

CHROMOSOME NUMBERS IN SOUTH AMERICAN ANDEAN SPECIES OF *LUPINUS* (LEGUMINOSAE)

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Chromosome numbers were determined in 22 accessions of 16 *Lupinus* L. species from the Andean region. All had $2n=48$ chromosomes, except *L. bandelierae* C. P. Smith ($2n=36$). These are the first chromosome countings for *L. arvensis* Benth., *L. chilensis* C. P. Smith, *L. chlorolepsis* C. P. Smith, *L. chrysanthus* Ulbr., *L. lindleyanus* Agardh, *L. mantaroensis* C. P. Smith, *L. piurensis* C. P. Smith, *L. proculastrinus* C. P. Smith, *L. prostratus* Agardh, *L. pulvinaris* Ulbr., *L. pycnostachys* C. P. Smith, *L. smithianus* Kunth and *L. tominensis* Wedd. The present study also confirmed literature data on chromosome numbers of *L. mutabilis* Sweet, *L. semperflorens* Benth. ($2n=48$) and *L. bandelierae* ($2n=36$). The results clearly confirm that, cytologically, the vast majority of Andean species are closer to the North American than to the eastern South American taxa.

Key words: cytotaxonomy, evolution, ploidy levels.

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Se determinó el número cromosómico en 22 poblaciones de 16 especies del género *Lupinus* L. de la región Andina. Todos presentaron $2n=48$ cromosomas con excepción de *L. bandelierae* C. P. Smith ($2n=36$). Estos son los primeros recuentos cromosómicos para *L. arvensis* Benth., *L. chilensis* C. P. Smith, *L. chlorolepsis* C. P. Smith, *L. chrysanthus* Ulbr., *L. lindleyanus* Agardh, *L. mantaroensis* C. P. Smith, *L. piurensis* C. P. Smith, *L. proculastrinus* C. P. Smith, *L. prostratus* Agardh, *L. pulvinaris* Ulbr., *L. pycnostachys* C. P. Smith, *L. smithianus* Kunth y *L. tominensis* Wedd. Este trabajo también confirmó recuentos cromosómicos para *L. mutabilis* Sweet, *L. semperflorens* Benth. ($2n=48$) y *L. bandelierae* ($2n=36$). Los resultados claramente confirman que, citológicamente, la mayoría de los *Lupinus* andinos son más próximos de las especies norteamericanas que a las del este de América del Sur.

Palabras clave: citotaxonomía, evolución, niveles de ploidía.

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Introduction

The genus *Lupinus* L. (Leguminosae, Faboideae, Genisteae) comprises around 200 species (Plittmann, 1981), an estimate which, depending on the author, ranges from 150 (Burkart, 1952) up to 500 (Dunn, 1984) taxa. The most recent estimate is of 275 species (Hughes & Eastwood, 2006). They inhabit a wide climatic range, from sub-arctic to semi-desertic and sub-tropical regions, and may be uni- or multifoliolate (predominantly), herbaceous or shrubby, annual, biennial or perennial.

The species are geographically separated in two big groups: 12 species in the Old World and the majority of the genus components in the Americas. Some of them have been cultivated by mankind since a long time, especially *L. albus* L., *L. angustifolius* L. and *L. luteus* L., in the Old World, and in the Americas, *L. mutabilis* Sweet (the only American species cultivated as a grain crop). For human consumption, seeds should be previously boiled or soaked in water, processes that remove their alkaloids. Other species are also used for other finalities such as green manure, forage, ornamentals and dune stabilization (Hoveland & Townsend, 1985; Gladstones, 1998).

A monophyletic origin for the genus is supported by several approaches including biochemical and molecular analyses (Cristofolini, 1989; Badr & al., 1994; Ainouche & Bayer, 1999; Ainouche & al., 2004) but its center of origin is still debatable. Suggestions range from North American (Plittmann, 1981), South American (Dunn, 1984; Gross, 1986) and Old World origins, the last one being the most supported hypothesis (Cristofolini, 1989; Wolko & Weeden, 1990). Gladstones (1998), suggested a Northern Hemisphere origin with a further and progressive branching to eastern South America, North Africa, the Mediterranean and finally North America and western South America. However, a very recent work by Hughes & Eastwood (2006) showed that the eastern South America species and most of the North America and Andean taxa form two

sister clades, therefore of same age.

The 12 Old World species, multifoliolate annuals and mostly autogamous, are divided into rough-seeded (*L. pilosus* Murr., *L. cosentinii* Guss., *L. digitatus* Forsk., *L. atlanticus* Gladst., *L. princei* Harms, *L. somaliensis* Baker) and smooth-seeded taxa (*L. albus*, *L. angustifolius*, *L. micranthus* Guss., *L. luteus*, *L. hispanicus* Boiss. & Reut.).

Most of the American species are multifoliolate except for the unifoliolate group of 13 species from sub-tropical Brazil and the four unifoliolate species from southeast North America (Dunn, 1971; Planchuelo & Dunn, 1984, 1989; Monteiro & Gibbs, 1986).

In South America, considered as a biological center of speciation in the genus (Planchuelo-Ravelo, 1984) there are two main geographical distribution areas, the Atlantic and the Andean regions (Planchuelo-Ravelo, 1984; Gross, 1986). Molecular data support the general separation of eastern and western South American species (Käss & Wink, 1997; Ainouche & Bayer, 1999), but the eastern South American species group to a few North American taxa (Hughes & Eastwood, 2006).

The 12 Old World species fall into distinct cytotoxic groups ($2n=52, 50, 42, 40, 38, 36$ and 32), some of them monospecific (Gladstones, 1998). Cytogenetic information on American species, except North American taxa, was, as a whole, rather limited. Most of the around 50 North American species analysed, have $2n=48$, occasionally $2n=96$ (generally as polyploid races of diploid taxa), $2n=50$ (as intraspecific variation of two $2n=48$ species), $2n=36$ (three species) or $2n=34$ (as intraspecific variation of two $2n=36$ species). Regarding the South American Andean species, until very recently, literature data were found for only four species: *L. microphylus* Desr., *L. mutabilis*, *L. paniculatus* Desr. and *L. pubescens* Benth., all with $2n=48$ (Darlington, 1955; Fedorov, 1969; Cox, 1972; Dunn, 1984; Gladstones, 1998; IPCN). It is suggested that $x=6$ is the basic number in the genus and that evolution was accompanied by aneuploidy and polyploidy (Dunn, 1984; Gladstones, 1998).

The first chromosome number determinations for south-eastern South American taxa were published in 2002 (Maciel & Schifino-Wittmann, 2002) for nine species: *L. gibertianus* C. P. Smith, *L. lanatus* Benth., *L. magnistipulatus* Planchuelo & Dunn, *L. multiflorus* Desr., *L. rubriflorus* Planchuelo, *L. uleanus* C. P. Smith and *L. reitzii* Pinheiro & Miotto, with $2n=36$, and *L. bracteolaris* Desr. and *L. linearis* Desr., both with $2n=32$ and $2n=34$. Conterato & Schifino-Wittmann (2006) further analysed *L. paranensis* C. P. Smith, *L. paraguariensis* Chod. & Hassl. and the unifoliolate *L. guaraniticus* (Hassl.) C. P. Smith, *L. crotalarioides* Mart. ex Benth, and *L. velutinus* Benth., all collected in Brazil and with $2n=36$. The same authors determined the number of chromosomes for the Andean *L. ballianus* C. P. Smith, *L. eanophyllus* C. P. Smith, *L. huaronensis* Macbride and *L. semperflorens* Benth. from Peru and Bolivia, all with $2n=48$, *L. bandeliera* ($2n = 36$) from Bolivia and for two unifoliolate species from Florida, *L. cumulicola* Small. and *L. villosus* Willd., both with $2n=52$, a chromosome number previously unknown among American taxa. These results supported the suggestions of Maciel & Schifino-Wittmann (2002) that southeastern South-American species are a group cytologically differentiated from the Andean as well as from most other American ones, and indicated that the Brazilian and the North American unifoliolate *Lupinus* had independent origins.

Data on nuclear DNA amounts for a few Old World and New World species (Naganowska & al., 2003a, 2006; Bennet & Leitch, 2004) showed no correlation between chromosome number and DNA amount, suggesting that evolution was accompanied by gain and/or loss of DNA. FISH (fluorescent *in situ* hybridization) for rRNA genes has been recently employed in a few Old World species (Naganowska & Zielinska, 2002; Naganowska & al., 2003b) but comparative studies with American taxa are still lacking.

The objective of the present work was to determine chromosome numbers in a larger sample of species and accessions of *Lupinus* from the Andean region, in order to have a more representative sample of the region.

Material and Methods

Seeds were obtained from Dr. Colin E. Hughes and Ruth E. Eastwood, Department of Plant Sciences, University of Oxford, United Kingdom. A list of the species and accessions analyzed, as well as information on the collection places are shown in Table 1. Voucher specimens are deposited at the Daubeny Herbarium (FHO), University of Oxford.

The work was conducted at the Cytogenetics Laboratory, Departamento de Plantas Forrageiras e Agrometeorologia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul.

Chromosome numbers were determined in root-tip cells. Seeds were scarified with sandpaper and germinated at room temperature in petri dishes with moist filter paper. When the roots were about 2-5 mm long they were pretreated in a saturated solution of 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. For slide preparation, the roots were washed in distilled water, hydrolysed in 1N HCl at 60°C for 20 min., stained with Feulgen, treated for less than 1 min. in 2% pectinase and squashed in 2% propionic carmine. At least ten metaphase plates per accession (intact cells, well spread chromosomes, no chromosome overlapping) were analysed.

Results

Chromosome numbers were determined in 22 accessions of 16 *Lupinus* species from Bolivia, Ecuador and Peru (Table 1) and all had $2n=48$ chromosomes, except *L. bandeliera* ($2n=36$). These are the first chromosome determinations for *L. arvensis* Benth., *L. chilensis* C. P. Smith, *L. chlorolepsis* C. P. Smith, *L. chrysanthus* Ulbr., *L. lindleyanus* Agardh, *L. mantaroensis* C. P. Smith, *L. piurensis* C. P. Smith, *L. proculastrinus* C. P. Smith, *L. prostratus* Agardh, *L. pulvinaris* Ulbr., *L. pycnostachys* C. P. Smith, *L. smithianus* Kunth and *L. tominensis* Wedd. The present study confirmed literature data on chromosome numbers of *L. bandeliera*, *L. mutabilis* and *L. semperflorens*.

Table 1. Somatic chromosome numbers (2n) and list of the *Lupinus* species and accessions examined.

| Species | 2n | Collector number | Place of collection | Lat. (S) | Long. (W) | Alt. (m) |
|--------------------------------------|----|------------------|-----------------------------------------------|----------|-----------|----------|
| <i>L. arvensis</i> Benth. | 48 | RJE 221 | Ecuador. Prov. Loja, Loja. | 03° 59' | 79° 11' | 2200 |
| <i>L. bandelierae</i> C. P. Smith | 36 | CEH 2318 | Bolivia. Dep. Potosí, Saavedra. | 19° 34' | 65° 25' | 3290 |
| <i>L. chilensis</i> C. P. Smith | 48 | CEH 2319 | Bolivia. Dep. Potosí, T. Frias. | 19° 37' | 65° 44' | 4340 |
| <i>L. chlorolepsis</i> C. P. Smith | 48 | CEH 2261 | Bolivia. Dep. La Paz, Larecaja. | 15° 50' | 68° 38' | 3770 |
| <i>L. chrysanthus</i> Ulbr. | 48 | CEH 2264 | Bolivia. Dep. La Paz, Murillo. | 16° 17' | 68° 07' | 4690 |
| <i>L. lindleyanus</i> Agardh | 48 | RJE 141 | Peru. Dep. Lima, Yauyos. | 12° 46' | 75° 49' | 2640 |
| <i>L. mantaroensis</i> C. P. Smith | 48 | CEH 2000 | Peru. Dep. La Libertad, Santiago de Chuco. | 07° 12' | 78° 24' | 2720 |
| | 48 | RJE 54 | Peru. Dep. La Libertad, Santiago de Chuco. | 08° 09' | 78° 12' | 3400 |
| <i>L. mutabilis</i> Sweet | 48 | CEH 1999 | Peru. Dep. Cajamarca, Cajamarca. | 07° 11' | 78° 27' | 2610 |
| | 48 | CEH 2009 | Peru. Dep. Cajamarca, Cajamarca. | 07° 05' | 78° 34' | 2920 |
| | 48 | CEH 2302 | Bolivia. Dep. Cochabamba, Arani. | 17° 37' | 65° 40' | 3550 |
| | 48 | CEH 2323 | Bolivia. Dep. La Paz, Manco Kapac. | 16° 09' | 69° 05' | 3845 |
| | 48 | CEH 2326 | Bolivia. Dep. La Paz, Manco Kapac. | 16° 00' | 69° 11' | 3840 |
| <i>L. piurenensis</i> C. P. Smith | 48 | RJE 113 | Peru. Dep. Cajamarca, Cajamarca. | 07° 16' | 78° 30' | 2600 |
| <i>L. proclaustrinus</i> C. P. Smith | 48 | RJE 142 | Peru. Dep. Lima, Yauvos. | 12° 46' | 75° 49' | 2780 |
| <i>L. prostratus</i> Agardh | 48 | RJE 126 | Peru. Dep. Piura, Huancabamba. | 05° 12' | 79° 28' | 2760 |
| <i>L. pulvinaris</i> Ulbr. | 48 | CEH 2333 | Bolivia. Dep. La Paz, B. Saavedra. | 15° 14' | 68° 57' | 4150 |
| <i>L. pycnostachys</i> C. P. Smith | 48 | CEH 2444 | Bolivia. Dep. La Paz, Los Andes. | 16° 15' | 68° 34' | 3860 |
| | 48 | CEH 2250 | Bolivia. Dep. La Paz, Los Andes. | 16° 22' | 68° 24' | 3950 |
| <i>L. semperflorens</i> Benth. | 48 | RJE 219 | Ecuador, Prov. Loja, Loja. | 04° 00' | 79° 15' | 2550 |
| <i>L. smithianus</i> Kunth | 48 | RJE 201 | Ecuador. Prov. Chimborazo, Volcan Chimborazo. | 01° 30' | 78° 52' | 4350 |
| <i>L. tominensis</i> Wedd. | 48 | CEH 2294 | Bolivia. Dep. Cochabamba, Quillacollo. | 17° 16' | 66° 20' | 3790 |

CEH: C. E. Hughes; **RJE:** Ruth E. Eastwood.

Discussion

Considering the present results and literature data, there are now chromosome number determinations for 22 Andean *Lupinus* species, 21 of which with $2n=48$. In their paper, Conterato & Schifino-Wittmann (2006) reported chromosome numbers of $2n=48$ for another eight taxa with pending taxonomic identification. We have also examined five additional *Lupinus* accessions, all with $2n=48$ but that could not be taxonomically clearly identified (data not shown). This information supports the predominance of $2n=48$ chromosomes among Andean *Lupinus* species as well as an apparent lack of intraspecific variability. The only species with $2n=36$, *L. bandelierae*, occurs widely across Bolivia and probably in NW Argentina, and has some morphological similarities with Brazilian/Paraguayan species such as *L. bracteolaris* Desr. and *L. gibertianus* C. P. Smith (C. E. Hughes, pers. com.).

The prevalence of $2n=48$ ($8x$, accepting that $x=6$) chromosomes among the Andean and North American species and $2n=36$ ($6x$) among the southeastern South American taxa supports the suggestion that, in the latter region, lower ploidy levels would have been involved in the speciation processes (Maciel & Schifino-Wittmann, 2002; Conterato & Schifino-Wittmann, 2006). In the molecular analyses of Aïnouche & Bayer (1999) and Aïnouche & al. (2004), the North American species *L. texensis* Hook., one of the few with $2n=36$, grouped with the southeastern South American *L. paraguariensis*, *L. multiflorus* Desr. and *L. bracteolaris*, and the Andean *L. mutabilis* ($2n=48$) grouped with the $2n=48$ North American taxa analysed. The molecular analyses of Hughes and co-workers (Hughes & Eastwood, 2006; C. E. Hughes, pers. com.) on a large sample of American *Lupinus* clearly showed the grouping of American *Lupinus* species according to their chromosome number. These works suggest that the different chromosome numbers between the two big American *Lupinus* groups reflect more profound genomic

differences and that chromosome evolution has been very important in the genus evolution.

From literature data, and the present results, it can be concluded that, while in the Old World, polyploidy and aneuploidy have played important roles in speciation and evolution, in the New World polyploidy was the dominant cytogenetic alteration.

However, many questions still needed to be answered, such as the real extent (if any) of intraspecific variability in chromosome number among American taxa. As generally few accessions per species have been so far examined, this question could only be solved by increasing the sample size and covering the species distribution area. Another important aspect, as pointed out in the introduction, is the apparent lack of relationship between chromosome number and DNA content, probably related to differences in chromosome size. This point should be further investigated by further DNA amount measures in a larger number of species as well as by detailed karyotypic analyses and chromosome size measurements. Even if no accurate measurements were made, we have observed during our continued *Lupinus* cytogenetic work that there are indeed some differences in chromosome size among different *Lupinus* species [as shown in the photographs of Conterato & Schifino-Wittmann (2006)]. Another intriguing point is the chromosome number and ploidy level of the ancestral that gave origin to the $2n=36$ and $2n=48$ lines. Conterato & Schifino-Wittmann (2006) suggested that the ancestral of all *Lupinus* could have been a $2n=24$ taxon.

Finally, additional cytogenetic techniques, such as chromosome banding, FISH and GISH (genomic *in situ* hybridization), would help to clarify which chromosomal changes, other than variation in number, happened during the genus evolution.

Conclusions

The predominant chromosome number among Andean species of *Lupinus* is $2n=48$.

The cytological separation between eastern and the majority of western South American and North American species is evident. An improved understanding of *Lupinus* chromosome evolution would require analyses of more species and accessions as well as the use of additional techniques, such as chromosome banding, FISH and GISH, and more determinations of nuclear DNA amounts, comparing Old World and the two major groups of New World species.

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