

Chromosomal criteria and taxonomic relationships in the genus *Lathyrus* (Fabaceae)

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Taxonomic relationships in eight species belonging to five of the 13 currently recognized sections of the genus *Lathyrus* have been discussed and their phylogeny based on chromosomal criteria is suggested. A diploid number of $2n = 14$ ($x = 7$) has been found in all species studied and thus $x = 7$ is confirmed as the basic number in the genus. Karyotypes of the examined species have metacentric and submetacentric chromosomes that are variable in length. The retention of *L. pratensis* in the section Pratensis is confirmed. The assignment of *L. hirsutus* to section Lathyrus and of *L. cicera* and *L. sativus* to section Clymenum are supported. The present data revealed that *L. pratensis* is the most likely ancestral type in the genus and that *Lathyrus* represents a natural monophyletic group in the tribe Viciae.

Key words: Chromosomes, karyotype, *Lathyrus*, Taxonomy

Introduction

The genus *Lathyrus* is well known legume in agriculture, its species are used as ornamentals, green manure, fodder plants and for erosion control as well as soil improvement (Whyte *et al.*, 1953; Dogan *et al.* 1992). Species of the genus are distributed in the temperate regions of Northern Hemisphere with center of diversity in Mediterranean countries (Goyder, 1986). The genus belongs to the tribe Viciae of family Fabaceae and comprises about 160 annual and perennial herbaceous creeping or climbing species (Schifino-Wittmann *et al.* 1994; Asmussen and Liston, 1998). The species of *Lathyrus* are classified into two subgenera i.e. Lathyrus and Orobus with a variable number of sections (Davis, 1970; Kupicha, 1983; Dagan *et al.*, 1992; Asmussen and Liston, 1998).

The chromosome count of $2n=14$ was reported in most of *Lathyrus* species. (see, Fedorov, 1969). However, alternative numbers have been observed within the genus. A diploid number of $2n=12$ and 16 was recorded in each of *L. subrotundus* and *L. davidii* respectively. Polyploid numbers have also been recorded in some *Lathyrus* species. A tetraploid number of $2n=4x=28$ was scored in each of *L. maritimus*, *L. odoratus*, *L. venosus* (Fedorov, 1969) and *L. pratensis* (Gutierrez *et al.*, 1994) whereas a hexaploid number of $2n=6x=42$ was observed in *L. palustris* (Sybenga, 1995). From these and other reports (e.g. Goldblatt, 1984; 1985; 1988; Goldblatt and Johnson, 1991), $x = 7$ has been encountered in almost all species of the genus.

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A number of studies have been focused on the role of chromosomal criteria for classification and phylogeny in *Lathyrus*. Yamamoto *et al.* (1984) recorded three karyotype groups in section *Cicercula*; as defined by Davis (1970); based on variation in satellited chromosomes. Wallace and Callow (1993) reported a considerable variation in genome length and the content of moderately repetitive DNA in some species of section *Lathyrus*. El-Shanshoury (1997) pointed out that, species of sections *Clymenum*, *Nissolia* and *Aphaca* have larger chromosome complement than those in section *Cicercula*. These studies and others (e.g. Datta, 1955; Chaudhari, 1966; Rees and Hazarika, 1969; Schifino-Wittmann *et al.*, 1994) revealed that, karyotype of most species of *Lathyrus* have metacentric- and submetacentric chromosomes.

Phylogenetic hypotheses have also been concluded for *Lathyrus* species based on karyological criteria; especially the chromosome number and the mean chromosome length. Goldblatt (1981a) suggested $x=7$ or 8 for the tribe *Vicieae*. He supported the view of Kupicha (1977) that $x=7$ of *Lathyrus* is derived through hypoploidy from ancestral $x=8$. On the other hand, Rees and Hazarika (1969), Broich (1989) and El-Shanshoury (1997) concluded that evolution of the genus *Lathyrus*, has resulted in large increase in chromosome length. However, Wallace and Callow, (1993) and Ahmad and Narayan (1994) argued that evolution has resulted in a reduction in chromosome length and DNA content.

Chromosomal studies on the interspecific and phylogenetic relationships in the genus *Lathyrus* are still limited. The present work deals with using the chromosomal criteria to address the specific and phylogenetic relationships of eight species of *Lathyrus*.

Materials and Methods

Seeds of the species studied were provided by the Institute of Plant Genetics & Horticulture (IPK) gene bank, Gatersleben, Germany and the plant genetic resources conservation unit of the United States Department of Agriculture (USDA), Georgia, USA. The source, origin and sectional delimitation, as proposed by Kupicha (1983), of the species studied are given in table 1. For the cytological preparations, young and healthy root tips were taken from seedlings that had been germinated in Pertri-dishes, pretreated for 3-4hr in 0.05% colchicine solution at room temperature, washed and fixed in 3:1 absolute ethanol: glacial acetic acid overnight. The root tips were hydrolyzed for 6 min in 1 M HCl at 60°C, washed and stained in Feulgen's solution for 1-2hr. Stained tips were squashed in a drop of 1% acetocarmine and permanent cytological preparations were made by mounting in Euparal.

Cells with a good spread of chromosomes were photographed using a Carl-Zeiss Photomicroscope III and prints were enlarged to a magnification of 2500. Karyotypes of the species were made by cutting out individual chromosomes and arranging them in homologous pairs in order of their length and arm ratio. The chromosomes were classed by the arm ratio according to Levan *et al.* (1965). Karyotype criteria were measured from 5 to 10 chromosome complements; these are mean length in μm , mean arm ratio (M. r-value). The standard error (SE) for the means of these parameters was also calculated. Karyotype asymmetry has been estimated using the equation of Huziwara (1962) $[\text{TF}\% = \frac{\text{sum of short arm length}}{\text{sum of total chromosome length}} \times 100]$. The asymmetry based

on the ratio between the chromosome arms A_1 and length A_2 have been estimated for each species using the equations of Zarco (1986) as follows:

$$A_1 = 1 - \frac{\sum_{i=1}^n \frac{bi}{Bi}}{n_i} \quad \text{and} \quad A_2 = \frac{S}{x}$$

Where A_1 is the intrachromosomal asymmetry index that ranges from zero to one. The equation is formulated in order to obtain lower values when chromosomes tend to be metacentric. n_i is the number of homologous chromosome pairs or groups. bi is the average length for short arms in every homologous chromosome pair or group and Bi is the average length for their long arms. On the other hand, A_2 is the interchromosomal asymmetry index, where x = mean chromosome length (MCL) and S is its standard deviation.

Results

A summary of the cytological data of the species studied is given in table 1 and their karyotypes are illustrated in figs. 1-8. All species studied were found to have a somatic chromosome number of $2n=14$ with a base number of $x=7$. Measurements of the mean chromosome length (MCL) of the species studied have revealed that the highest value ($6.85 \pm 0.55 \mu\text{m}$) is recorded in *L. aphaca*, while the lowest value ($4.21 \pm 0.20 \mu\text{m}$) is found in *L. pratensis*. Relatively long chromosomes were recorded in the remaining species studied i.e. *L. hirsutus* (MCL= $6.70 \pm 0.59 \mu\text{m}$), *L. sativus* ($6.18 \pm 0.51 \mu\text{m}$), *L. clymenum* ($6.14 \pm 0.35 \mu\text{m}$), *L. cicera* ($5.86 \pm 0.60 \mu\text{m}$), *L. ochrus* ($5.65 \pm 0.43 \mu\text{m}$) and *L. nissolia* ($5.14 \pm 0.55 \mu\text{m}$). The most variable chromosomes in length are found in *L. cicera* (SE of MCL= $0.60 \mu\text{m}$), whereas the most similar chromosomes are scored in *L. pratensis* (SE of MCL= 0.20). The variation in length among chromosomes of the species studied is also reflected in the values of A_2 , lower A_2 values are scored in species with lower degree of variation in length.

Values of the mean arm ratio (M. r-ratio) indicate that the karyotypes of the species studied have metacentric to submetacentric chromosomes. This value ranges from a lowest of 1.36 ± 0.07 in *L. sativus* and a highest of 1.95 ± 0.19 in *L. clymenum*. The degree of karyotype asymmetry as indicated by TF%-values ranges between 42.19% in *L. sativus* and 34.05% in *L. clymenum*. A_1 values range between 0.896 in *L. sativus* and 0.926 in *L. clymenum* and indicate a high degree of karyotype symmetry in most of the species studied.

Table 1: Source, origin, and sectional delimitation of the studied species of *Lathyrus* and summary of their karyological criteria

No.	Species	Source	Origin	Section	2n	x	MCL±SE (µm)	M. r-ratio ± SE	Karyotype asymmetry			Chromosome type	
									TF%			m	sm
01	<i>L. aphaca</i> L.	IPK	Italy	Aphaca	14	7	6.85±0.55	1.54±0.07	39.07	0.908	0.212	6	1
02	<i>L. cicera</i> L.	USDA	Spain	Lathyrus	14	7	5.86±0.60	1.70±0.19	37.71	0.914	0.271	4	3
03	<i>L. clymenum</i> L.	IPK	Morocco	Clymenum	14	7	6.14±0.35	1.95±0.19	34.05	0.926	0.151	2	5
04	<i>L. hirsutus</i> L.	IPK	Egypt	Lathyrus	14	7	6.70±0.59	1.60±0.14	38.93	0.909	0.233	4	3
05	<i>L. nissolia</i> L.	IPK	Turkey	Nissolia	14	7	5.14±0.55	1.49±0.10	40.67	0.902	0.283	5	1
06	<i>L. ochrus</i> (L.) DC.	USDA	Portugal	Clymenum	14	7	5.65±0.43	1.50±0.08	40.75	0.902	0.201	5	1
07	<i>L. pratensis</i> L.	IPK	Austria	Pratensis	14	7	4.21±0.20	1.42±0.06	41.36	0.899	0.126	5	1
08	<i>L. sativus</i> L.	IPK	Egypt	Lathyrus	14	7	6.18±0.51	1.36±0.07	42.19	0.896	0.218	7	-

IPK = Institute of Plant Genetics & Horticulture gene bank, Germany; USDA = United States Department of Agriculture, USA; 2n = Diploid chromosome number; x = Basic chromosome number; MCL = Mean chromosome length, SE = Standard error; M. r-ratio = Mean arm ratio; TF% = Total form percentage, A_1 = Intrachromosomal asymmetry index, A_2 = Interchromosomal asymmetry index; m = Metacentric chromosomes and sm = Submetacentric chromosomes.

Discussion

The uniformity of the basic chromosome number ($x=7$) in the species studied confirms earlier reports on these species (Fedorov, 1969; Goldblatt; 1981b, 1984, 1985, 1988). This could also support the view of Broich (1989); Sin and Alton (1990); Wallace and Callow (1993) that $x=7$ is the primary basic number in the Old World *Lathyrus*. The similarity among the species studied in chromosomal criteria, particularly in karyotype symmetry, is in agreement with the results of some previous studies (Datta, 1955; Chaudhari, 1966; Rees and Hazarica, 1969; Yamamoto *et al.* 1984). The numerical constancy and morphological similarity in the chromosomes of *Lathyrus* is reflected in the successful hybridization between some species (Hammet *et al.*, 1994).

Davis (1970) and Kupicha (1983) placed the two species *L. aphaca* and *L. pratensis* in two different sections i.e. Aphaca and Pratensis, respectively. However, Dagan *et al.* (1992) grouped these two species in one section i.e. Aphaca of subgenus *Lathyrus*. The present data (Table 1; Figs. 1&7) confirms the separation of the two species in two different sections i.e. Aphaca and Pratensis as proposed by Davis (1970) and Kupicha (1983). The karyotype of *L. aphaca* is characterized from that of *L. pratensis* by longer chromosomes and higher degree of asymmetry due to variation in length of individual chromosomes. Morphologically, the angled glabrous stem, parallel venation and the glabrous pod characterize *L. aphaca*. The variation in the phytochemical structure (Reynaud *et al.*, 1981; Ranabahu and Harborne, 1993) between the two species could further support the latter view.

Davis (1970) included the species *L. cicera*, *L. hirsutus* and *L. sativus* in section Cicercula. However, Kupicha (1983), who merged this section in section *Lathyrus* treated these species as belonging to section *Lathyrus*. On the other hand, Dogan *et al.* (1992) retained *L. hirsutus* in the section Cicercula and treated *L. cicera* and *L. sativus* as belonging to section Clymenum. The present data (Table 1; Figs. 2,4 & 8) support the separation of *L. hirsutus* from the other two species i.e. *L. cicera* and *L. sativus* as proposed by Dogan *et al.* (1992). MCL values revealed that *L. hirsutus* has longer chromosomes than those in *L. cicera* and *L. sativus*. Morphologically, *L. hirsutus* is characterized from the other two species by the parallel leaflet venation, silky pods, tuberculate based hairs, sessile glandular indumentum and wingless seeds. The latter character distinguishes the species of section Cicercula. On the other hand, *L. cicera* and *L. sativus* are similar in the compressed, oblong-linear, beaked, reticulate-veined corolla and pods with prominent midrib on each valve. These are the characteristics of the species in section Clymenum.

Senn (1938) reported that the basic chromosome number of $x = 8$ is the most frequent number in the subfamily Papilionoideae. He postulated that other numbers arose as a result of aneuploid loss or gain or duplication of this number. However, Goldblatt (1981a) suggested $x=14$ for the subfamily Papilionoideae (Fabaceae) and $x=7$ for the tribe Viciae. Moreover, he supported the view of Kupicha (1977) that $x=7$ of *Lathyrus* is derived through hypoploidy from ancestral $x = 8$. In the present study, the recorded $x=7$ and the presence of $x=6,7&8$ as recorded in previous reports might indicate that aneuploidy is common in the genus. This observation is supported by the score of $2n=9, 14, 16, 21$ & 28 in *L. pratensis* (Fedorov, 1969; Gutierrez *et al.*, 1994) and could contradict the view of Kupicha (1977) and Goldblatt (1981a) that *Lathyrus* is derived

through aneuploid reduction from $x=8$. These data might indicate that $x=7$ is the primary basic number from which other numbers could have evolved.

Within the genus *Lathyrus*, the known chromosome counts are known for 64% of the total number of species; $x=7$ is found in the vast majority of species (86.00%). Polyploids derived from this number are recorded in 12.6 % of the species whereas the haploid numbers of $n=6$ & 8 are reported in the remaining species. From this statistics and the results of the present study, $x=7$ is confirmed as the basic number from which other numbers are derived by aneuploid changes. On the other hand, the high frequency of $x=7$ in the genus supports the presence of environmental selection pressure favoring higher number of linkage groups in the karyotype. This view has previously been expressed, based on the similarity in DNA content, either in *Lathyrus* (Narayan and Durrant, 1983; Ahmad, 1993) or in other genera e.g. *Orchid* (Cox *et al.*, 1998).

Stebbins (1974) and Moore (1978) discussed karyotype variation in the degree of symmetry among the plant genera and species. Symmetrical karyotypes are considered more primitive than asymmetrical ones. The evolution of the latter from the former has been recorded in several genera, for example *Crotalaria* (Gupta and Gupta, 1978), *Phaseolus* (Sarbhoy, 1980), *Sesbania* (Abou-El-Enain *et al.*, 1998) and several others. In the present study, high degrees of karyotype asymmetry; as indicated by A_1 value; are recorded in the annual species having longer chromosomes i.e. *L. aphaca*, *L. hirsutus* and *L. sativus*. This might support the view of Rees and Hazarika (1969), Broich (1989) and El-Shanshoury (1997) that evolution has resulted in large increase in chromosome size in *Lathyrus* species. Consequently, it can be concluded that *L. pratensis* of the subgenus *Orobus* is most likely ancestral type in the genus since it has the shortest genome length among the species studied (Table 1 & Fig. 7). The primitiveness of species in the subgenus *Orobus* have previously been evidenced by Kupicha (1983) who reported that, the primitive ancestral stock of *Lathyrus* has had the characters of section *Orobus* that originated at high latitudes in the Old World and migrated to North America.

In the light of the interspecific similarity in karyotype asymmetry among the species studied herein, as well as in previous reports (Datta, 1955; Chaudhary, 1966; Rees and Hazarika, 1969; Schifino-Wittmann *et al.*, 1994) and the interspecific crossing ability reported by Hammet *et al.* (1994), it can be concluded that the genus *Lathyrus* might be considered as a natural monophyletic group within the tribe Viciae. Similar view has previously been concluded by Asmussen and Liston (1998) based on the cladistic analysis of cpDNA restriction site characters in the genus *Lathyrus*.

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Figs. 1-8: Karyotype in *Lathyrus aphaca* (1), *L. cicera* (2), *L. clymenum* (3), *L. hirsutus* (4), *L. nissolia* (5), *L. ochrus* (6), *L. pratensis* (7) and *L. sativus* (8). Bar = 5 μ m