164 and ICARDA Genetic Resource (IG). The accessions represent 13 species belonging to four sections. For cytological preparations, seeds were germinated on moist filter paper in petri dishes at room temperature (18-22 C).1-2 cm long roots were detached and pretreated with a saturated solution of 1,4-dichlorobenzene for 3-4 h. Roots were then washed briefly in water and fixed in a mixture of 3:1 (v/v) ethyl alcohol: glacial acetic acid for 24 h and kept in a refrigerator until use.

Cytological preparation were carried out using the Feulgen squash technique for Feulgen staining root tips were hydrolyzed in 1N HCl at 60°C for 8-10 min, washed in distilled water and stained in Leuco-basic fuchsin for at least 1 h. The terminal 1-2 mm of the root tips were squashed in a drop of 45% acetic acid on a clean slide. Cover slips were separated by the freeze-drying method. samples were then dehydrated in absolute ethanol for 2-3 min and made as permanent preparation by mounting in Depex slides were allowed to dry at room temperature for few days. Cells with good spreading of chromosomes were photographed using a zeiss Ultraphoto microscope equipped with automatic camera. The nomenclature used for the description of the chromosome morphology is that proposed by Levan *et al*. (1965). The abbreviations m, sm and st designate metacentric, submetacentric and subtelocentric chromosomes, respectively. Idiograms were drawn based on mean centromeric index and arranged in order of decreasing size.

DATA ANALYSIS

For the numerical characterization of the karyotypes the following parameters were calculated: (1) total chromosome length of the haploid complement (TCL); (2) mean Chromosome Length (CL); (3) mean Centromeric Index (CI); Comparisons of chromosome morphological features were made by arranging the chromosomes of each karyotype in pairs in order of their arm ratio and length as determined from the photographic prints. An idiogram for each sample was constructed using the total length of each pair of homologous chromosomes to represent the haploid chromosome number. The relative position of the centromere and their variation within the karyotype has been expressed. A cluster analysis of the karyotype data was coned out to examine karyotype similarity among species and sections. A data matrix 21 OTUs (operational taxonomic units) × 7 variables was constructed. The TCL, CI, number of m, sm and st chromosomes as well as the life cycle were considered. The SYSTAT ver. 7 program was used to standardize the data matrix, to calculate the average taxonomic distance and to generate a phenogram. Clustering was performed using the unweighted pair-group method (UPGMA).

RESULTS

All the studied samples have 2n = 14 chromosomes. Karyotype formulae and parameters for the studied species are summarized in Table 1. Figure 1 shows the mitotic metapbases and Fig. 2 the respective idiograms.

As a whole, karyotypes of the analyzed species, in the four sections, have a predominance of m chromosomes. The most common formula among section *Cicercula* species is 12 m+2 sm (five species), followed by 14 m (two species). In the section *Lathyrus* the dominance formula is 8 m+6 sm (four species) and the second formula is

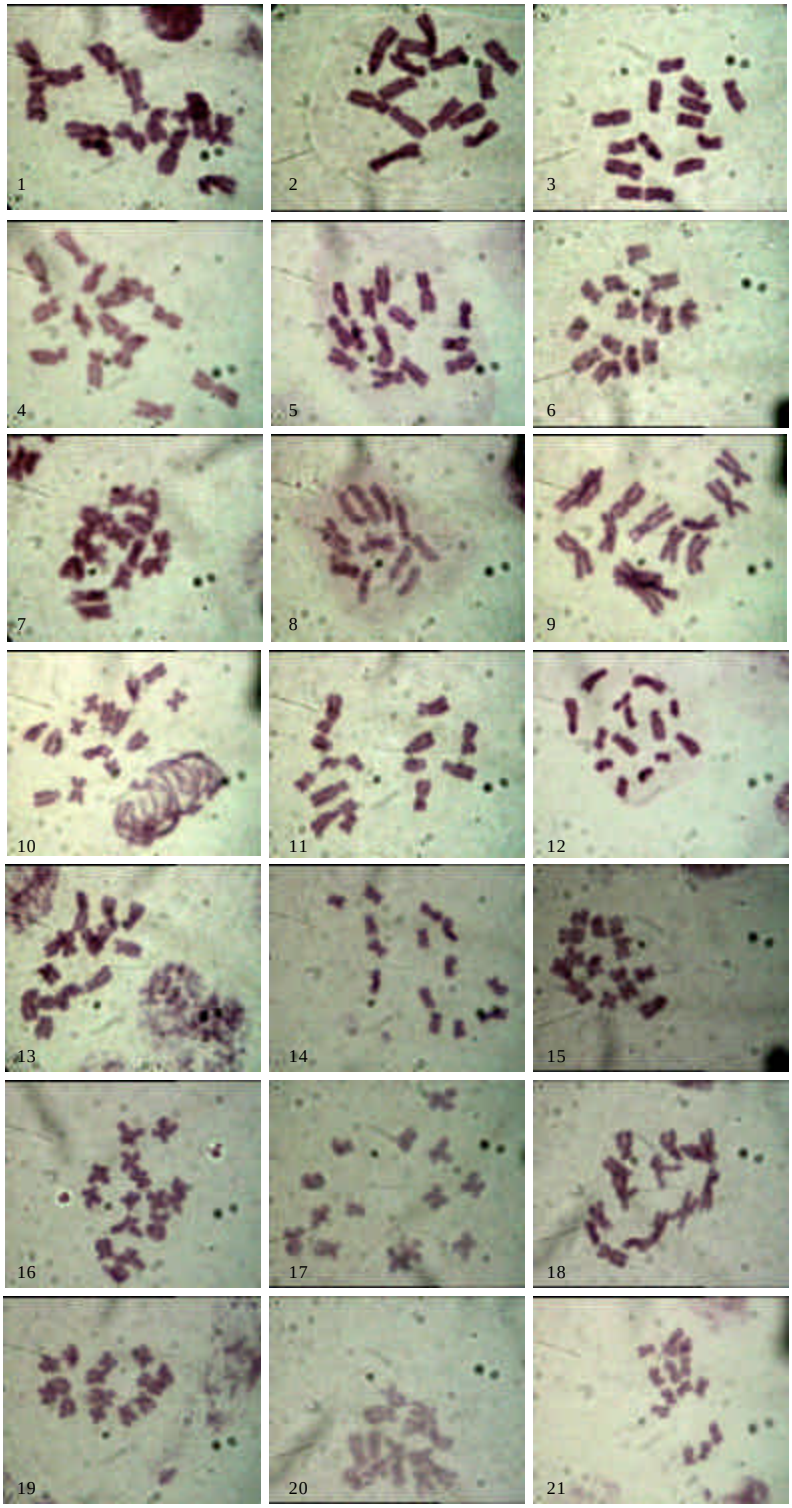

Fig. 1: Somatic chromosomes of Lathyrus 1: *L. sylvestris*$_1$ 2: *L. sylvestris*$_2$ 3: *L. hirsutus*$_1$ 4: *L. hirsutus*$_2$ 5: *L. hirsutus*$_3$ 6: *L. hirsutus*$_4$ 7: *L. annus* 8: *L. sphaerious* 9: *L. latifolius* 10: *L. ochrus* 11: *L. clymenum* 12: *L. articulatus* 13: *L. aphaca* 14: *L. inconspicus* 15: *L. blepharicarpus* 16: *L. cicera*$_1$ 17: *L. cicera*$_2$ 18: *L. cicera*$_3$ 19: *L. cicera*$_4$ 20: *L. cicera*$_5$ 21: *L. marmoratus*

Table 1: Locality, Accession number (A No.), Karyotype Formula (KF), total length of the haploid complement (TCL), mean Centromeric Index (CI), life cycle (CY) and of the studied *Lathyrus* species (m) metacentric (sm) sub metacentric (st) subteleocentric (P) perennial (A) annual

Section and species	Locality	A. No.	KF	TCL	MCL	MCI	CY
Section: *Lathyrus*							
1-L. sylvestris$_1$ L.(A)	Yugoslavia	PI 358879	6 m+8 sm	14.2	2.02	0.38	P
2-L. sylvestris$_2$ L.(B)	USA	PI 358893	6 m+8 sm	14.5	2.07	0.37	P
3-L. hirsutus$_1$ L.(C)	Egypt	IG 64766	8 m+6 sm	11.2	1.60	0.38	A
4-L. hirsutus$_2$ L.(D)	Tunisia	IG 64764	8 m+6 sm	12.8	1.82	0.35	A
5-L. hirsutus$_3$ L.(E)	Turkey	PI 358860	8 m+6 sm	11.7	1.67	0.38	A
6-L. hirsutus$_4$ L.(F)	USA	PI 358886	8 m+6 sm	11.3	1.19	0.38	A
7-L.annus L.*(G)*	Turkey	IG 65725	10 m+4 sm	10.1	1.44	0.40	A
8-L. sphaerious Rtez.(H)	Turkey	IG 65848	10 m+4 sm	13.4	1.91	0.44	A
9-L. latifolius L.(I)	USA	PI 477009	10 m+4 sm	15.6	2.23	0.41	P
Section: *Clymenum*							
10-L. ochrus L.(J)	Cyprus	PI 283540	8 m+4 sm+2 st	7.7	1.10	0.37	A
11-L.clymenum L(k).	Turkey	PI 344076	8 m+4 sm+2 st	10.5	1.50	0.35	A
12-L.articulatus L.(L)	Portugal	IG 64735	10 m+2 sm+2 st	9.2	1.31	0.37	A
Section *Aphaca*							
13-L. aphaca L.(M)	Greece	IG 64767	10 m+4 sm	9.6	1.37	0.39	A
Section *Cicercula*							
14-L. inconspicus L.(N)	Turkey	PI 407631	14 m	7.9	1.13	0.42	A
15-L. blepharicarpus Bioss(Q)	Turkey	IG 65716	14 m	7.9	1.13	0.44	A
16-L. cicera$_1$ L.(P)	Cyprus	PI 283506	12 m+2 sm	7.6	1.09	0.41	A
17-L. cicera$_2$ L.(Q)	Pakistan	IG 65664	12 m+12 sm	1.20	0.43	08.4	A
18-L. cicera$_3$ L.*(R)*	Norway	IG 64734	12 m+2 sm	7.8	1.53	0.38	A
19-L. cicera$_4$ L.(S)	Syria	PI 237639	12 m+2 sm	7.6	1.28	0.44	A
20-L. cicera$_5$ L.*(T)*	Turkey	PI 174236	8 m+6 sm	7.6	1.07	0.38	A
21-L. marmoratus Bioss.(Y)	Syria	IG 65280	10 m+4 sm	9.6	0.94	0.44	P

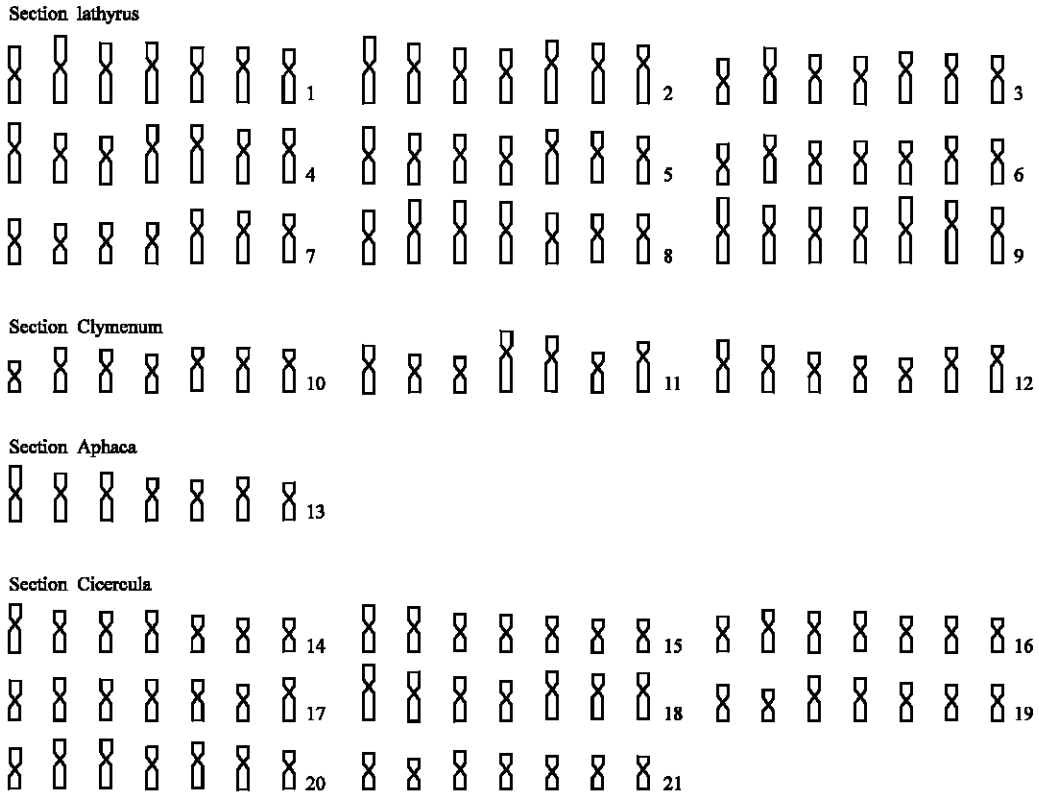

Fig. 2: Idiograms of *Lathyrus* Somatic chromosomes of *Lathyrus*, 1: *L. sylvestris*$_1$, 2: *L. sylvestris*$_2$, 3: *L. hirsutus*$_1$, 4: *L. hirsutus*$_2$, 5: *L. hirsutus*$_3$, 6: *L. hirsutus*$_4$, 7: *L. annus*, 8: *L. sphaerious*, 9: *L. latifolius*, 10: *L. ochrus*, 11: *L. clymenum*, 12: *L. articulatus*, 13: *L. aphaca*, 14: *L. inconspicus*, 15: *L. blepharicarpus*, 16: *L. cicera*$_1$, 17: *L. cicera*$_2$, 18: *L. cicera*$_3$, 19: *L. cicera*$_4$, 20: *L. cicera*$_5$ and 21: *L. marmoratus*

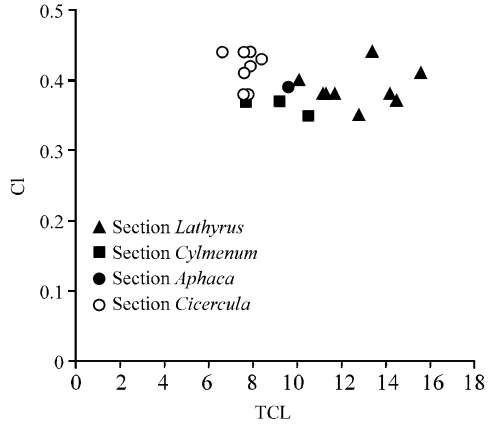

Fig. 3: Relationship between the total lengths of the haploid complement (TCL) and the mean Centromeric Index (CI). Values of TCL and CI are summarized in Table l. These variables grouped species mainly by sections. Each symbol in the plot represents once species

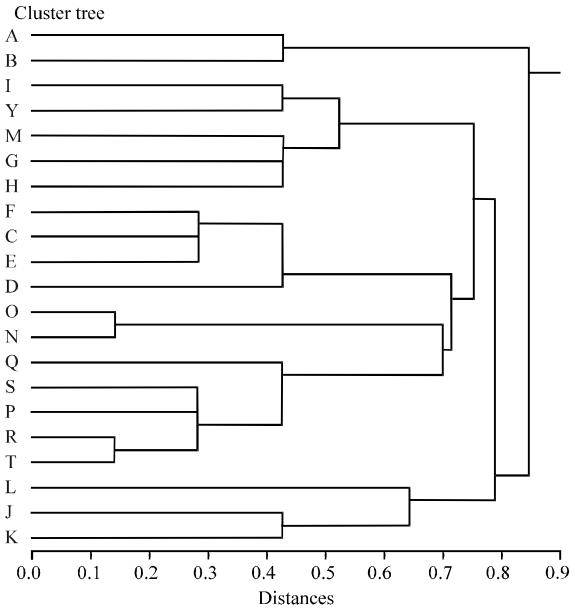

Fig. 4: Dendrogram showing the phenetic relationships among the studied species of *Lathyrus*, constructed using the matrix of karyotype similarities with UPGMA

10 m+4 sm (three species). The section *Clymenum* characterized by the presence of two subtelcentric chromosomes and different number of m and sm chromosomes. Section *Aphaca* has a formula 10 m+4 sm. Considering the life cycle, perennial species have a greater TCL than the annual. Considering the mean value of TCL and CI among the studied section, the section *Lathyrus* has the longest Total Chromosome Length (TCL) and but the centromeric index is almost the lower than those in section *Cicercula*. The idiograms as well as the parameters presented in this paper represent the means of the populations analyzed for each species. However, at the interspecific level, TCL and CI significantly differentiate some species (Table 1).

The relationship between TCL and CI of each species is plotted in Fig. 3. ANOVA of TCL discriminated among species of the four sections analyzed ($F = 20.140$, $p<0.01$) and the CI ($F = 4.201$, $p<0.0021$).

The UPGMA phenogram constracted on the basis of karyotype similarities shows two major clusters (Fig. 4). The first cluster is comprised the two samples of *L. sylvestris* in section *Lathyrus*, which are characterized by the highest CI, TCL and perennial species and the second cluster include the rest of studied samples. This cluster comprised two major groups, the first one includes the samples of section *Clymenum*, which characterized by the presence of two subtelcentric and the second groups separated into three subgroups. The samples of *L. cicera*, *L. inconspicus* and *L. blepharicarpus*, which are belonging to section *Cicercula* are delimited in one subgroup. The second subgroup includes the samples of *L hirsutus*. The species *L. annus*, *L. sphaercus*, *L. latifolius*, *L. aphaca* and *L. marmoratus* are delimited in the last subgroup. *L. marmoratus* is delimited with *L. latifolius* due to the similarities in the CI and life cycle.

DISCUSSION

The results of this study revealed a detailed picture of the chromosome features of some *Lathyrus* species belonging to four sections and of their pattern of variation in relation to their systematic position and life cycle. Like most species of *Lathyrus*, all the species analyzed here were diploids with 2n = 14. The chromosome numbers of the species agree with those published previously (Senn, 1938; Lavania and Sharma, 1980; Yamamoto *et al*., 1984; Battistin and Feranández, 1994; Klamt and Schifino-Wittmann, 2000). In addition to this numerical chromosome constancy species displayed uniformity in chromosome morphology (all chromosomes were either metacentric or submetacentric) and in karotype arrangements. Evolution, nevertheless, has resulted in a large increase in chromosome size which is associated with a five-fold increase in 2C nuclear DNA amounts (Rees and Hazarika, 1969). Cytological and molecular investigations into the organization of chromosomes and the composition and distribution of nuclear DNA within and between chromosome complements have given

evidence that there are strong constraints controlling evolutionary changes in genome organization (Narayan, 1982; Narayan and Durrant, 1983; Narayan and Rees, 1976).

L. clymenum and *L. ochrus* belonging to the section *Clymenum*, these two species are separate in cluster tree from *L. articulatus*. The lower degree of similarity among them may be attributed to a considerable difference in karyotype, including differences in chromosome length between individual chromosomes of both species and also difference in some morphological variations such as pod shape and structure, these agree with protein bands reported by El-Shanshoury (1997).

Karyotype formulae and quantitative analysis have a great uniformity among populations of any species, except those that correspond to different taxonomic varieties. These results support the hypothesis that claims infraspecific stability of karyotypes in *Lathyrus* species (Murray *et al*., 1992b). At the interspecific level, quantitative and qualitative data allowed the differentiation of several of the taxa studied. Among species of section *Lathyrus* and *Cicercula*, the most variable characters were the number of m chromosomes, as well as the karyotype formulae were more similar, but species still could be differentiated mainly by the number and type of chromosomes. These facts show that the karyotypes of *Lathyrus* species are not as fully constant as has been postulated (Narayan and Durrant, 1983; Klamt and Schifino-Wittmann, 2000) and that entities may be characterized by their chromosome features as was suggested by other authors (Yamamoto *et al*., 1984; Murray *et al*., 1992a; Battistin and Fernández, 1994).

In relation to the genome size variation, there are differences in the chromosome length among the studied taxa. These differences among complement length of diploid species are in accordance with that cited for nuclear DNA amounts of Old World species of *Lathyrus* (Rees and Hazarika, 1969; Narayan, 1982) and support the statement that variation in genome size is, perhaps, one of the more striking changes that have occurred during the divergence and evolution of the chromosome complements of this genus (Narayen and Rees, 1976). As a whole, *Lathyrus* is characterized by symmetrical karyotypes, with a predominance of sm chromosomes (Yamamoto *et al*., 1984), but the sections under this study have a predominance of m chromosomes and the presence of a st pair is characterized to section *Clymenum*. In spite of the observed interspecific variation, the bulk of karyotype data-formula, TCL and CI showed a conservative tendency toward the maintenance of the general structure of the karyotypes among different clusters of species, in accordance with observations by Yamamoto *et al*. (1984). Analysis of karyotype formulae showed that, in general, species of section *Clymenum* have a pair of st. chromosome; they differ from the other sections, *Lathyrus*, *Aphaca* and *Cicercula*. Therefore, karyotype features may become good taxonomic characters to define members of *Clymenum*.

The bulk of available karyotype data showed that most of karyotype groups found in *Lathyrus* could be related either to different sections sensu Kupicha (1983) or to the life cycle of the entities. The phenctic analyses of karyotype characters support this postulate. At the interspecific level, within section *Lathyrus* and *Cicercula*, some species can be distinguished clearly by their karyotype formulae and when quantitative karyotype data are added, the majority of entities can be differentiated. The constancy in chromosome number observed in the species studied here and in those cited in the literature (Senn, 1938; Battistin and Fernández, 1994; Klamt and Schifino-Wittmann, 2000; Seijo and Fernández, 2003) indicates that numerical changes have not been important in the evolution of South American species, as noted for most of the entities of *Lathyrus* (Hitchcock, 1952; Yamamoto *et al*., 1984; Sahin *et al*., 2000). However, this constancy differs from the situation described for North America, where several endemic polyploid species were found, so that North America was considered as a center of polyploid origin for *Lathyrus* (Broich, 1989).

Present findings that TCL varies without significant changes in karyotype formula, as seen among annual and perennial species of sections *Cicercula* and *Lathyrus*, suggest that changes in genome size may have been nonrandom and that the variation in DNA amounts is equally distributed among all chromosomes of the complements. These observations agree with those cited for Old World species of *Lathyrus*, in which variation of genome size was attributed to proportional distribution of mainly moderately repetitive DNA throughout the complement (Narayan and Durrant, 1983). Data obtained from banding patterns also support the nonrandomness of genomic change in *Lathyrus* because bands with similar base composition tend to have equilocal disposition in the karyotypes (Unal *et al*., 1995; Seijo, 2002). This pattern of evolution at molecular and subchromosomal levels suggests that species within each group evolved in a concerted fashion, maintaining the karyotype morphology.

The reduction of genome size that accompanied the evolutionary change from perennial to annual in sections *Lathyrus* and *Cicercula* species coincides with different reports on angiosperm groups (Price and Baehmanm, 1976; Greilhuber and Ehrendorfr 1988; Seijo and Fernández, 2003). Moreover, annual species of this

section, in addition to having lower TCL, present smaller pollen grains and lighter seed than perennials (Seijo, 2002). These observations are in agreement with a considerable amount of data that show that the size of reproductive organs may be related to the genome size (Choi, 1971; Chung *et al*., 1998); as was postulated in the nucleotype hypothesis (Bennett, 1972).

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