

The cytogenetics and evolution of forage legumes from Rio Grande do Sul: a review

Maria Teresa Schifino-Wittmann

Departamento de Plantas Forrageiras e Agrometeorologia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Caixa Postal 776, 91501-970 Porto Alegre, RS, Brasil. Fax: +55-51-316-6045. E-mail: mtschif@vortex.ufrgs.br

Abstract

The work developed by the Cytogenetics Group of the Department of Forage Plants and Agrometeorology (Departamento de Plantas Forrageiras e Agrometeorologia - DPFA), Agronomy Faculty, Federal University of Rio Grande do Sul (UFRGS), are reviewed in the present study. Topics discussed include: the chromosome numbers and meiotic behavior of *Desmodium* and *Vigna*; the application of cytogenetic methods (e.g., polyploidy induction in *Trifolium riograndense*) to plant breeding; the genetic control of chromosome pairing in autopolyploids of *T. riograndense*; karyotypes of the *Vicia sativa* aggregate in Southern Brazil as an example of a founder effect leading to a reduction in karyotype but not to morphological variability; data on the karyotypes of four *Lathyrus* species which show that the evolution of these species has been accompanied by a decrease in chromosome size, and the results of an investigation of variability in chromosome number in a complete genus, *Leucaena*. The main objectives of the group for the near future are also outlined.

INTRODUCTION

Native pastures and cytogenetics

The native pastures of Rio Grande do Sul, the southernmost Brazilian state, contain a rich diversity of native grass and legume species, many of them described as potentially good forages (Barreto and Kappel, 1964; Grossman *et al.*, 1965). Before proceeding to agronomic evaluation and genetic breeding programs, the use of some of these taxa as forage plants adapted to regional conditions first of all requires, among other things, the gathering of basic information on their taxonomy, cytogenetics and mode of reproduction. Many of these groups may also provide interesting evolutionary case studies.

The taxonomy of many of the native grass and legume genera of Rio Grande do Sul has been elucidated by several research programs undertaken mainly by researchers at the Botany Department of UFRGS.

Regarding native grasses there is, in the university, a long tradition of cytogenetic and evolutionary research in which a great volume of data have been built up on grasses of various genera, e.g., *Paspalum* L. (Moraes Fernandes *et al.*, 1968, 1973, 1974; Hickenbick *et al.*, 1992), *Axonopus* Pal. (Hickenbick *et al.*, 1975), *Briza* L. (Sampaio *et al.*, 1979;

Schifino and Winge, 1983), *Setaria* Beauv. (Freitas-Sacchet *et al.*, 1984) and *Sorghastrum* Nash. (Flores and Valls, 1987).

The cytogenetics of native legumes started with the work of Mohrdieck (1950), who first counted the chromosomes of *Trifolium riograndense*, but this work was discontinued for three decades until, in the early 80's, the cytogenetics group at Department of Forage Plants and Agrometeorology (DPFA) began to be formed.

In this study we present a review of our work on the cytogenetics of forage legumes, along with a more detailed description of some specific cases in which cytogenetic information has been linked to plant breeding and evolutionary studies.

The Cytogenetics Group of the DPFA

The objective of the first work on the cytogenetics of forage species, begun in the early 80's, was to provide basic information for developing future breeding programs. The first studies conducted were on native or introduced grasses and legumes and included the investigation and transmission of chromosome number instability in species of *Phalaris* L. and its hybrids (Schifino *et al.*, 1985, 1986; Schifino and Gus, 1986), the determination of chromosome numbers in *Desmodium* Desv. species (Schifino, 1983), and chromosome number confirmation in species of *Paspalum*, *Hemarthria* R. Br., *Coelorachys* Brongn. and *Lolium* L. (Schifino, M.T., unpublished results) that were being evaluated agronomically.

Later efforts were concentrated on legume species of immediate or future interest to plant breeding and other approaches to germplasm characterization were adopted, including isoenzyme characterization (Gonzales and Schifino-Wittmann, 1996; Schifino-Wittmann *et al.*, 1996; Schifino-Wittmann and Lange, 1997; Lange and Schifino-Wittmann, 2000), phenology and reproductive studies (Becker *et al.*, 1987; Klamt, 1997; Tedesco *et al.*, 1998) and, more recently, DNA markers. However, the main line is still cytogenetics. Besides the intimate link to plant breeding projects, some of the studies also allow the utilization of cytogenetical information for taxonomical delimitation as well as in evolutionary studies. The group has one permanent senior researcher and a varying number of co-workers, mainly undergraduate and graduate students.

Recently, other authors, outside the group, have also published work on *Lathyrus* L. (Battistin and Fernandez, 1994; Battistin *et al.*, 1999) and *Adesmia* DC. (Miotto and Forni-Martins, 1995; Coelho, 1996; Coelho and Battistin, 1998).

RESULTS OF SOME SELECTED RESEARCH PROGRAMS

Desmodium, *Vigna* and *Arachis* - basic information

Chromosome number determinations in four *Desmodium* species occurring in Rio Grande do Sul, *D. uncinatum* (Jacq.) DC. (yellow and violet-flowered bio-types), *D. affine* Schlecht., *D. incanum* DC. and *D. triarticulatum* Malme, confirmed the diploid number ($2n = 22$) for all taxa and provided an original count for *D. triarticulatum*.

Populational studies in three species of *Vigna* Savi, that is, *V. luteola* (Jacq.) Benth, *V. adenantha* (G.F. Meyer) Maréchal, Mascherpa & Satainier and *V. longifolia* (Bentham) Verdcourt, showed that all species were diploid with $2n = 22$, and with regular meiosis and high meiotic indexes (92-96%) and pollen fertility (92-98%) (Senff *et al.*, 1992).

Senff *et al.* (1995) has investigated chromosome number, meiotic behavior and pollen fertility in 22 populations of *Arachis pintoi* Krapovicas & Gregory ($2n = 20$), *Desmodium incanum*, *D. uncinatum*, *D. triarticulatum*, *D. pachyrrizum* Vog., *D. cunetaum* Hook et Arn., *Vigna adenantha*, *V. luteola* and *V. longifolia* (all $2n = 22$). All populations displayed regular meiosis, and meiotic indexes and pollen fertility over 96%.

Trifolium - cytogenetics in plant breeding and a model for chromosome pairing

Three native *Trifolium* species are described for Rio Grande do Sul: *T. riograndense* Burkart, *T. polymorphum* Poir., and *T. argentinense* Speg. *T. riograndense* is very well adapted to the acid, aluminum-rich soils of Rio Grande do Sul. From the plant breeding point of view this species could be a source of acid-soil tolerance genes which could be introduced into the widely cultivated white clover (*T. repens* L.). As was mentioned above, the first chromosome count for *T. riograndense* ($2n = 16$) was made by Mohrdieck (1950).

The cytogenetic studies with the native *T. riograndense*, *T. polymorphum* and *T. repens* were originally linked to a breeding project aimed at inducing polyploidy in *T. riograndense* with the subsequent crossing of the artificial autopolyploid with the exotic natural polyploid *T. repens* ($2n = 32$) in order to produce hybrids which combined the high yield of *T. repens* with the acid-soil tolerance of *T. riograndense* (Becker *et al.*, 1986; Schifino and Moraes-Fernandes, 1987a; Souza *et al.*, 1988).

Karyotypic as well as population studies of chromosome numbers and meiotic behavior were performed not only in *T. riograndense* (Figure 1a and b) but also in *T. polymorphum* (Figure 1c). The results showed that all the populations studied were formed only of diploid ($2n = 16$) individuals, the karyotypes of both species being very similar, differing only in the position of the satellited pair. Very little intraspecific variability was observed.

Meiosis in *T. riograndense* was regular and meiotic indexes and pollen fertility were high. In *T. polymorphum*, however, about 50% of the individuals examined presented quadrivalents, trivalents and univalents in metaphase one and varying pollen fertility, unusual for a diploid inbreeding species (Schifino and Moraes-Fernandes, 1988).

Due to the smaller than expected number of quadrivalents in the autotetraploids, the comparison of diploids and induced autotetraploids of *T. riograndense* (Schifino and Moraes-Fernandes, 1986, 1987a) led to the suggestion that there exists a genetic control mechanism for bivalent formation in this species (Schifino and Moraes-Fernandes, 1987b).

Vicia - a founder effect leading to a bottleneck in karyotype variability

Seven native *Vicia* species and two *Vicia* varieties have been described for southern Brazil (Bastos and Miotto, 1996); *V. sativa* L. *sensu str.* is cultivated in backyards and fields while *V. angustifolia* L. (= *V. sativa* subsp. *nigra*), a highly polymorphic and morphologically variable naturalized ruderal, is very common in disturbed habitats (Miotto, S., personal communication).

A preliminary cytogenetic investigation of native, exotic and naturalized *Vicia* species (Schifino-Wittmann *et al.*, 1994) showed all species to be diploid: the native *V. epetiolearis* Burk. (syn. *V. graminea* Sm.), *V. linearifolia* Hook. et Arn., *V. macrograminea* Burk. (syn. *V. stenophylla* Vog.) and *V. nana* Vog. with $2n = 14$; the exotic *V. faba* L., *V. sativa* L. and *V. panonica* L. with $2n = 12$, and *V. villosa* Roth and *V. hirsuta* (L.) S.F. Gray with $2n = 14$. Interesting results were found in the naturalized *V. angustifolia*, with most plants having $2n = 12$ but some $2n = 14$ plants were detected. Meiosis was regular in all species and meiotic indexes and pollen fertility just over 90%.

More comprehensive work has been carried out with populations of the *V. sativa* aggregate in Rio Grande do Sul (Weber and Schifino-Wittmann, 1999). There are several references in the literature to the so-called *Vicia sativa* aggregate in the Old World. This aggregate of taxa is morphologically, karyologically and ecologically variable (Hanelt and Mettin, 1989) and its formation could be explained by the hybridization which commonly occurs in the genus (Yamamoto, 1986) and the anthropogenic disturbance of primary habitats (Zohary and Plitmann, 1979). Maxted (1993, 1995) recognizes one species, *V. sativa*

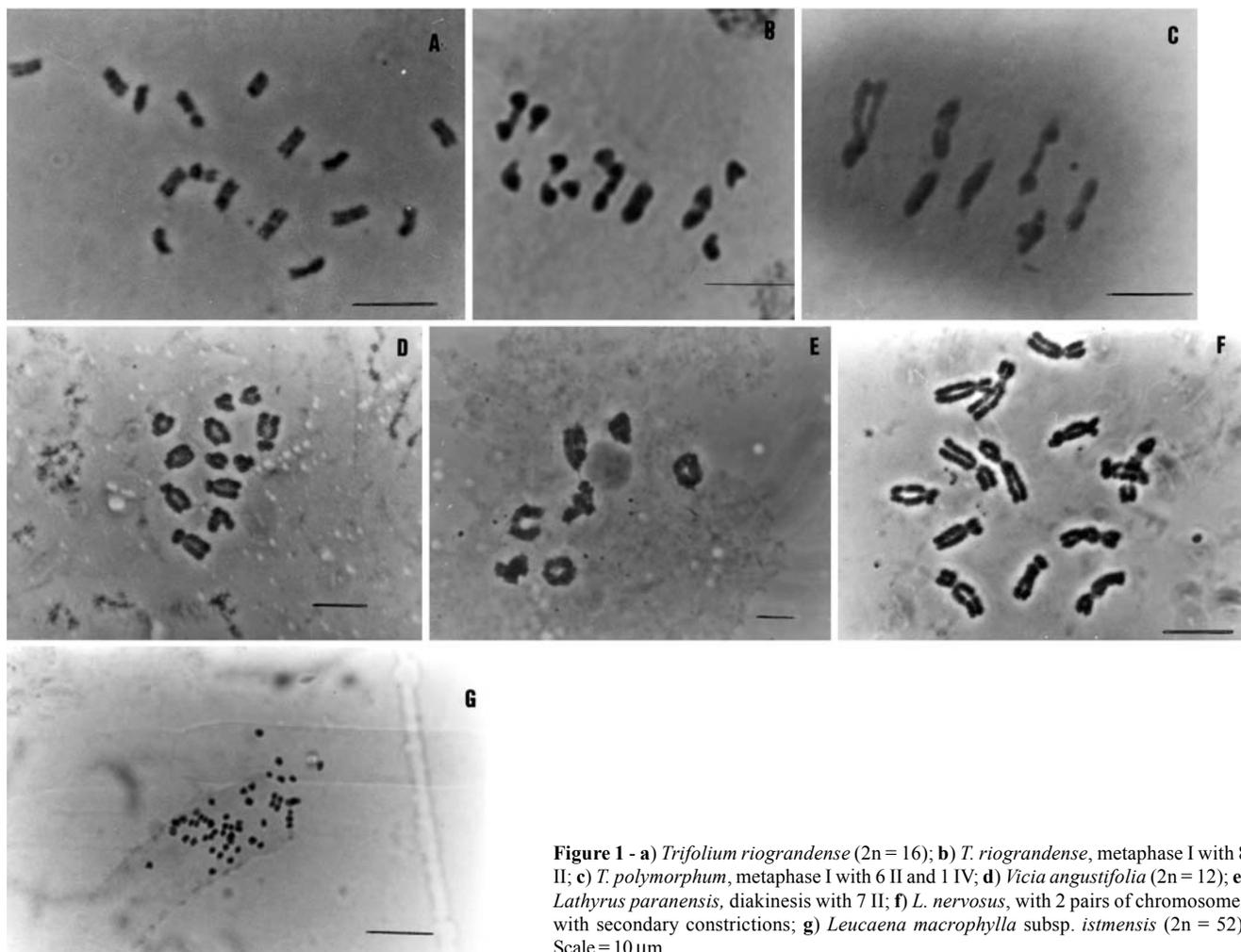


Figure 1 - a) *Trifolium riograndense* ($2n = 16$); b) *T. riograndense*, metaphase I with 8 II; c) *T. polymorphum*, metaphase I with 6 II and 1 IV; d) *Vicia angustifolia* ($2n = 12$); e) *Lathyrus paranensis*, diakinesis with 7 II; f) *L. nervosus*, with 2 pairs of chromosomes with secondary constrictions; g) *Leucaena macrophylla* subsp. *istmensis* ($2n = 52$). Scale = 10 μm .

L., and six subspecies, while Potokina (1997) recognizes seven separate species. The different forms coexist sympatrically, and present high morphological and karyotypical variability (Hollings and Stace, 1974; Ladizinsky, 1978, 1981; Ladizinsky and Temkim, 1978; Zohary and Plitmann, 1979; Ladizinsky and Shefer, 1982; Hanelt and Mettin, 1989). The taxa are reproductively compatible and the hybrids partially fertile (Ladizinsky, 1978). It is known that the still cultivated *V. sativa* was brought to Rio Grande do Sul by Italian settlers during the XIX century and it is thought that *V. angustifolia* probably came along with it as a weed, since both taxa are very similar and difficult to separate during harvest. *V. angustifolia* expanded around the areas of *V. sativa* cultivation and became a widespread ruderal in the state.

The investigation of the karyotypes of taxa of the *Vicia sativa* aggregate in southern Brazil (Weber and Schifino-Wittmann, 1999) provides an example of a founder effect, which although not leading to loss of morphological variability did produce a reduction in karyotype variability. The analyses of 37 accessions of *V. sativa*, *V. angustifolia*, intermediate types, and *V. cordata* Wulf. ex. Hoppe from the

States of Rio Grande do Sul and Santa Catarina allowed the separation of three taxa according to their karyotypes (Figure 2). *V. sativa* had $2n = 12$ with five acrocentric chromosome pairs, one with a secondary constriction in the long arm and one marker metacentric chromosome pair. *V. cordata* had $2n = 10$ acrocentric chromosomes, and the smallest pair had a secondary constriction in the long arm. All accessions of *V. angustifolia* had $2n = 12$ acrocentric chromosomes, one with a secondary constriction in the long arm (Figure 1d). Schifino-Wittmann *et al.* (1994) had previously described *V. angustifolia* from Rio Grande do Sul as having $2n = 14$ but this chromosome number was not found in the accessions included in the study described in the present paper, where the karyotypic data ($2n = 12$) agrees with what was found by Hanelt and Mettin (1966) and Ladizinsky (1978).

These results show a relation between karyotype and taxonomy, the presence of a marker metacentric chromosome in *V. sativa* providing a clear-cut distinction between the taxa. Despite the sympatry of different taxa of the *V. sativa* aggregate in southern Brazil, no mixed or different cytotypes were found. One possible explanation for this is

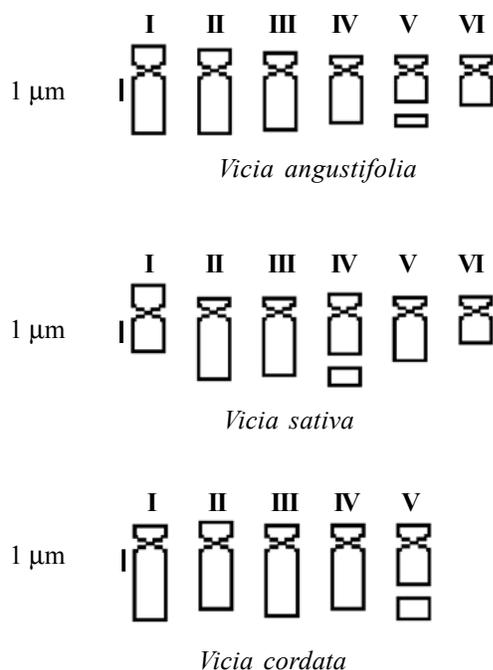


Figure 2 - Idiograms of the *Vicia sativa* aggregate taxa (from Weber and Schifino-Wittmann, 1999).

some type of “founder effect”, where the introduced *V. sativa* were probably stable cultivated forms while *V. angustifolia* seeds that came as weeds with these introductions, having been collected in the same localities, did not contain wide karyotypic variation.

Lathyrus - an evolutionary trend in chromosome size decrease

The 23 *Lathyrus* species endemic to South America were included in the section *Notholathyrus* by Kupicha (1983). Of these, 12 (*L. crassipes* Gill ap. Hook. et Arn., *L. hasslerianus* Burk., *L. hookeri* G. Don., *L. linearifolius* Vog., *L. macrostachys* Vog., *L. nervosus* Lam., *L. nitens* Vog., *L. paranensis* Burk., *L. paraguariensis* Hass., *L. parodii* Burk., *L. pubescens* Hook. et Arn., and *L. subulatus* Lam.) occur in Rio Grande do Sul (Neubert, E., personal communication).

Chromosome number and meiotic behavior were studied in the native species *L. crassipes* (syn. *L. pusillus*), *L. subulatus*, *L. paranensis* (Figure 1e), *L. pubescens*, *L. nervosus*, *L. sp.* (later defined as *L. linearifolius*), and in the exotics *L. sativus* L. and *L. latifolius* L. Meiotic behavior was normal, and meiotic indexes and pollen fertility were generally over 90%. All species were diploid, with $2n = 14$ chromosomes (Schifino-Wittmann *et al.*, 1994). This agrees with previous studies of *Lathyrus* species, including South American taxa (Federov, 1969; Kupicha, 1983; Yamamoto *et al.*, 1984; Broich, 1989; Battistin and Fernandez, 1994).

Polyploidy is uncommon in the genus (Federov, 1969) and all known species in the section *Notholathyrus* are diploid.

The karyotypes of 38 accessions of *L. nervosus*, *L. pubescens*, *L. paranensis* and *L. crassipes* have been analyzed (Klamt, 1997; Klamt and Schifino-Wittmann, 2000), the karyotypic formulae being $2m + 12sm$ for *L. nervosus*, *L. pubescens* and *L. paranensis* and $4m + 10sm$ for *L. crassipes*. In all these species the smallest chromosome pair bore a secondary constriction with a satellite in the long arm. Intraspecific variability in the position and number of secondary constrictions was observed in *L. nervosus* (Figure 1f) and *L. pubescens*. All of the species had a conservative karyotype morphology but differed in total complement size by as much as 20% between the highest (*L. nervosus*, 43.6 μm) and the lowest (*L. crassipes*, 35.54 μm) values. There was symmetrical variation in the size of the chromosomes in the different species suggesting that changes in chromosome size during evolution have been homogeneous along the complement. Equal amounts of DNA had been lost (or acquired) by all the chromosomes as suggested by Narayan and Durrant (1983) for some *Lathyrus* species.

Total chromosome length in perennial *Lathyrus* species is generally greater than for annual species (Rees and Hazarika, 1969; Yamamoto *et al.*, 1984). *L. nervosus* and *L. pubescens* are outbreeding perennials, *L. crassipes* an inbreeding annual, and *L. paranensis* an annual (Burkart, 1935, 1987). Generally annual species are derived from perennial species and inbreeding species from outbreeding species. Our data suggest that the evolution of the *Lathyrus* species studied was accompanied by a decrease in chromosome size and that *L. crassipes* is a derived species. This interpretation is based on the total chromosome complement length, life cycle and mode of reproduction of the four species examined.

Leucaena

The Central American genus *Leucaena* Benth (Leguminosae) is one of the most well known and widespread genera of nitrogen-fixing multipurpose trees. Nowadays two species, *L. leucocephala* (Lam.) de Wit. and *L. diversifolia* (Schltdl.) Benth., are widely grown in the tropical regions of the world where they have great economic and social importance (Hughes, 1993). *Leucaena* taxonomy has been a controversial subject for many years until the comprehensive and complete work of Hughes (1998a and b), recognizing 22 species, 4 subspecies, 2 varieties and 2 named hybrid taxa. The evolution of the genus is complex, possibly involving speciation by reticulate evolution. It has been suggested that *L. leucocephala* may have arisen as a result of indigenous domestication (Hughes and Harris, 1995; Harris *et al.*, 1996). If adapted plants are selected, there is great potential for *Leucaena* utilization in Rio Grande do Sul as forage, as a dietary supplement or as a protein bank, and this explains the interest of our group in this genus.

Hybrids

Studies in *L. leucocephala* ($2n = 4x = 104$), *L. trichandra* (Zucc.) Urban ($2n = 2x = 52$, formerly known as *L. diversifolia* subsp. *stenocarpa*) and their F1, F2 and F3 hybrids, selected for tolerance to acid soils, showed a variation from diploid to tetraploid numbers and a tendency to high chromosome numbers in advanced generations (Freitas *et al.*, 1988). Hybrids between *L. leucocephala* and *L. diversifolia*, both $2n = 4x = 104$, were all $2n = 104$ with regular meiosis and high pollen fertility (Freitas *et al.*, 1991a,b). Unreduced gametes were observed in these crosses, up to 30% in some individuals (Schifino-Wittmann *et al.*, 1997; Schifino-Wittmann and Simioni, 1998).

Chromosome number determinations in the whole genus

There is still a gap in the cytogenetic data of this genus since most of the available information is restricted to chromosome counts of a few plants per species and meiotic studies in some species and hybrid populations (Gonzales *et al.*, 1967; Pan and Brewbaker, 1988; Palomino *et al.*, 1995) and also because many of the chromosome counts are imprecise or not confirmed (Hughes, 1998), with even the chromosome number unknown for some species. The main reasons for this scarcity of information include small chromosome size (about $1 \mu\text{m}$), the high number of chromosomes ($2n = 52, 56, 104$ and 112 , according to the literature), which makes chromosome counting difficult, and the lack, until recently, of a defined taxonomy. To fill the gaps in the cytogenetic information available for the genus our group, in collaboration with the Oxford Forestry Institute (OFI - University of Oxford, UK), is developing a program of cytological analysis based on the OFI germplasm collection of the genus.

Somatic chromosome counts in 51 provenances (Schifino-Wittmann *et al.*, 1999 and in press) gave chromosome numbers of $2n = 52$ for *L. esculenta* (Sesse & Moc. ex DC.) Benth., *L. lanceolata* S. Watson var. *lanceolata* and var. *sousae* (S. Zárate) C.E. Hughes, *L. magnifica* (C.E. Hughes) C.E. Hughes, *L. pueblana* Britton & Rose and *L. shanonii* J.D. Smith, $2n = 56$ for *L. collinsii* Britton & Rose subsp. *zacapana* C.E. Hughes, *L. greggii* S. Watson and *L. matudae* (S. Zárate) C.E. Hughes, $2n = 104$ for *L. confertiflora* S. Zárate var. *confertiflora*, *L. diversifolia*, *L. leucocephala* subsp. *leucocephala* and subsp. *glabrata* (Rose) S. Zárate and *L. x spontanea*, and $2n = 112$ for *L. involucrata* S. Zárate, therefore increasing the number of known tetraploid species in the genus. Intrataxon variability was found in *L. macrophylla* Benth. subsp. *istmensis* C.E. Hughes (Figure 1g) ($2n = 52$ and 56), *L. confertiflora* var. *adenotheloidea* (S. Zárate) C.E. Hughes ($2n = 104$ and 112), *L. pallida* Britton & Rose ($2n = 104$ and 112) and *L. trichandra* ($2n = 52$ and 104). These results support the theory of complex evolutionary patterns in the genus and

the multiple origins of tetraploid *L. confertiflora* and *L. pallida*. Most of the 25 provenances analyzed had pollen fertility of over 90%. A varying percentage of unreduced gametes was found, up to 12% in the diploid *L. trichandra* 3/91, opening the possibility of utilizing unreduced gametes in genetic breeding of the genus.

PERSPECTIVES

The cytogenetic study of the genus *Leucaena* is still going on, including a thorough meiotic analysis of all taxa in the OFI collection.

Regarding native legumes, a detailed meiotic analysis of about 90 accessions of the 17 Brazilian species of *Adesmia* is currently being developed as well as chromosome number determination for those taxa for which this information is lacking, and this work will be repeated with the genus *Lupinus* L.

A comprehensive project on the occurrence of unreduced gametes in native and cultivated forage legumes is underway due to its importance in plant breeding programs.

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