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CHROMOSOMAL CHARACTERIZATION OF THREE NATIVE AND ONE CULTIVATED SPECIES OF *Lathyrus* L. IN SOUTHERN BRAZIL

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ABSTRACT

Mitotic metaphase chromosomes and interphase nuclei of nine populations of three South American species of *Lathyrus* (*L. pubescens, L. nervosus* and *L. crassipes*) and six populations of the cultivated species *L. odoratus* were analyzed. All populations had 2n = 2x = 14 chromosomes. There were significant differences among populations within each species and among species in the number of metacentric, submetacentric and subtelocentric chromosomes, the number and location of secondary constrictions, chromosome length (longest and shortest), total haploid complement, arm ratio, and centromeric index. *L. odoratus* showed the highest tendency towards karyotype symmetry whereas the three South American species showed a moderate tendency towards asymmetry, with *L. pubescens* being the most asymmetrical. Silver staining was used to identify the nucleolar organizer regions (NORs) and the number of nucleoli per interphase nucleus in each species. In *L. pubescens* and *L. nervosus,* the NORs were located on the secondary constriction of the long arm of pair 7, in *L. crassipes*, the NOR was proximal being located in the pair of metacentric chromosomes, and in *L. odoratus* there were four terminal NORs on the short arms of pairs 4 and 5. The four species had a maximum of four nucleoli per interphase nucleus, indicating the presence of four regions with active ribosomal genes in each case.

INTRODUCTION

The genus *Lathyrus* L. of the family Leguminosae consists of annual and perennial species, most of which are self-pollinating (Rees and Narayan, 1977; Narayan and McIntyre, 1989). According to Allkin *et al.* (1983), the genus comprises approximately 190 species and varieties, with centers of diversification in the Old and New Worlds. These are located in temperate zones. All species have 2n = 2x = 14 chromosomes, with the basic number being n = x = 7. Although there is no variation in chromosome number, there are variations in chromosome size, in centromere location, and in the number, size and location of secondary constrictions (Sharma and Datta, 1959; Roy and Singh, 1967; Federov, 1969; Fouzard and Tandon, 1975; Broich, 1989; Battistin and Fernández, 1994), and in DNA content, involving euchromatin and heterochromatin, as well as repetitive and non-repetitive DNA sequences (Lavania and Sharma, 1980; Narayan and Durrant, 1983; Kuryan and Narayan, 1987; Murray *et al*., 1992). Another important variation observed in these species is in the number and location of nucleolar organizer regions (NORs) (Nazeer *et al*., 1982; Murray *et al*., 1992; Battistin and Fernández, 1993).

The objective of the present study was to compare the chromosomal characteristics within and between four species of *Lathyrus*.

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MATERIAL AND METHODS

Fifteen populations of four species of *Lathyrus* L. were collected (Table I). Four mitotic metaphases were selected at random from plants of each population. Metaphases were obtained by the method of Battistin and Fernández (1994). The number of chromosomes, karyotype formula, mean chromosome length (μm), total length of the haploid complement, mean long/short arm ratio, and centromeric index were determined. These were compared among populations within each species by analysis of variance and by the *t*-test, and between species by the Tukey test (Steel and Torrie, 1960). The karyogram was mounted by arranging the chromosomes in pairs in decreasing order of size according to the method of Singh (1993). Chromosome nomenclature based on centromere location followed that proposed by Levan *et al*. (1964), i.e., metacentric (m), submetacentric (sm) and subtelocentric (st).

Eight metaphases and 300 interphase nuclei selected at random from four plants of each species were analyzed for NOR bands. The NORs and nucleoli were identified by $AgNO_3$ staining as described by Howell and Black (1980) for *L. odoratus*, and Herickoff *et al*. (1992) for the remaining species.

RESULTS AND DISCUSSION

The 15 populations of the four *Lathyrus* species studied were diploid, with 2n = 2x = 14 chromosomes (Table II). All species of *Lathyrus* from Europe, North America, Australia, New Zealand and South America, including polyploid and aneuploid individuals, have the same basic number x = 7 (Khawaja, 1988; Broich, 1989; Murray *et al*., 1992; Battistin and Fernández, 1994; Schifino-

Table I - Details of the species, collection sites and number of plants studied in 15 populations of four species of *Lathyrus* L.

Species	Voucher No.*	Pop. No.	Origin	Number of plants
Lathyrus pubescens Hook et Arn.	SMDB 3493	1	Caçapava do Sul/ RS - Brazil	5
		2	Bagé/RS - Brazil	4
		20	São Gabriel/RS - Brazil	8
		21	Lavras do Sul/RS - Brazil	7
Lathyrus nervosus Lam.	SMDB 3825	13	Caçapava do Sul/RS - Brazil	4
		14	Bagé/RS - Brazil	6
		15	Torres/RS - Brazil	4
Lathyrus crassipes Hook et Arn.	SMDB 3813	11	Caçapava do Sul/RS - Brazil	8
		13	Santa Maria/RS - Brazil	10
Lathyrus odoratus L.	SMDB 4117	1	ISLA SA, Lote 1293, Porto Alegre/RS - Brazil	5
		2	ISLA SA, Lote 1635, Porto Alegre/RS - Brazil	5
		3	Royal Fleur, Lote 76, France	5
		4	Royal Fleur, Lote 74, France	5
		5	Royal Fleur, Lote 73, France	5
		6	Feltrin, Lote 9014, Farroupilha/RS - Brazil	5

* Specimens deposited in the Santa Maria Department of Biology (SMDB), Federal University of Santa Maria, Rio Grande do Sul, Brazil.

Table II - Chromosome number (2n), karyotype formula, total length of the longest chromosome (TLLC), total length of the shortest chromosome (TLSC), total haploid complement (THC), ratio long arm/short arm (LA/SA) and centromeric index (CI), in 10 populations (Pop.) of four *Lathyrus* species.

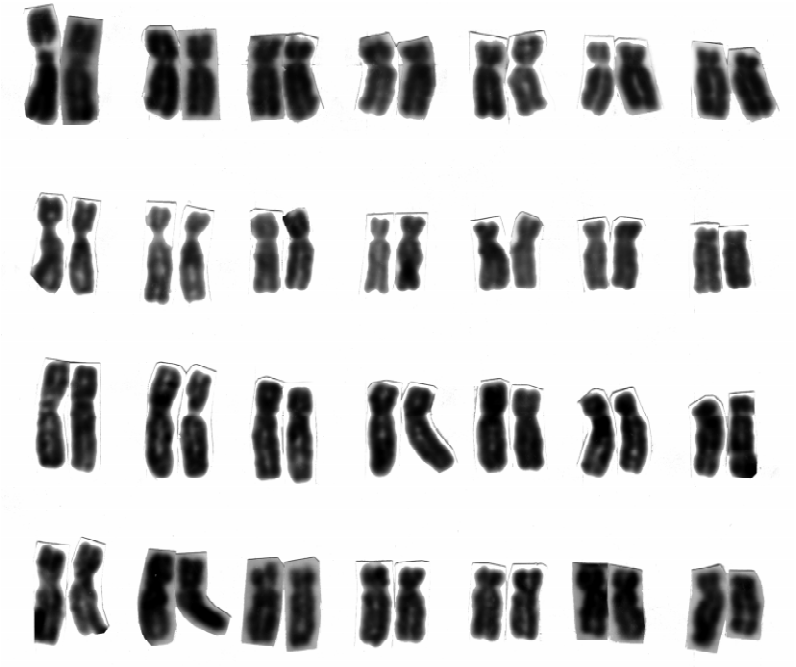
			Chromosome length (μm)				
Species/pop.	2n	Karyotype	TLLC	TLSC	THC	LA/SA	CI
L. pubescens							
Pop. 1	14	10sm+4st	$12.45^{a} \pm 0.41$	$8.30^{a} \pm 1.15$	$34.95^{ab} \pm 0.99$	$2.53^{a} \pm 0.26$	$29.53^{a} \pm 1.27$
Pop. 2	14	10sm+4st	$11.83^{a} \pm 1.26$	$8.33^{a} \pm 0.49$	$35.63^{ab} \pm 3.20$	$2.48^{a} \pm 0.10$	$29.50^{a} \pm 0.91$
Pop. 20	14	2m+8sm+4st	$13.60^{a} \pm 0.89$	$9.23^{a} \pm 1.64$	$38.93^{a} \pm 1.42$	$2.53^{a} \pm 0.05$	$29.18^{a} \pm 0.29$
Pop. 21	14	2m+8sm+4st	$11.93^{a} \pm 1.15$	$7.93^{a} \pm 0.69$	$34.50^{b} \pm 1.75$	$2.58^{a} \pm 0.10$	$28.75^{a} \pm 0.45$
L. nervosus							
Pop. 13	14	2m+10sm+2st	$12.48^{a} \pm 2.82$	$8.28^{a} \pm 0.90$	$38.50^{a} \pm 4.28$	$2.23^{a} \pm 0.05$	$31.65^{a} \pm 0.71$
Pop. 14	14	2m+10sm+2st	$10.10^{a} \pm 1.66$	$7.28^{a} \pm 0.81$	$31.35^{b} \pm 2.86$	$2.15^{a} \pm 0.13$	$32.38^{a} \pm 1.42$
Pop. 15	14	2m+10sm+2st	$10.85^{a} \pm 2.16$	$7.80^{a} \pm 0.96$	$32.85^{ab} \pm 5.97$	$2.13^{a} \pm 0.10$	$32.85^{a} \pm 0.72$
L. crassipes							
Pop. 11	14	6m+8sm	$8.38^{a} \pm 1.41$	$6.45^{a} \pm 1.39$	$26.10^{a} \pm 4.88$	$2.25^{a} \pm 0.06$	$31.85^{b} \pm 0.86$
Pop. 13	14	4m+8sm+2st	$9.50^{a} \pm 1.19$	$7.10^{a} \pm 0.92$	$29.03^{a} \pm 3.14$	$2.03^{b} \pm 0.05$	$34.03^{a} \pm 0.66$
L. odoratus							
Pop. 1	14	4m+10sm	$9.28^{a} \pm 1.07$	$6.25^{c} \pm 0.25$	$27.58^{bc} \pm 2.69$	$1.80^{c} \pm 0.14$	$37.10^{a} \pm 2.07$
Pop. 2	14	2m+12sm	$8.80^{a} \pm 1.79$	$5.85^{c} \pm 0.44$	$25.70^{c} \pm 3.63$	$2.00^{ab} \pm 0.12$	$34.53^{bc} \pm 1.96$
Pop. 3	14	6m+8sm	$12.00^{a} \pm 0.91$	$7.90^{ab} \pm 0.70$	$34.08^{a} \pm 3.18$	$2.07^{ab} \pm 0.26$	$34.05^{c} \pm 2.29$
Pop. 4	14	4m+10sm	$11.00^{a} \pm 2.73$	$7.20^{bc} \pm 1.19$	$32.03^{ab} \pm 6.38$	$1.90^{bc} \pm 0.12$	$34.45^{ab} \pm 1.23$
Pop. 5	14	2m+12sm	$9.83^{a} \pm 1.29$	$6.38^{bc} \pm 1.02$	$27.15^{bc} \pm 3.64$	$2.15^{a} \pm 0.13$	$33.40^{c} \pm 1.13$
Pop. 6	14	2m+12sm	$11.55^{a} \pm 2.63$	$8.88^{a} \pm 2.16$	$36.13^{a} \pm 6.96$	$2.10^{a} \pm 0.14$	$33.93^{c} \pm 1.32$

Lowercase letters indicate significant differences ($P < 0.05$, *t*-test). The results are shown as the mean ± SD when appropriate.

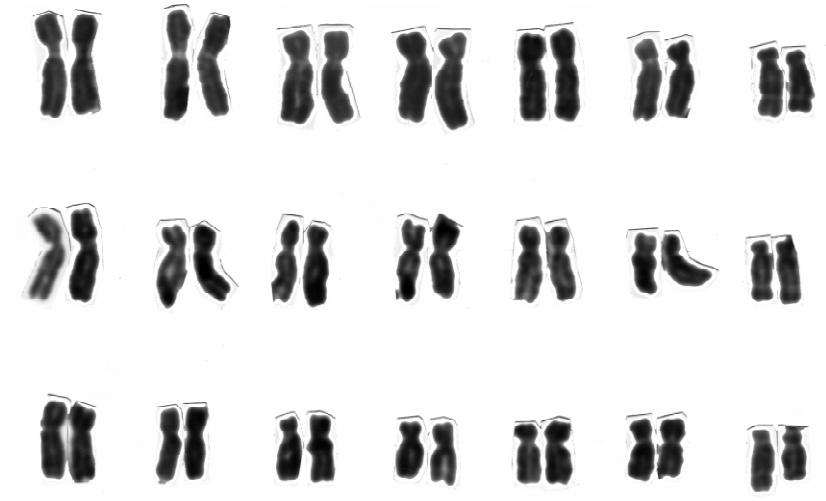
Wittmann *et al*., 1994). A conserved chromosome number is a common phenomenon in the species of this genus.

Populations 1 and 2 (Table II) of *L. pubescens* had similar karyotypes, with only submetacentric and subtelocentric chromosomes. However, they diverged in the position of secondary constrictions (Figure 1). In the karyotype of population 1, the secondary constriction was proximal on the short arm of pair 1, followed by a macrosatellite, whereas in population 2, the constriction was on the long arm of pair 7, followed by a macrosatellite. Populations 20 and 21 of *L. pubescenes*, all populations of *L. nervosus* and population 13 of *L. crassipes* also showed similarities in their karyotypes, with metacentric, submetacentric and subtelocentric chromosomes, which differed only in num-

Lathyrus pubescens



Lathyrus nervosus



Lathyrus crassipes

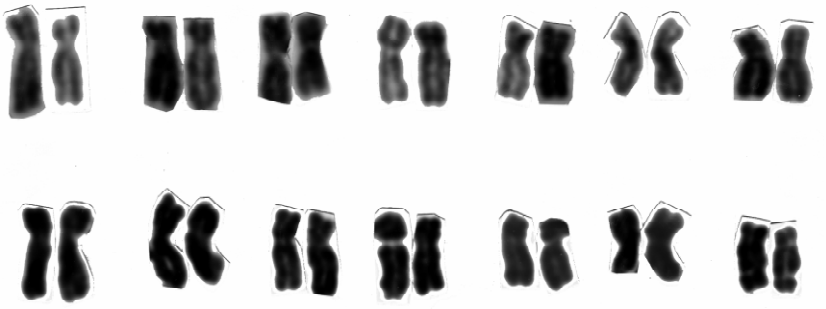


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Figure 1 continued

Lathyrus odoratus

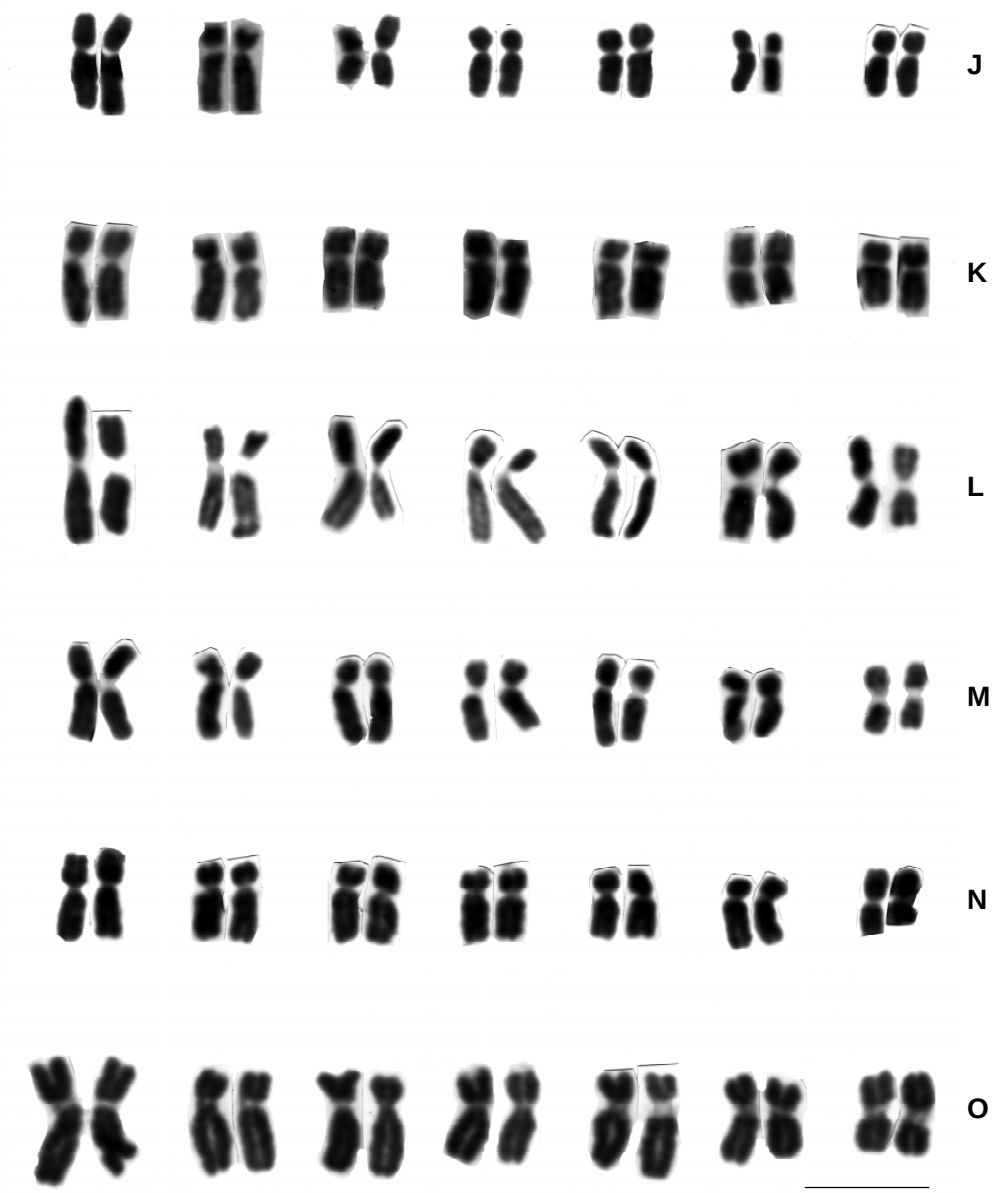

Figure 1 - Karyograms: *Lathyrus pubescens*: **A** - pop. 1; **B** - pop. 2; **C** - pop. 20; **D** - pop. 21. *Lathyrus nervosus*: **E** - pop. 13; **F** - pop. 14; **G** - pop. 15. *Lathyrus crassipes*: **H** - pop. 11; **I** - pop. 13. *Lathyrus odoratus*: **J** - pop. 1; **K** - pop. 2; **L** - pop. 3; **M** - pop. 4; **N** - pop. 5; **O** - pop. 6. Scale = 10 µm.

ber. This finding suggests a more restricted degree of homology among these populations compared to the others. The similarity between the karyotypes of *L. pubescens* and *L. nervosus* was reported by Battistin and Fernández (1994). Another characteristic shared by the above populations was the location of secondary constrictions on the long arm of pair 7, followed by a macrosatellite. An exception was population 20 of *L. pubescens* which, in addition to the secondary constriction on pair 7, had a proximal secondary constriction on the short arm of pair 1, similar to population 1.

Population 11 of *L. crassipes* (Table II and Figure 1H) and all the populations of *L. odoratus* (Table II and Figure 1J-O) had metacentric and submetacentric chromosomes which differed in number. Two distal secondary constrictions were identified on the short arms of pair 4 in population 6 of *L. odoratus*. No secondary constrictions were seen in the remaining populations.

Although the material originated from different locations, the karyotypes of the *L. odoratus* populations maintained a stable composition of chromosome types (metacentric and submetacentric), in contrast to popula-

tions of the native species, *L. pubescens*, *L. nervosus* and *L. crassipes*. This difference may indicate that during evolution *L. odoratus* reached greater stability, with a higher symmetry present in its chromosomes than in the three South American species.

Another morphological trait that showed significant variation among populations within and among species was chromosome length (Tables II and III). The mean lengths of the largest chromosomes did not differ significantly among species (Table III); the highest value was found in *L. pubescens* (12.45 μm) and the lowest in *L. crassipes* (8.94 μm). There were only slight differences in the mean length of the smallest chromosome among the populations of *L. odoratus*. *L. pubescens* had the greatest mean length (8.44 μm), which was significantly different from that of *L. crassipes* (6.78 μm) and *L. odoratus*. For the total haploid length (μm), which reflects the size of the karyotype, there were significant differences among populations within each species, except for *L. crassipes*. The largest mean was that of *L. pubescens* (36 μm), which differed significantly from the smallest mean, found in *L. crassipes* (27.56 μm), and also from *L. odoratus*. The mean for *L. nervosus* differed significantly from that for *L. crassipes*. Variation in chromosome size is often the result of the amplification or deletion of a chromatin segment during species diversification. According to Rees and Narayan (1977), the within- and between-species variation in chromosome size in *Lathyrus* indicates marked differences in the amount of DNA affecting complement size; a high percentage of this DNA is moderately repetitive. Murray *et al*. (1992) indicated that at the molecular level the changes in the amount of DNA occurred over a very short time during formation of the species.

Comparative analyses of the arm ratios allowed us to characterize each karyotype. The populations of *L. pubescens* and *L. nervosus* showed no important variations, whereas the populations of *L. crassipes* and *L. odoratus* diverged in their means. The arm ratios varied (Table III), the highest value being found in *L. pubescens* and the lowest in *L. odoratus*.

Table III - Chromosome characteristics in the four species of *Lathyrus* studied.

	Chromosome length (μm)				
Species	TLLC	TLSC	THC	LA/SA	CI
L. pubescens	12.45^{a}	8.44^{a}	36.00^{a}	2.53^{a}	29.24^{c}
L. nervosus	11.14^{ab}	7.78^{ab}	34.23^{ab}	2.17^{b}	32.29^{b}
L. crassipes	8.94^{c}	6.78^{b}	27.56^{c}	2.14^{bc}	32.94^{b}
L. odoratus	10.41^{bc}	7.08^{b}	30.44^{bc}	2.00^{c}	34.74^{a}

Lowercase letters indicate significant differences at the 5% level (Tukey test). TLLC = Total length of the longest chromosome, TLSC = total length of the shortest chromosome, THC = total haploid complement, LA/AS = ratio long arm/short arm, CI = centromeric index.

The centromeric index (CI) was stable among the populations of *L. pubescens* and *L. nervosus*, whereas it differed significantly among populations of *L. crassipes* and *L. odoratus*. *L. odoratus* had the highest means for all populations when compared with the means for populations of the three South American species (Tables II and III). A similar relationship was observed when the means were compared among species (Table III). In the South American species, *L. pubescens* had the lowest means compared to *L. nervosus* and *L. crassipes* (Tables II and III), showing clearly that two opposite trends have occurred in karyotype symmetry within the genus.

The arm ratios and CI data revealed opposite trends in karyotype symmetry among the *Lathyrus* species studied. *L. odoratus*, with the highest mean CI and the lowest arm ratio, showed the greatest tendency towards karyotype symmetry, while *L. pubescens*, *L. nervosus* and *L. crassipes* tended towards karyotype asymmetry. *L. pubescens* was the most asymmetrical of these species and had the lowest CI and the highest arm ratio. Levan *et al*. (1964) described these karyotypes as having a moderately tendency towards asymmetry.

Based on the chromosomal traits studied here, the exotic species *L. odoratus* was the most symmetrical, a tendency towards more primitive species in this genus (Stebbins, 1971). On the other hand, karyotype assymetry in the three South American species suggests that evolutionarily they are more recent species. Of these three species, *L. crassipes* is considered to be the most primitive and *L. pubescens* the most recent. Rees and Narayan (1977) and Murray *et al*. (1992) indicated that *L. pubescens* is outstanding because of its greater mean karyotype length, indicative of a greater chromatin gain in its chromosome complement over a shorter period than in the other three species.

NOR bands

Silver nitrate staining of mitotic metaphases (Figure 2) allowed the identification of NORs in the chromosomes of the four species examined. Population 20 of *L. pubescens* and population 13 of *L. nervosus* were studied. Both showed NORs on the secondary constrictions of the long arm of pair 7. The NOR of *L. pubescens* stained deeply (Figure 2a), whereas that of *L. nervosus* stained weakly (Figure 2c). In population 13 of *L. crassipes*, the NOR was located in the proximal region of metacentric pair 3 (Figure 2e). In population 6 of *L. odoratus*, four deeply stained terminal NORs were seen on the short arms of pairs 4 and 5 (Figure 2g). Nazeer *et al*. (1982) and Murray *et al*. (1992) also identified two chromosome pairs with terminal NORs in *L. odoratus*. These investigators also found three NORs in the secondary constrictions of *L. sativus* and a pair of chromosomes with $AgNO_3$-positive secondary constrictions in *L. blepharicarpus*, *L. cassius* and *L. hirsutus*. Not all secondary constrictions are NOR sites,

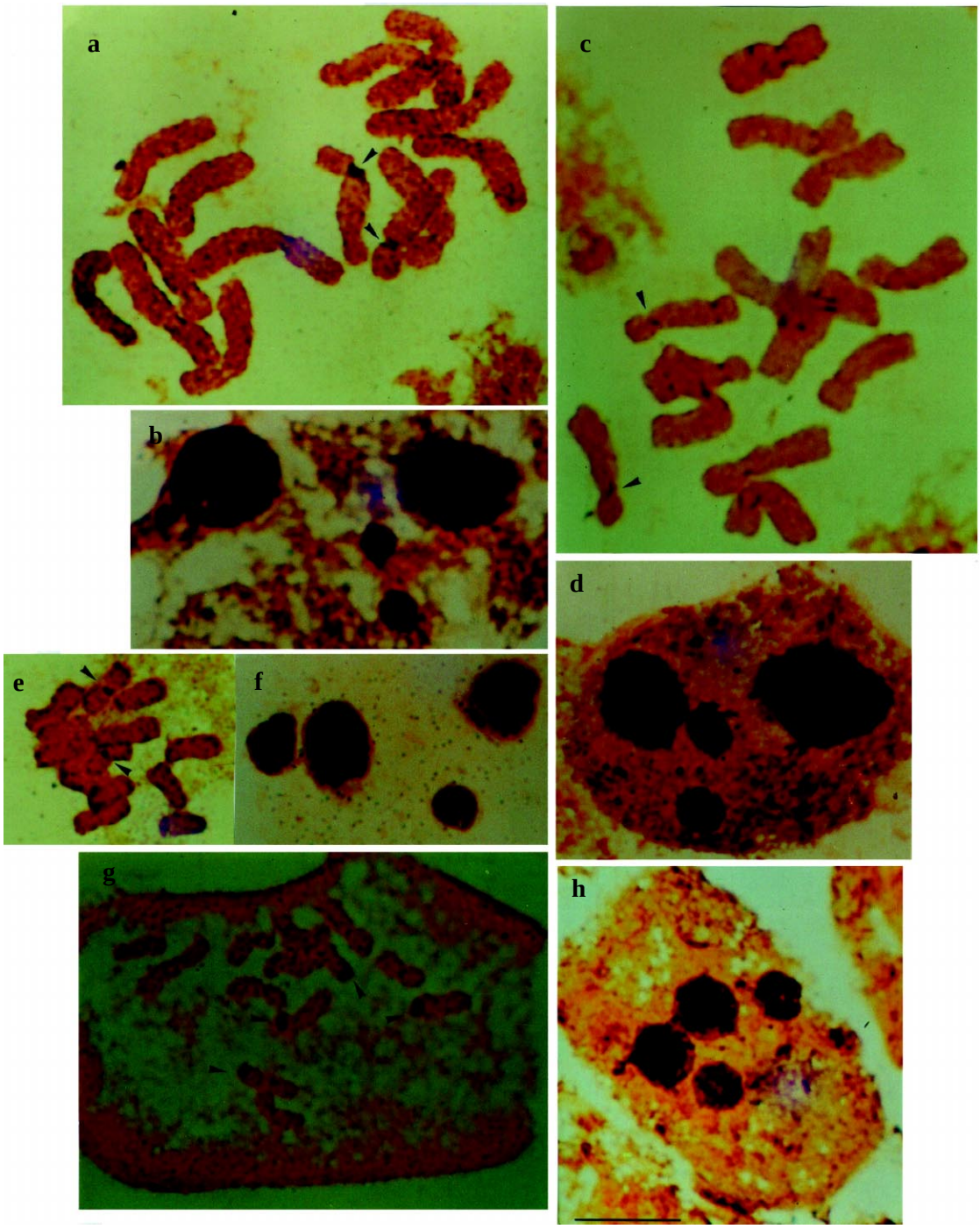

Figure 2 - Mitotic metaphases and maximum number of nucleoli in interphase cells. **a-b** *Lathyrus pubescens*; **c-d** *Lathyrus nervosus*; **e-f** *Lathyrus crassipes*; **g-h** *Lathyrus odoratus*. Arrowheads indicate NORs. Scale = 10 µm.

but all NORs are located in secondary constrictions. Each species has a characteristic number and size, which indicated the quantity of active ribosomal genes. Silver nitrate staining also showed the maximum number of nucleoli in interphase cells. Each species had a maximum of four nucleoli per cell, indicating the existence of four regions with active ribosomal genes. The fact that only one NOR pair was observed in metaphases from *L. pubescens* (Figure 2a,b), *L. nervosus* (Figure 2c,d) and *L. crassipes* (Figure 2e,f) may reflect the presence of small NORs represented by two smaller nucleoli in each cell, which may have impaired the detection of NORs in metaphase chromosomes. In *L. odoratus*, the four interphase nucleoli reflected the four NOR bands in the metaphases (Figure 2g,h).

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RESUMO

Cromossomos em metáfases mitóticas e núcleos interfásicos em 9 populações de 3 espécies sul-americanas de *Lathyrus* (*L. pubescens*, *L.nervosus* e *L.crassipes*) e 6 populações da espécie cultivada *L. odoratus* foram analisados. Todas as populações apresentaram 2n = 2x = 14 cromossomos. As diferenças significativas observadas entre as populações dentro de cada espécie e entre as espécies foram: número de cromossomos metacêntricos, submetacêntricos e subtelocêntricos; número e localização das constrições secundárias; comprimento dos cromossomos (maior e menor); complemento total haplóide; razão braço longo/braço curto e índice centromérico. *L. odoratus* é a espécie com maior tendência simétrica em seu cariótipo, enquanto que nas três espécies sul-americanas os cariótipos têm tendência moderada para assimetria, sendo *L. pubescens* o mais assimétrico. Com nitrato de prata foi possível identificar as NORs e o número máximo de nucléolos por núcleo interfásico em cada espécie. Em *L. pubescens* e *L. nervosus* as NORs estão localizadas na constrição secundária do braço longo do par 7, em *L. crassipes* a NOR é proximal, localizada no par de cromossomos metacêntricos, e em *L. odoratus* foram observadas quatro NORs terminais nos braços curtos dos pares 4 e 5. As quatro espécies possuem número máximo de quatro nucléolos em cada núcleo interfásico, indicando quatro regiões com genes ribossomais ativos em cada espécie.

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