

New chromosome numbers, meiotic behaviour and pollen fertility in American taxa of *Lupinus* (Leguminosae): contributions to taxonomic and evolutionary studies

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Original chromosome determinations are presented for 20 American *Lupinus* taxa, including, for the first time, unifoliolate species, together with first data on meiotic behaviour and pollen fertility for some South American species. Most of the Brazilian multifoliolate *L. lanatus*, *L. rubriflorus*, *L. multiflorus*, *L. paranensis*, *L. bracteolaris* and *L. reitzii* and unifoliolate *L. crotalarioides*, *L. guaraniticus* and *L. velutinus* accessions analysed presented regular chromosome pairing. Meiotic indexes and estimations of pollen viability were higher than 90% for all species and accessions analysed, reflecting the generally regular meiotic behaviour of these plants. Chromosome numbers were determined for the first time for the eastern South-American species *L. guaraniticus*, *L. crotalarioides*, *L. paranensis*, *L. paraguariensis* and *L. velutinus* ($n = 18$ or $2n = 36$) and for the Andean *L. ballianus*, *L. eanophyllus*, *L. huaronensis*, *L. semperflorens*, plus another eight taxa ($2n = 48$) from Peru and Bolivia, and *L. bandelierae* ($2n = 36$) from Bolivia. Chromosome numbers were confirmed for *L. lanatus*, *L. rubriflorus* ($2n = 36$), *L. bracteolaris* ($2n = 34$) and *L. microphyllus* ($2n = 48$). In the three accessions of the North American unifoliolate species, *L. cumulicola* and *L. villosus*, a chromosome number ($2n = 52$) previously unknown among American taxa was found. The results of the study, plus published data, support the suggestions that south-eastern South American species are a group cytologically differentiated from the Andean as well as from most other American ones, and that the Brazilian and the North American unifoliolate *Lupinus* had independent origins. © 2006 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2006, 150, 229–240.

ADDITIONAL KEYWORDS: cytological separation – cytotaxonomy – evolution – geographical distribution – polyploidy – unifoliolate *Lupinus*.

INTRODUCTION

The species of the genus *Lupinus* L. (Leguminosae), widely distributed in the Old and New Worlds, inhabit a wide climatic range, from subarctic to semidesert and subtropical regions (Gladstones, 1998). Most of the 200 (Plittmann, 1981) to 500 (Dunn, 1984) species occur in North America, from Alaska to Mexico, and in Central and South America (Hill, 1995; Gladstones, 1998). The species may be uni- or multifoliolate, herbaceous or shrubby, annual, biennial or perennial.

Twelve species are recognized in the Old World, found from the Mediterranean region to East Africa. They are multifoliolate annuals and mostly auto-

gamous, commonly divided into rough-seeded ($2n = 32, 26, 38, 42$) and smooth-seeded ($2n = 40, 50, 52$) taxa. Three species, *L. albus* L., *L. angustifolius* L. and *L. luteus* L. have long been cultivated as grain and forage crops and as green manure (Plittmann, 1981; Gladstones, 1998; Zohary & Hopf, 2000). The nuclear DNA content ($2C$) ranges from 0.97 pg (*L. princei* Harms, $2n = 38$) to 2.44 pg (*L. luteus*, $2n = 52$) (Nagajowska *et al.*, 2003).

The number of American taxa is still not yet clearly established. Regional surveys such as those of Barneby (1989) and Dunn & Gillet (1966) have been performed for the North American taxa. In South America, there are two main geographical distribution areas, the Atlantic and Andean regions. Most of the American species are multifoliolate except for the unifoliolate group of species typical of subtropical Brazil

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and four unifoliolate species from North America (Dunn, 1971). The taxonomy of South American taxa has been reviewed by, e.g., Planchuelo & Dunn (1984, 1989) and a review of the unifoliolate Brazilian group has been produced by Monteiro & Gibbs (1986), but there is still a need to integrate all these taxonomic works into a general taxonomy for the American taxa. An interesting point that remains to be clarified is the possible relationship between the unifoliolate Brazilian and North American species. From the American species, *L. mutabilis* Sweet is the only one cultivated as a grain crop. *Lupinus arboreus* Sim. is used for coastal dune stabilization in places such as Chile and New Zealand and the Russel Lupin (a putative hybrid between *L. polyphyllus* Lindl. and *L. arboreus*) is grown as an ornamental in Europe and is used in New Zealand as a forage plant for sheep in poor soils (Hoveland & Townsend, 1985).

A monophyletic origin for the genus is supported by seed protein patterns (Cristofolini, 1989), chloroplast DNA (Badr, Martin & Jensen, 1994) and ITS sequences (Ainouche & Bayer, 1999), but the centre of origin is a controversial issue. A North American origin (Plittmann, 1981) with further migration to the Mediterranean and South America, as well as an Old World origin based on serology (Cristofolini, 1989), isozymes (Wolko & Weeden, 1990a, 1990b; Wolko, 1995) and DNA sequences (Käss & Wink, 1997; Ainouche & Bayer, 1999), with further migration to the Mediterranean and South American regions, have been suggested. Other authors (Dunn, 1984; Gross, 1986) have proposed South America as the centre of origin and the unifoliolate species from Brazil as the ancestral form. Gladstones (1998), reviewing the existing data, suggested an evolutionary process that began in the Northern Hemisphere with a further progressive development and branching into eastern South America, North Africa, the Mediterranean and, finally, North America and western South America.

There are limited cytogenetic data on American species as a whole. Most of the studied North American species, as well as the Andean *L. microphyllus* Desr., *L. mutabilis*, *L. paniculatus* Desr. and *L. pubescens* Benth. have $2n = 48$, occasionally 96, 50, 36 or 34 (Darlington, 1955; Fedorov, 1969; Cox, 1972; reviewed by Gladstones, 1998). Nuclear DNA content (2C) has been determined only for *L. mutabilis* (1.90 pg) (Nagawska *et al.*, 2003). Just recently, chromosome numbers have been determined for nine south-eastern South American species ($2n = 32, 34$ and 36), with $2n = 36$ being the rule (Maciel & Schifino-Wittmann, 2002).

The present work is part of a broader project on the cytogenetics of the South American *Lupinus*. Besides determining chromosome numbers for several multifoliolate and unifoliolate Brazilian and North Ameri-

can species and for several multifoliolate Andean taxa for the first time, we present data on meiotic behaviour and pollen fertility for South American species, also for the first time, and confirm chromosome numbers for others.

MATERIAL AND METHODS

Seeds or flower buds of ten south-eastern South American species (the multifoliolate *L. bracteolaris* Desr., *L. lanatus* Benth., *L. multiflorus* Desr., *L. paraguayensis* Chod & Hassl., *L. paranensis* C.P. Sm., *L. rubriflorus* Planchuelo and *L. reitzii* Pinheiro & Miotto, and the unifoliolate *L. crotalarioides* Mart. ex Benth., *L. guaraniticus* (Hassl.) C.P. Sm. and *L. velutinus* Benth.) were collected in several regions of Rio Grande do Sul, southern Brazil and Central Brazil. Seeds from 14 Andean taxa (*L. ballianus* C.P. Sm., *L. bandelierae* C.P. Sm., *L. eanophyllus* C.P. Sm., *L. huaronensis* Macbride, *L. microphyllus*, *L. semperflorens* Benth., plus eight other clearly distinct taxa (some as yet unidentified or not yet formally named) from Bolivia and Peru, and two North American unifoliolate species (*L. cumulicola* Small. and *L. villosus* Willd.) were collected by Dr Colin Hughes (University of Oxford, UK), who is currently revising the taxonomy of Andean species (Table 1). Taxonomic vouchers of the mother plants are kept at the ICN (Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Brazil) and FHO (University of Oxford, UK) Herbaria.

For meiotic analysis, young flower buds were fixed in 3:1 ethanol-acetic acid for 24 h at room temperature and afterwards transferred to 70% ethanol and kept at 4 °C until required. Slides were prepared by squashing the anthers in 2% propionic carmine. All available phases of meiosis were analysed and special emphasis was given to chromosome associations at diakinesis and metaphase I and chromosome segregation at anaphase and telophase I and II. Meiotic indexes (mi) were calculated following Love (1949), from 400 pollen tetrads per plant. Those tetrads with four equal-sized cells were considered as normal and any deviation from this pattern as abnormal. Pollen fertility was estimated from 800 mature grains per plant. Full, well-stained grains were considered as potentially fertile, whereas empty or weakly stained grains as sterile.

Somatic chromosome numbers were determined in root-tip cells. Seeds were scarified with sandpaper and germinated in Petri dishes lined with moist filter paper at room temperature. Roots about 2–5 mm long were pre-treated in saturated aqueous paradichlorobenzene at 4 °C for 18–20 h, fixed in absolute ethanol: glacial acetic acid (3:1) for 12–24 h, and stored in 70% ethanol at 4 °C until required. Prior to analyses, the material was washed in distilled water, hydrolysed

Table 1. *Lupinus* species and accessions examined

Species	Accession	Place of collection ^c
<i>L. ballianus</i>	CEH 1989 ^b	Peru, Huarochiri, Lima
<i>L. bandelierae</i> C.P. Sm.	CEH 2301 ^b	Bolivia, Arani, Cochabamba
	CEH 2321 ^b	Bolivia, Manco Kapak, La Paz
<i>L. bracteolaris</i> Desr.	I. Conterato s/n = ICN 128464 ^a	Brazil, Porto Alegre, RS
	I. Conterato s/n = ICN 130222 ^b	Brazil, Porto Alegre, RS
<i>L. crotalarioides</i> Mart. ex Benth.	CEH 2482 a ^a	Brazil, São João da Aliança, Goiás
	CEH 2482 b ^a	Brazil, São João da Aliança, Goiás
<i>L. cumulicola</i> Small.	CEH 1984 ^b	USA, Highlands, Florida
	CEH 1985 ^b	USA, Polk, Florida
<i>L. eanophyllus</i>	CEH 1997 ^b	Peru, San Juan, Cajamarca
<i>L. guaraniticus</i> (Hassl.) C.P. Sm.	S.T.S. Miotto & M.T.P. Santos 1841 ^b	Brazil, Cambará do Sul, RS
	S. T. S. Miotto 2107 ^a	Brazil, São Francisco de Paula, RS
	S. T. S. Miotto 2109 ^a	Brazil, Tainhas, RS
	S. T. S. Miotto 2111 ^a	Brazil, Cambará do Sul, RS
	S. T. S. Miotto 2125 ^a	Brazil, Jaquirana, RS
	I. Conterato s/n = ICN 129985 ^b	Brazil, Jaquirana, RS
	I. Conterato s/n = ICN 130223 ^b	Brazil, Cambará do Sul, RS
<i>L. huaronensis</i>	CEH 2241 ^b	Peru, Ancash, Recuay
<i>L. lanatus</i> Benth.	S. T. S. Miotto 2110 ^a	Brazil, Tainhas, RS
	S. T. S. Miotto 2119 ^a	Brazil, Cambará do Sul, RS
	Valls <i>et al.</i> 1985 = BRA 00710 ^b	Brazil, Bagé, RS
<i>L. microphyllus</i>	CEH 2272 ^b	Bolivia, Tapacari, Cochabamba
<i>L. multiflorus</i> Desr.	S. T. S. Miotto 2122 ^a	Brazil, Jaquirana, RS
<i>L. paraguariensis</i> Chod. & Hassl.	Valls <i>et al.</i> 10688 = BRA 002861 ^b	Brazil, Cruz Alta, RS
<i>L. paranensis</i> C.P. Sm.	I. Conterato s/n = ICN 129986 ^a	Brazil, São José dos Ausentes, RS
<i>L. rubriflorus</i> Planchuelo	S. T. S. Miotto 2102 ^a	Brazil, São Francisco de Paula, RS
	S. T. S. Miotto 2104 ^a	Brazil, Tainhas, RS
	S. T. S. Miotto 2108 ^a	Brazil, São Francisco de Paula, RS
	S. T. S. Miotto 2114 ^a	Brazil, Cambará do Sul, RS
	S. T. S. Miotto 2117 ^a	Brazil, Cambará do Sul, RS
	S. T. S. Miotto 2123 ^a	Brazil, Jaquirana, RS
	S. T. S. Miotto 2136 ^a	Brazil, São Francisco de Paula, RS
	S. T. S. Miotto 2139 ^a	Brazil, São Francisco de Paula, RS
	I. Conterato s/n = ICN 129995 ^b	Brazil, Cambará do Sul, RS
<i>L. reitzii</i> Pinheiro & Miotto	S. T. S. Miotto 2098 ^a	Brazil, São Francisco de Paula, RS
	S. T. S. Miotto 2099 ^a	Brazil, Tainhas, RS
	S. T. S. Miotto 2105 ^a	Brazil, Tainhas, RS
	S. T. S. Miotto 2113 ^a	Brazil, Cambará do Sul, RS
	S. T. S. Miotto 2126 ^a	Brazil, Bom Jesus, RS
	S. T. S. Miotto 2131 ^a	Brazil, Lajeado Grande, RS
<i>L. semperflorens</i>	CEH 2012 ^b	Peru, Cajamarca, Cajamarca
<i>L. velutinus</i> Benth.	CEH 2477 ^a	Brazil, Chapada da Contagem, DF
	CEH 2478 ^a	Brazil, Sobradinho, DF
<i>L. villosus</i> Willd.	CEH 1986 ^b	USA, Leon, Florida
<i>Lupinus</i> sp. 1	CEH 2001 ^b	Peru, Cajamarca, Cajamarca
<i>Lupinus</i> sp. 2	CEH 2002 ^b	Peru, Cajamarca, Cajamarca
<i>Lupinus</i> sp. 3	CEH 2037 ^b	Peru, Huancabamba, Cajamarca
<i>Lupinus</i> sp. 4	CEH 2218 ^b	Peru, Calendin, Cajamarca
<i>Lupinus</i> sp. 5	CEH 2248 ^b	Peru, Pasco, Pasco
<i>Lupinus</i> sp. 6	CEH 2296 ^b	Bolivia, Quillacollo, Cochabamba
<i>Lupinus</i> sp. 7	CEH 2325 ^b	Peru, Manco Kapak, La Paz
<i>Lupinus</i> sp. 8	CEH 2332 ^b	Bolivia, B. Saavedra, La Paz

^aUsed in meiotic analyses. ^bUsed in somatic number determinations. ^cRS, Rio Grande do Sul; DF, Distrito Federal.

in 1 N HCl at 60 °C for 20 min, stained with Feulgen and squashed on slides in 2% propionic carmine. At least ten metaphase plates per accession (intact cells, well-spread chromosomes, no chromosome overlapping) were analysed.

Semi-permanent slides were examined by light microscopy and the work recorded by photomicrographs and digital image capturing.

RESULTS

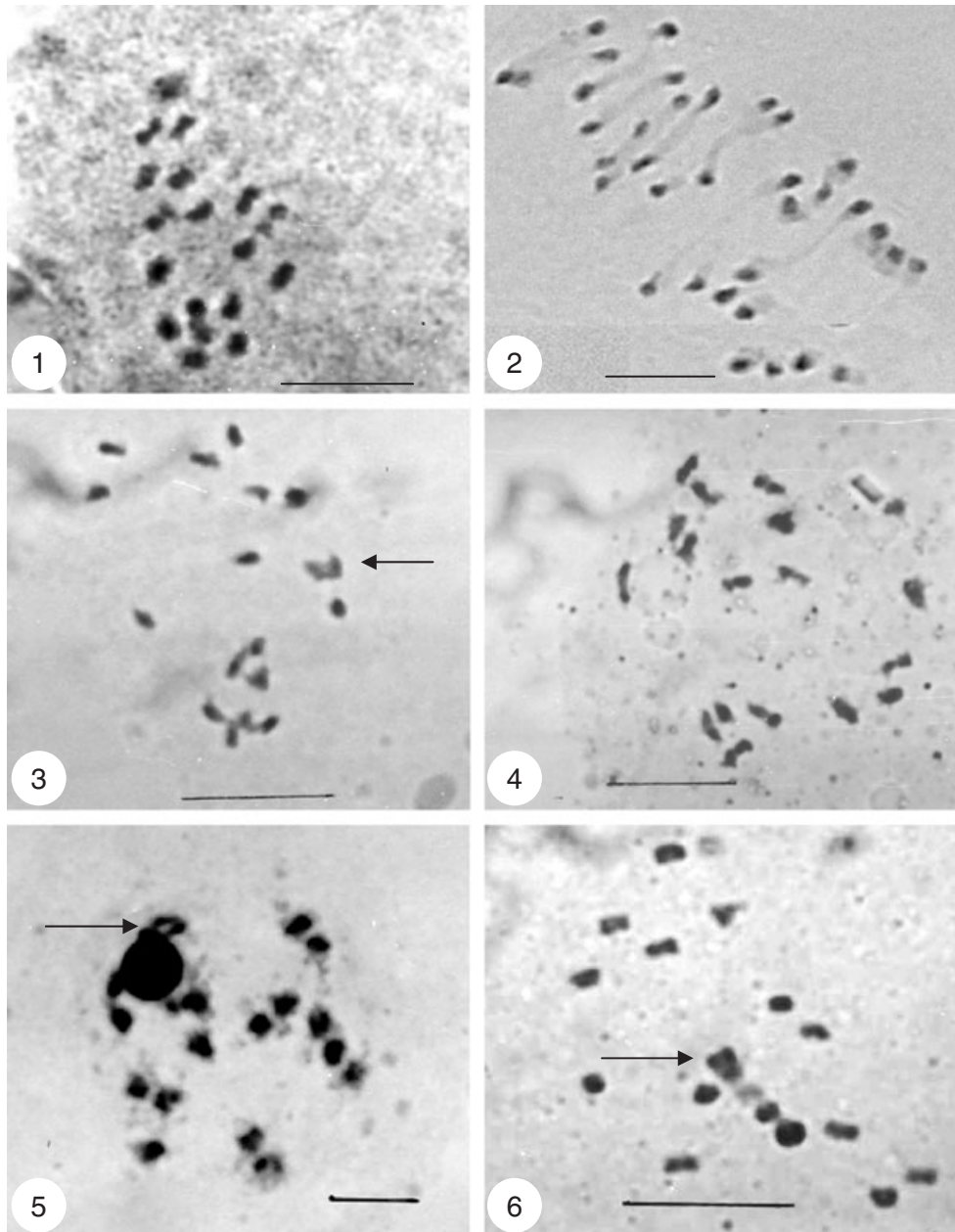
Meiotic behaviour, meiotic indexes and pollen fertility were studied in 27 accessions of *L. lanatus*,

L. rubriflorus, *L. multiflorus*, *L. paranensis*, *L. bracteolaris*, *L. reitzii*, *L. crotalarioides*, *L. velutinus* and *L. guaraniticus* (Table 2). Most of the plants examined had a very regular meiotic behaviour, with predominance of bivalents (II) at diakinesis and metaphase I (17 II in *L. bracteolaris* and 18 II in the other species) and regular disjunction at anaphase I (Figs 1–4). Quadrivalents were found in a few cells of *L. reitzii* (accession 2098), and quadrivalents (IV) (Fig. 5), other multivalents and chromosome stickiness were observed in accession 2125 of *L. guaraniticus* (Fig. 6). Meiotic indexes and estimations of pollen viability were higher than 90% for all

Table 2. Meiotic behaviour, meiotic indexes and pollen fertility in eastern South American species and accessions of *Lupinus*

Species	Accession ^a	n	Meiosis I		Meiosis II		
			Chromosome associations, diakinesis and metaphase I ^{b,c}	Chromosome segregation, anaphase I ^b	Chromosome segregation, anaphase and telophase II ^b	Meiotic index (%)	Pollen viability (%)
<i>L. bracteolaris</i>	ICN 128464 (2)	17	17 II (32)	17–17 (23)	Regular (9)	98.80	97.34
<i>L. crotalarioides</i>	CEH 2482 ^a	18	18 II (19)	–	–	100.00	99.10
	CEH 2482 ^b	18	18 II (111)	–	–	–	98.07
<i>L. guaraniticus</i>	S.T.S. Miotto 2107 (1)	18	18 II (15)	–	–	95.52	94.95
	S.T.S. Miotto 2109 (2)	–	–	–	–	98.25	94.63
	S.T.S. Miotto 2111 (2)	–	–	–	–	97.41	94.70
	S.T.S. Miotto 2125 (3)	18	18 II (55), 1–2 IV (8), M (7), A (4)	18–18 (10)	Regular (7)	96.70	95.13
<i>L. lanatus</i>	S.T.S. Miotto 2110 (3)	18	18 II (45)	–	–	99.12	96.22
	S.T.S. Miotto 2119 (3)	18	18 II (38)	–	–	99.13	95.10
<i>L. multiflorus</i>	S.T.S. Miotto 2122 (5)	18	18 II (49)	18–18 (4)	Regular (8)	99.30	97.25
<i>L. paranensis</i>	ICN 129986 (1)	18	18 II (5)	18–18 (5)	Regular (2)	98.28	98.25
<i>L. rubriflorus</i>	S.T.S. Miotto 2102 (1)	–	–	–	–	–	98.51
	S.T.S. Miotto 2104 (2)	18	18 II (18)	–	–	98.76	99.00
	S.T.S. Miotto 2108 (2)	–	–	–	–	99.52	97.46
	S.T.S. Miotto 2114 (2)	18	18 II (7)	18–18 (17)	Regular (7)	99.60	97.08
	S.T.S. Miotto 2117 (3)	18	18 II (14)	18–18 (7)	Regular (8)	99.60	97.30
	S.T.S. Miotto 2123 (3)	18	18 II (32)	18–18 (8)	Regular (7)	99.10	97.27
	S.T.S. Miotto 2136 (2)	–	–	–	–	98.92	96.48
	S.T.S. Miotto 2139 (3)	18	18 II (13)	18–18 (24)	Regular (6)	99.50	97.36
	S.T.S. Miotto 2098 (2)	18	18 II (26), 1 IV (2)	18–18 (1)	–	99.20	97.30
<i>L. reitzii</i>	S.T.S. Miotto 2099 (3)	18	18 II (4)	–	–	98.93	97.84
	S.T.S. Miotto 2105 (3)	18	18 II (20)	18–18 (6)	Regular (6)	99.17	98.29
	S.T.S. Miotto 2113 (3)	18	18 II (8)	18–18 (10)	Regular (6)	99.17	97.51
	S.T.S. Miotto 2126 (3)	18	18 II (20)	18–18 (2)	–	99.28	97.77
	S.T.S. Miotto 2131 (1)	18	18 II (9)	18–18 (12)	Regular (6)	99.03	98.72
	<i>L. velutinus</i>	CEH 2477	18	18 II (121)	–	–	–
CEH 2478		18	18 II (11)	–	–	–	99.02

^aNumber of individuals analysed in parentheses. ^bNumber of cells in parentheses. ^cTypes of chromosome associations: II, bivalent; IV, quadrivalent; M, multiple associations not clearly identified; A, adhesions.



Figures 1–6. Meiosis in *Lupinus* spp. Scale bars = 10 μ m. Fig. 1. Metaphase I, polar view, in *L. bracteolaris* 128464 ($n = 17$). Fig. 2. Late metaphase/early anaphase I in *L. lanatus* 2119 ($n = 18$). Fig. 3. Metaphase I in *L. rubriflorus* 2104 ($n = 18$), arrow points to a secondary association between two bivalents. Fig. 4. Metaphase I, polar view, in *L. reitzii* 2131 ($n = 18$). Fig. 5. Diakinesis with 16 bivalents and one quadrivalent (arrow) in *L. reitzii* 2098. Fig. 6. Metaphase I with 16 bivalents and one quadrivalent (arrow) in *L. guaraniticus* 2125.

species and accessions analysed, reflecting their generally regular meiotic behaviour and indicating that these plants are meiotically stable and potentially male-fertile (Table 2). To our knowledge, these are the first studies of meiotic behaviour and pollen fertility for South American *Lupinus* species.

Data for somatic chromosome number determinations are presented in Table 3. Chromosome numbers (gametic and/or somatic; Tables 2, 3) are presented for the first time for the eastern South American multifoliolate *L. paraguariensis* (Figs 7, 15), *L. paransensis*, and for the unifoliolate *L. crotalarioides*,

Table 3. Somatic chromosome numbers in species and accessions of American *Lupinus*

Species	Accessions	Number of cells and individuals analysed ^a	2n
South American			
<i>L. ballianus</i> ^b	CEH 1989	70 (2)	48
<i>L. bandelierae</i> ^{b,c}	CEH 2301	80 (3)	36
	CEH 2321	20 (3)	36
<i>L. bracteolaris</i>	I. Conterato s/n = ICN 130222	58 (4)	34
<i>L. eanophyllus</i> ^b	CEH 1997	12 (1)	48
<i>L. guaraniticus</i>	S.T.S. Miotto & M.T.P. Santos 1841	18 (2)	36
	I. Conterato s/n = ICN 129985	82 (8)	36
	I. Conterato s/n = ICN 130223	101 (4)	36
<i>L. huaronensis</i> ^b	CEH 2241	28 (3)	48
<i>L. lanatus</i>	Valls <i>et al.</i> 9525 = BRA 000710	36 (3)	36
<i>L. microphyllus</i> ^b	CEH 2272	8 (1)	48
<i>L. paraguayensis</i>	Valls <i>et al.</i> 002861 = BRA 10689	22 (2)	36
<i>L. rubriflorus</i>	I. Conterato s/n = ICN 129995	32 (3)	36
<i>L. semperflorens</i> ^b	CEH 2012	35 (3)	48
<i>Lupinus</i> sp. 1	CEH 2001	48 (5)	48
<i>Lupinus</i> sp. 2	CEH 2002	18 (2)	48
<i>Lupinus</i> sp. 3	CEH 2037	56 (3)	48
<i>Lupinus</i> sp. 4	CEH 2218	43 (2)	48
<i>Lupinus</i> sp. 5	CEH 2248	21 (1)	48
<i>Lupinus</i> sp. 6	CEH 2296	10 (1)	48
<i>Lupinus</i> sp. 7	CEH 2325	51 (4)	48
<i>Lupinus</i> sp. 8	CEH 2332	42 (3)	48
North American			
<i>L. cumulicola</i>	CEH 1984	63 (4)	52
	CEH 1985	3 (1)	52
<i>L. villosus</i>	CEH 1986	19 (3)	52

^aNumber of individuals (seedlings) per accession in parentheses. ^bAndean region. ^cSee text for the species distribution.

L. guaraniticus (Figs 8, 16) and *L. velutinus*, both with $2n = 36$. Chromosome numbers of $2n = 36$ (or $n = 18$) were confirmed for *L. lanatus*, *L. rubriflorus* and *L. multiflorus*, and $2n = 34$ ($n = 17$) for *L. bracteolaris*.

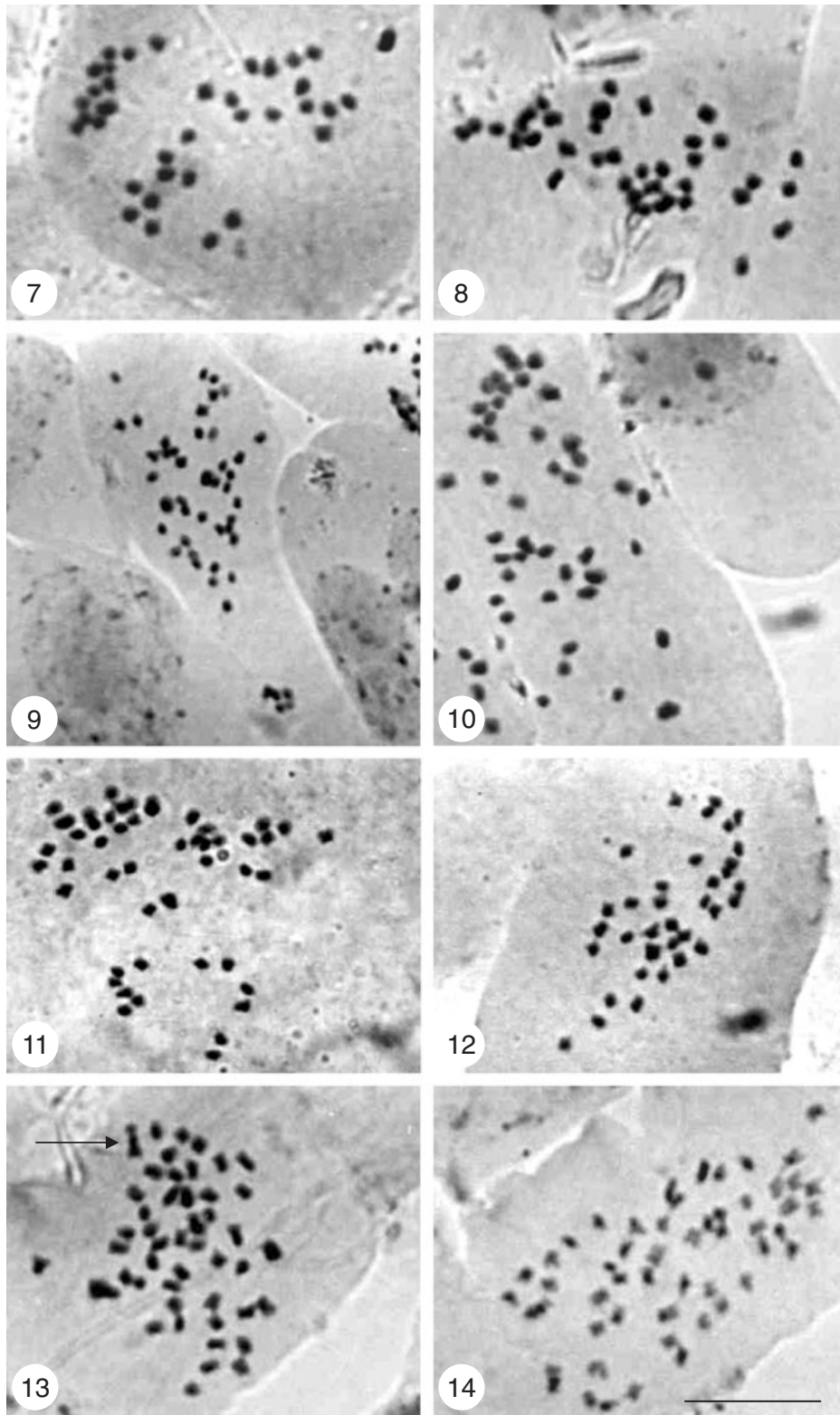
All of the Andean taxa analysed had $2n = 48$ (Table 3, Figs 9–11, 17–19), with the exception of the two *L. bandelierae* accessions, which had $2n = 36$ (Table 3, Figs 12, 20). Our determinations of chromosome number are original for *L. ballianus*, *L. bandelierae*, *L. eanophyllus*, *L. huaronensis* and *L. semperflorens* and confirm data for *L. microphyllus*, increasing the number of Andean species with known chromosome number, as previously published data for species from that geographical region exists only for *L. microphyllus*, *L. paniculatus*, *L. pubescens* and the cultivated *L. mutabilis* (all with $2n = 48$).

Chromosome numbers were determined also for the first time for the unifoliolate North American species *L. cumulicola* and *L. villosus*, both with $2n = 52$ (Table 3, Figs 13, 14, 21, 22), a number not previously reported for any North American species.

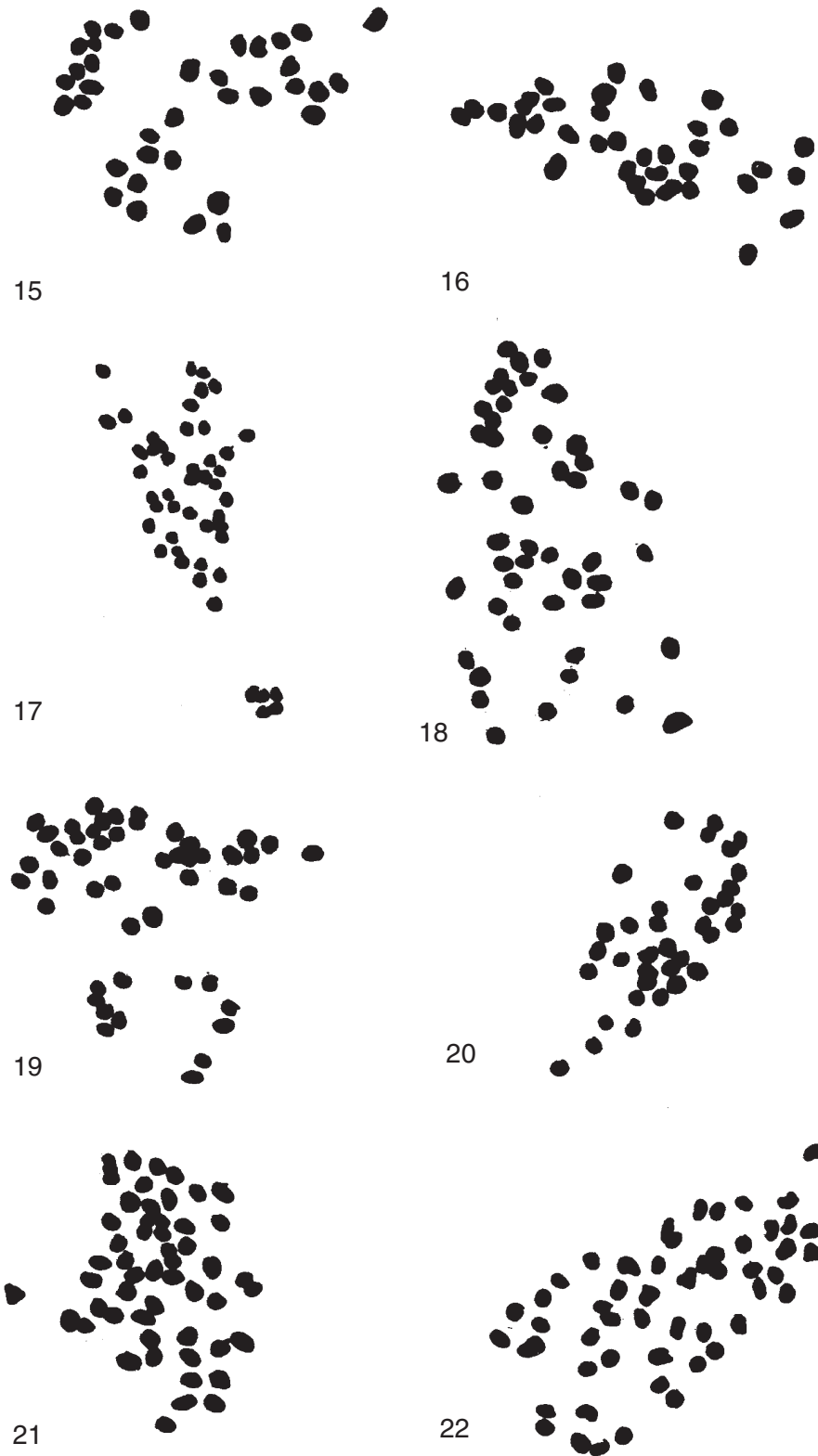
DISCUSSION

MEIOTIC BEHAVIOUR

There are no published data on meiotic behaviour or pollen fertility in any other South American *Lupinus* species. Literature data have reported a mostly regular meiotic behaviour in the Old World species *L. albus*, *L. angustifolius*, *L. luteus*, *L. micranthus* Guss (Pazy *et al.*, 1977), *L. princei*, *L. atlanticus* Gladst. and *L. digitatus* Forsk. (Carstairs, Buirchell & Cowling, 1992). Quadrivalents have been observed in three populations of the Old World *L. palaestinus* Boiss. (Pazy *et al.*, 1977), a situation similar to that found for one accession each of *L. guaraniticus* and *L. reitzii*. Multivalents are expected to occur occasionally in these polyploids, provided the pairing chromosomes are large enough to pair with two others to produce associations of three or more, a process which requires the formation of two chiasmata along the lengths of some of them. If the pairing chromosomes are too short to produce more than one chiasma at



Figures 7–14. Somatic chromosomes of *Lupinus* species. Scale bars = 10 μm . Fig. 7. *L. paraguariensis* 2861 ($2n = 36$). Fig. 8. *L. guaraniticus* 129985 ($2n = 36$). Fig. 9. *Lupinus* sp. 3 CEH 2037 ($2n = 48$). Fig. 10. *L. microphyllus* CEH 2272 ($2n = 48$). Fig. 11. *Lupinus* sp.1 CEH 2001 ($2n = 48$). Fig. 12. *L. bandelierae* CEH 2301 ($2n = 36$). Fig. 13. *L. cumulicola* CEH 1984 ($2n = 52$), arrow points to one satellited chromosome. Fig. 14. *L. villosus* CEH 1986 ($2n = 52$).



Figures 15–22. Schematic drawings of Figures 7–14, illustrating somatic chromosomes in *Lupinus* species. Fig. 15. *L. paraguariensis* 2861 ($2n = 36$). Fig. 16. *L. guaraniticus* 129985 ($2n = 36$). Fig. 17. *Lupinus* sp. 3 CEH 2037 ($2n = 48$). Fig. 18. *L. microphyllus* CEH 2272 ($2n = 48$). Fig. 19. *Lupinus* sp.1 CEH 2001 ($2n = 48$). Fig. 20. *L. bandelierae* CEH 2301 ($2n = 36$). Fig. 21. *L. cumulicola* CEH 1984 ($2n = 52$). Fig. 22. *L. villosus* CEH 1986 ($2n = 52$).

meiosis, multivalents will never occur, even in autopolyploids. High meiotic indexes and pollen fertility, such as those found for the *Lupinus* species analysed in this work, were also described for Old World *Lupinus* species (Carstairs *et al.*, 1992). Natural populations are normally expected to be meiotically stable and male-fertile, such as in *Adesmia* D.C. (Tedesco, Schifino-Wittmann & Dall'Agnol, 2002), *Lathyrus* L. and *Vicia* L. (Schifino-Wittmann, Lau & Simioni, 1994) and *Leucaena* Benth (Boff & Schifino-Wittmann, 2002, 2003), to cite just a few examples.

DISTRIBUTION OF CHROMOSOME NUMBERS AMONG AMERICAN *LUPINUS*

Interesting conclusions about chromosome number distribution may be drawn from analysis of the literature together with our data on *Lupinus* (Table 4), even considering that a limited sample of all American species has been examined.

The new data on chromosome numbers of *L. paraguayensis*, *L. paranensis*, *L. crotalariodes*, *L. velutinus* and *L. guaraniticus* support the suggestions of Maciel & Schifino-Wittmann (2002) that $2n = 36$ is the most common chromosome number among south-eastern South American *Lupinus* species and that this group of species is cytologically differentiated from the other American *Lupinus* studied so far. Numbers lower than $2n = 36$ are rare among south-eastern South American *Lupinus* species and have so far been reported only for *L. bracteolaris* and *L. linearis*, both with $2n = 34$ and $2n = 32$ (Table 4). These lower chromosome numbers were possibly derived by dysploidy from $2n = 36$. We could tentatively suggest that the $2n = 34$ plants were formed via aneuploid gametes, at first producing a $2n = 35$ plant, which later stabilized as a $2n = 34$ dysploid that would remain bivalent-forming, because two copies of the same chromosome are missing in the latter. The $2n = 32$ plants could have been produced through similar steps from the $2n = 34$ ones.

The chromosome numbers of the Andean *Lupinus* taxa analysed, all with $2n = 48$ except for *L. bandelierae*, support a close relationship between these species and the North American ones. *Lupinus bandelierae* occurs widely across Bolivia and probably in north-west Argentina, but had not been found further north in the Andes so far, suggesting its possible affinities with species from further south rather than to the main Andean species radiation. This is supported by morphological similarities between *L. bandelierae* and some of the southern Brazilian/Paraguayan species such as *L. bracteolaris* and *L. gibertianus* C.P. Smith (C.E. Hughes, pers. comm.).

The results support the suggestion of $x = 6$ as the basic number for American *Lupinus* species (Dunn,

1984; Gladstones, 1998), with different ploidy levels prevalent among South American taxa ($2n = 6x = 36$) and Andean and North American ones ($2n = 8x = 48$). How these different numbers and ploidy levels could have arisen is still not clear, but it is very unlikely that the octoploid species with $2n = 48$ chromosome could have been formed from the hexaploids with $2n = 36$. The most likely supposition, even if speculative, would be that both were formed independently from an ancient ancestor, a $2n = 24$ tetraploid that is unknown or extinct. From the latter, the octoploid could appear through chromosome number doubling and the hexaploid as a product of the fusion of a non-reduced gamete with a normal one.

NORTH AMERICAN × BRAZILIAN UNIFOLIOLATE SPECIES

The chromosome number $2n = 52$, found for *L. cumulicola* and *L. villosus*, two of the four unifoliolate North American species, does not occur among any other American *Lupinus* species studied so far. It has been observed in just three Old World smooth-seeded species: *L. micranthus*, *L. luteus* and *L. hispanicus* Boiss. & Reuter (Gladstones, 1998). These surprising results raise several questions about the connections of these species to the other American taxa, as well as to the Old World ones. On the other hand, they may help to clarify the relationship between the Brazilian and the North American unifoliolate species. Dunn (1971) suggested that the North American unifoliolate taxa represent a single and recent speciation event from the ancestral unifoliolate species from Brazil, through long-distance seed dispersal. As the three analysed Brazilian unifoliolate species, *L. crotalariodes*, *L. guaraniticus* and *L. velutinus*, all have $2n = 36$ chromosomes, the difference in chromosome numbers between the two unifoliolate groups, supports an independent origin rather than a direct relationship between South and North American unifoliolate taxa. Given the complexity of evolutionary patterns in *Lupinus*, it is difficult to suggest a likely explanation for the origin of the $2n = 52$ chromosome number in the unifoliolate North American species. Theoretically, they could have arisen by dysploid increase from the $2n = 48$ species or maybe from the group of Old World species with $2n = 50$ and 52 , but these suggestions are rather speculative. Additional cytogenetical data, including comparative nuclear DNA content determination, as well as the definition of molecular phylogenetic relationships, are needed to clarify the situation.

Molecular analyses performed so far (Käss & Wink, 1997; Ainouche & Bayer, 1999), even considering that just a few of the eastern South American and Andean species were included, have suggested a separation of these two groups. It is interesting to note in the ITS

Table 4. Chromosome numbers in *Lupinus* species

Species	2n	Reference	Species	2n	Reference
Old World			<i>L. ornatus</i>	48	Darlington (1955); Fedorov (1969)
Smooth-seeded			<i>L. paniculatus</i>	48	Fedorov (1969)
<i>L. albus</i>	50	Gladstones (1998)	<i>L. perennis</i>	48, 96	Fedorov (1969); IPCN
<i>L. angustifolius</i>	40	Gladstones (1998)	<i>L. platamodes</i>	48	IPCN
<i>L. hispanicus</i>	52	Gladstones (1998)	<i>L. polyphyllus</i>	48	Darlington (1955); Fedorov (1969); IPCN
<i>L. luteus</i>	52	Gladstones (1998)			
<i>L. micranthus</i>	52	Gladstones (1998)			
Rough-seeded			<i>L. pubescens</i>	48	Darlington (1955); Fedorov (1969)
<i>L. atlanticus</i>	38	Gladstones (1998)	<i>L. pusillus</i>	48	Fedorov (1969)
<i>L. cosentinii</i>	32	Gladstones (1998)	<i>L. reticulatus</i>	40	Fedorov (1969)
<i>L. digitatus</i>	36	Gladstones (1998)	<i>L. russellianus</i>	36	Fedorov (1969)
<i>L. palaestinus</i>	42	Gladstones (1998)	<i>L. saxosus</i>	96	Fedorov (1969)
<i>L. pilosus</i>	42	Gladstones (1998)	<i>L. semperflorans</i>	48	This paper
<i>L. princei</i>	38	Gladstones (1998)	<i>L. sericeus</i>	48	Fedorov (1969)
North America and Andean region			<i>L. subcarnosus</i>	34, 36, 48	Darlington (1955); Fedorov (1969)
<i>L. albicaulis</i>	48	Fedorov (1969)	<i>L. succulentus</i>	48	Fedorov (1969)
<i>L. albicoccineus</i>	48	Fedorov (1969)	<i>L. suksdorfii</i>	96	Fedorov (1969)
<i>L. ananeanus</i>	48	Fedorov (1969)	<i>L. sulphureis</i>	48, 96	Fedorov (1969)
<i>L. arboreus</i>	48	Fedorov (1969)	<i>L. superbus</i>	48	Fedorov (1969)
<i>L. arcticus</i>	48	Fedorov (1969); IPCN	<i>L. texensis</i>	34, 36	Fedorov (1969)
<i>L. argenteus</i>	48	Fedorov (1969); IPCN	<i>L. varius</i>	48	Fedorov (1969)
<i>L. aridus</i>	48	Fedorov (1969)	<i>L. verustus</i>	48	Fedorov (1969)
<i>L. ballianus</i>	48	This paper	<i>L. villosus</i> ^a	52	This paper
<i>L. bandelierae</i>	36	This paper	8 other Andean taxa ^b	48	This paper
<i>L. barkeri</i>	48, 50	Darlington (1955); Fedorov (1969)	South-eastern South America		
<i>L. caballoanus</i>	48	IPCN	<i>L. bracteolaris</i>	32,34	Maciel & Schifino- Wittmann (2002); This paper
<i>L. caespitosus</i>	48	Fedorov (1969)	<i>L. crotalarioides</i> ^a	36	This paper
<i>L. caudatus</i>	48, 96	Fedorov (1969)	<i>L. gibertianus</i>	36	Maciel & Schifino- Wittmann (2002)
<i>L. cumulicola</i> ^a	52	This paper	<i>L. guaraniticus</i> ^a	36	This paper
<i>L. cytisoidse</i>	48	Fedorov (1969)	<i>L. lanatus</i>	36	Maciel & Schifino- Wittmann (2002); This paper
<i>L. densiflorus</i>	48	Darlington (1955); Fedorov (1969)	<i>L. linearis</i>	32,34	Maciel & Schifino- Wittmann (2002)
<i>L. douglasii</i>	48	Darlington (1955); Fedorov (1969)	<i>L. magnistipulatus</i>	36	Maciel & Schifino- Wittmann (2002)
<i>L. eanophyllus</i>	48	This paper	<i>L. multiflorus</i>	36	Maciel & Schifino- Wittmann (2002); This paper
<i>L. elegans</i>	48	Darlington (1955); Fedorov (1969)	<i>L. paraguariensis</i>	36	This paper
<i>L. hartwegii</i>	48–50	Darlington (1955); Fedorov (1969)	<i>L. paranensis</i>	36	This paper
<i>L. hilarianus</i>	48	Fedorov (1969)	<i>L. reitzii</i>	36	Maciel & Schifino- Wittmann (2002); This paper
<i>L. huaronensis</i>	48	This paper	<i>L. rubriflorus</i>	36	Maciel & Schifino- Wittmann (2002); This paper
<i>L. humulicula</i>	48, 96	Fedorov (1969)	<i>L. uleanus</i>	36	Maciel & Schifino- Wittmann (2002)
<i>L. laxiflorus</i>	48, 96	Fedorov (1969)	<i>L. velutinus</i> ^a	36	This paper
<i>L. lepidus</i>	48	Fedorov (1969)			
<i>L. leptophyllus</i>	48	Fedorov (1969)			
<i>L. leucophyllus</i>	48, 96	Fedorov (1969)			
<i>L. littoralis</i>	48	Fedorov (1969)			
<i>L. mexicanus</i>	48	Fedorov (1969)			
<i>L. microphyllus</i>	48	Fedorov (1969); This paper			
<i>L. montanus</i>	48	Fedorov (1969)			
<i>L. mutabilis</i>	48	Darlington (1955); Fedorov (1969)			
<i>L. nanus</i>	48	Darlington (1955); Fedorov (1969)			
<i>L. nootkatensis</i>	48	Darlington (1955); Fedorov (1969)			

^aUnifoliate. ^bSee text for details.

data of Ainouche & Bayer (1999) that the three eastern South American species that they analysed (*L. multiflorus*, *L. paraguariensis* and *L. bracteolaris*) grouped with *L. texensis* Hook, one of the two North American species with $2n = 36$, while the Andean *L. mutabilis* ($2n = 48$) grouped with other North American taxa, presumably all $2n = 48$. In the work of Käss & Wink (1997), the three eastern South American species analysed, *L. albescens* Hooker & Arnott, *L. aureonitens* Gilles and *L. paraguariensis* formed a separate group, but chromosome numbers are known just for *L. paraguariensis*.

CONCLUDING REMARKS

A more comprehensive survey of chromosome numbers among North, Central and South American *Lupinus* species needs to be carried out. Points that still need to be clarified are: (a) the apparent cytological separation between eastern and Andean South American *Lupinus*; (b) how often chromosome numbers other than $2n = 48$ occur among North American taxa; (c) the difference in chromosome numbers between the North American and the Brazilian unifoliolate *Lupinus*. Analyses to date have shown that cytogenetic information is a powerful tool in the study of *Lupinus* taxonomy and evolution and, if coupled with additional data such as those from morphological and molecular approaches, will help us to shed light on the evolutionary history of this fascinating genus.

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