Cytogenetics and Cytotaxonomy of Brazilian Species of *Senna* Mill. (Cassieae - Caesalpinioideae - Leguminosae).

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Abstract — Chromosome numbers, meiotic behaviour, meiotic indexes and pollen fertility are reported for 17 *Senna* Mill. species occurring in Southern Brazil. Haploid numbers were n=11, 12, 13, 14 and 28. No intraspecific variability was detected; n=14 predominated. The only accession of *S. rugosa* showed to be tetraploid, in contrast to the a previous reference for this species. Meiotic behaviour was generally regular for all species and accessions, but some irregularities such as uni and multivalents and unequal segregation were occasionally observed. Meiotic indexes were high, over 95%. Mean pollen fertility was over 81% for all species, but in some taxa much lower values were found in some accessions. All species had prolate-spheroidal pollen grains. No relation between grain size, taxonomic position and chromosome number could be established. Our results, together with literature data, suggest that x=14 is the basic number for the genus, probably a secondary number derived from the x=7 suggested for all Caesalpinioideae, and that the other numbers in the genus represent a disploid series. A broader cytogenetic survey of a more representative number of species, along the geographic distribution of the genus, should be done addressing questions such as the extent of intra and inter-specific variability in chromosome number, occurrence of polyploidy and the real role of disploidy in *Senna* evolution

Key words: chromosome numbers, disploid chromosome evolution, meiotic behaviour, pollen, *Senna*

INTRODUCTION

The pantropical genus *Senna* Mill. (Cassiinae), included in Tribe Cassieae, is considered to be paraphyletic (Irwin and Barneby 1981; 1982; Käss and Wink 1996; Lewis and Polhill 1998; Doyle *et al.* 1997; 2000; Herendeen 2000; Bruneau *et al.* 2001). The genus is taxonomically complex and was previously considered as a subgenus of *Cassia* L. (*s.l.*), which is presently divided in three genus: *Senna*, *Chamaecrista* Moench and *Cassia* (*s.s.*). This separation is supported by several studies based on morphological, vegetative and reproductive characters (Irwin and Barneby 1981; 1982; Gottsberger and Silberbauer-Gottsberger 1988; Owens and Lewis 1989; Dulberger *et al.* 1994), ontogenetic characteristics (Tucker 1996), seed proteins (Guareeb *et al.* 1999), DNA fragments (Mondal *et al.* 2000), molecular systematics (Doyle *et al.* 1997; 2000; Bruneau *et al.* 2001) and cytogenetics (Goldblatt 1981; Biondo 2004). In spite of that, works classifying *Senna* species as *Cassia* (*s.l.*) are still found. Irwin and Barneby (1982) divided the genus in six sections: *Psilorhegma*, *Chamaefistula*, *Senna*, *Peiranisia*, *Paradyction* and *Astroites*.

Around 80% of the nearly 260 *Senna* species occur in the neotropical region (Irwin and Barneby 1981; 1982). They present a wide variety of habits, but most species are of the tree-shrubby type and occur in fields and several forest-like formations, mainly around wood borders and wood openings, being also common in disturbed areas like roadsides and degraded lands. Bracteoles absence, filets larger than anthers and the frequent presence of staminodes are the main characters allowing the distinction of *Senna* from the other genera of the subtribe Cassiinae (Irwin and Barneby 1981; 1982; Bortoluzzi *et al.* 2002). The economic importance of *Senna* species is manyfold: many have a great ornamental potential, being widely used (Santos and Teixeira

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2001), may also be employed for wood production (Marchiori 1997; Backes and Irgang 2002), in the reclamation of degraded areas (Resende and Kondo 2001), and as natural medicines (Lorenzi and Abreu 2002; Silva *et al.* 2002; Barbosa *et al.* 2004), besides their unquestionable ecologic importance (Lorenzi 1992; Marchiori 1997; Lima 2000; Backes and Irgang 2002).

According to the literature, chromosome numbers in *Senna* species are 2n = 22, 24, 26 and 28 (Irwin and Turner 1960; Bandel 1974; Coleman and Demenezes 1980; Goldblatt 1981; Irwin and Barneby 1982; Biondo *et al.* 2002; Souza and Benko-Iseppon 2004). The basic number for the genus is accepted to be x = 14, however considered as a polyploid derivative of x = 7 which is the basic number for the subfamily Caesalpinioideae. The additional basic numbers of x = 13, 12 and 11, would have been derived by disploidy during the evolutionary process (Goldblatt, 1981).

Twenty four native, sub-spontaneous and cultivated species, belonging to sections *Chamaefistula*, *Senna* and *Peiranisia,* are found in the three most southern states of Brazil (Rio Grande do Sul, Santa Catarina and Paraná, spanning a latitude interval from 33° to 22° S). Taxonomy and cytogenetics of these taxa need further investigation, aiming to broaden the systematic knowledge of the group, therefore contributing to a better understanding of their taxonomic and evolutionary relations.

In this paper we report chromosome numbers, meiotic behaviour and pollen fertility in 17 native and sub-spontaneous *Senna* species from Southern Brazil.

MATERIALS AND METHODS

Flower buds from 135 accessions (each represented by a single speciment) of 15 native and two sub-spontaneous *Senna* species were collected in several localities of Rio Grande do Sul, Santa Catarina and Paraná (Table 1, Figure 1). A voucher from each plant is deposited at the ICN Herbarium, Botany Department, Universidade Federal do Rio Grande do Sul. The buds were fixed with Carnoy 9:3:1 (ethanol-chloroformium-acetic) for 24h at room temperature and afterwards stored below 0^{0}C until required.

Table 1 — List of the *Senna* species and accessions examined

Species	Place of collection	Accessions/type of analyses
SECTION *CHAMAEFISTULA*		
Series Baccilares		
S. angulata (Vogel) H. S. Irwin & Barneby	PR, Morretes	R.L.C.Bortoluzzi & E.Biondo, 1190 / a,b
S. macranthera (DC. ex. Collad.) H.S. Irwin & Barneby	SC, Orleans	E.Biondo, 194 / a,b
	SC, Ilhota	E.Biondo, 196 / a
S. rugosa (G.Don) H.S. Irwin & Barneby	PR, Jaguariaíva	E.Biondo, 266 / c
	PR, Jaguariaíva	E.Biondo, 438 / c
	PR, Jaguariaíva	E.Biondo, 442 / a
S. splendida (Vogel) H. S. Irwin & Barneby	PR,Imbituva	E.Biondo, 252 / a,c
	PR, Prudentópolis	E.Biondo, 254 / c
Series Basiglandulosae		
S. cernua (Balb.) H.S. Irwin & Barneby	PR, Ponta Grossa	E.Biondo, 256 / b,c
	PR, Ponta Grossa	E.Biondo, 255 / a,b,c
	PR, Jaguariaíva	E.Biondo, 264 / b,c
	PR, Castro	E.Biondo, 433 / b,c
	PR, Castro	E.Biondo, 434 / b,c
	PR, Jaguariaíva	E.Biondo, 437 / b,c
S. hirsuta (L.) H. S. Irwin & Barneby	RS, São Borja	E.Biondo, 229 / c
	RS, Getúlio Vargas	E.Biondo, 245 / a,b,c
	RS, Passo Fundo	E.Biondo, 242 / c
	RS, Sertão	E.Biondo, 244 / a,c
	RS, Getúlio Vargas	E.Biondo, 246 / a,c
	PR, Paz	E.Biondo, 317 / b,c
	PR, Marióplis	E.Biondo, 319 / a,b,c
	PR, Juranda	E.Biondo, 304 / a,b,c
	SC, Seara	E.Biondo, 284 / a,b,c
	RS, São Miguel das Missões	E.Biondo, 357 / c
	RS, Entre Ijuís	E.Biondo, 347 / c
	RS, Ibirubá	E.Biondo, 332 / a,b,c

Species	Place of collection	Accessions/type of analyses
	RS, Entre Ijuís	E.Biondo, 244 / a,b,c
	RS, São Miguel das Missões	E.Biondo, 408 / c
S. neglecta (Vogel) H. S. Irwin & Barneby	RS, Bom Jesus	S.T.S. Miotto, 1933 / b,c
	RS, Bom Jesus	S.T.S. Miotto, 1941 / a,b,c
	RS, Bom Jesus	S.T.S. Miotto, 1940 / a,b,c
	RS, Jaquirana	E.Biondo, 176 / b,c
	SC, Luzerna	R.L.C.Bortoluzzi, 870 / b,c
	SC, Lages	R.L.C.Bortoluzzi, 853 / c
	RS, Galópolis	S.T.S. Miotto, 2042 / a,c
	SC, Monte Castelo	R.L.C.Bortoluzzi & S.T.S.Miotto, 887 / b,c
	SC, Brunópolis	S.T.S. Miotto, 2047 / a,b,c
	SC, Lontras	R.L.C.Bortoluzzi & S.T.S. Miotto,1027 / b,c
	RS, Jaguari	S.T.S. Miotto, 2039 / a,b,c
	RS, Cachoeira do Sul	S.T.S. Miotto, 1992 / a,b,c
	RS, Mariante	S.T.S. Miotto, 1991 / a,c
	RS, São Borja	S.T.S. Miotto, 2025 / a,b,c
	SC, São Bento do Sul	R.L.C.Bortoluzzi & A.Reis, 1115 / b,c
	SC, Campo Alegre	R.L.C.Bortoluzzi & A.Reis, 1114 / a,c
	SC, Joinville	R.L.C.Bortoluzzi *et al.*, 1071 / a,c
	SC, Joinville	R.L.C.Bortoluzzi *et al.*, 1070 / a,b,c
	PR, Ponta Grossa	E.Biondo, 257 / a,c
	RS, Pouso Novo	E.Biondo, 328 / c
	SC, Jaraguá do Sul	E.Biondo, 428 / b,c
S. occidentalis (L.) Link.**	RS, Espumoso	S.T.S. Miotto, 1882 / c
	RS, Marques de Souza	E.Biondo, 239 / c
	SC, Imbituba	E.Biondo, 277 / a,b,c
	RS, Marques de Souza	E.Biondo, 240 / b,c
	PR, Xambrê	E.Biondo, 305 / a,b,c
	PR, Realeza	R.L.C.Bortoluzzi & E.Biondo, 1230 / a,b,c
	SC, Chapecó	E.Biondo, 287 / a,c
	PR, Fênix	E.Biondo, 314 / a,b,c
	SC, Seara	E.Biondo, 285 / b,c
	PR, Capanema	E.Biondo, 292 / a,b,c
	PR, Toledo	* / c
	RS, Montenegro	E.Biondo, 322 / a,b,c
	RS, Ibirubá	E.Biondo, 335 / b,c
	RS, Tabaí	E.Biondo, 321 / c
Series Coluteoideae		
S. araucarietorum H. S. Irwin & Barneby	SC, Catanduvas	R.L.C.Bortoluzzi & S.T.S. Miotto, 868 / b,c
	SC, Catanduvas	S.T.S. Miotto, 2055 / b,c
	SC, Monte Castelo	R.L.C.Bortoluzzi & S.T.S.Miotto, 885 / a,b,c
	SC, Criciúma	R.L.C.Bortoluzzi, 1138 / a,b,c
	SC, Criciúma	R.L.C.Bortoluzzi, 1139 / a,b,c
	SC, Criciúma	R.L.C.Bortoluzzi, 1141 / c
	SC, Criciúma	R.L.C.Bortoluzzi, 1140 / a,b,c
	PR, União da Vitória	R.L.C.Bortoluzzi & E.Biondo, 1153 / c
	PR, Campina Grande do Sul	E.Biondo, 271 / a,b,c
	SC, Monte Castelo	R.L.C.Bortoluzzi & R.A.Camargo, 1295 / a, b
S. corymbosa (Lam.) H. S. Irwin & Barneby	RS, Tabaí	E.Biondo, 237 / a,c
	RS, Tabaí	E.Biondo, 238 / a,b,c
	RS, Santo Antônio das Missões	E.Biondo, 362 / b,c
	RS, São Sepé	E.Biondo, 382 / a,b,c
	RS, General Câmara	E.Biondo, 411 / b,c
S. hilariana (Benth.) H. S. Irwin & Barneby	RS, Entre Ijuís	E.Biondo, 351 / a,b,c
	RS, São Luís Gonzaga	E.Biondo, 403 / a,b,c
	RS, São Luís Gonzaga	E.Biondo, 400 / a,b,c

Species	Place of collection	Accessions/type of analyses
S. oblongifolia (Vogel) H. S. Irwin &Barneby	RS, Jaquirana	S.T.S.Miotto, 1943 / c
	RS, Bom Jesus	S.T.S.Miotto, 1933 / a,c
	RS, Bom Jesus	S.T.S.Miotto, 1934 / c
	RS, Cambará do Sul	E.Biondo, 177 / c
	SC, Capão Alto	R.L.C.Bortoluzzi & S.T.S.Miotto, 852 / b,c
	RS, São Marcos	* / a,b,c
S. pendula (Humb. & Bonpl. ex. Willd.) H. S. Irwin & Barneby	RS, Torres	R.L.C.Bortoluzzi & E.Biondo, 1040 / c
	PR, Paulo Frontim	E.Biondo, 247 / a,c
	PR, Doutor Ulisses	E.Biondo, 269 / a,b,c
	PR, Irati	E.Biondo, 249 / b,c
	PR, Prudentópolis	E.Biondo, 253 / a,b,c
	PR, Turvo	E.Biondo, 316 / a,b,c
	PR, Nova Esperança	E.Biondo, 308 / c
	PR, Pato Branco	E.Biondo, 318 / a,b,c
	RS, São Lourenço do Sul	E.Biondo, 288 / b,c
	RS, Erechim	E.Biondo, 282 / c
	SC, Concórdia	E.Biondo, 283 / b,c
	RS, Entre Ijuís	E.Biondo, 342 / b,c
	RS, Ijuí	* / c
	RS, Boqueirão do Butiá	E.Biondo, 331 / c
	RS, Marques de Souza	* / c
	RS, Lajeado	* / a,b,c
	RS, Entre Ijuís	E.Biondo, 348 / a,b,c
S. septemtrionalis (Viviani) H. S. Irwin & Barneby	SC, São Bento do Sul	R.L.C.Bortoluzzi & A.Reis, 1120 / a,b,c
	SC, São Bento do Sul	R.L.C.Bortoluzzi & A.Reis, 1117 / a
	SC, São Bento do Sul	R.L.C.Bortoluzzi & A.Reis, 1118 / a,b,c
	SC, São Bento do Sul	R.L.C.Bortoluzzi & A.Reis, 1119 / c
	SC, Concórdia	R.L.C.Bortoluzzi & E.Biondo, 1151/ b,c
	SC, São Bento do Sul	E.Biondo, 427/c
	SC, São Bento do Sul	E.Biondo, 428 /a,b,c
Series Trigonelloideae		
S. obtusifolia (L.) H. S. Irwin & Barneby**	RS, São Borja	E.Biondo, 228 / c
	PR, Toledo	E.Biondo, 298 / a,b,c
	RS, Ibirubá	E.Biondo, 333 / a,b,c
	RS, São Luís Gonzaga	E.Biondo, 359 / c
	RS, São José do Herval	E.Biondo, 330 / a,b,c
S. pilifera (Vogel) H. S. Irwin & Barneby	RS, São Borja	S.T.S. Miotto, 2029 / a
	RS, São Miguel das Missões	E.Biondo, 356 / c
	RS, São Luís Gonzaga	E.Biondo, 401 / c
	RS, São Lourenço das Missões	E.Biondo, 407 / a,c
	RS, Boa Vista do Cadeado	E.Biondo, 409 / c
	RS, São Luís Gonzaga	E.Biondo, 399 / a,b,c
SECTION *PEIRANISIA*		
Series Interglandulosae		
S. multijuga (L.C.Rich.) H. S. Irwin & Barneby	SC, Joaçaba	S.T.S. Miotto, 2054 / b
	SC, Trombudo	S.T.S. Miotto, 2064 / a
	SC, Orleans	S.T.S. Miotto, 2069 / b
	SC, São José	R.L.C. Bortoluzzi *et al.*, 911 / a,b
	SC, Ermo	R.L.C. Bortoluzzi & E. Biondo, 1038 /c
	SC, Jacinto Machado	E.Biondo, 218 / a,b
	SC, Ermo	R.L.C. Bortoluzzi & E. Biondo, 1038 / b
	SC, Corupá	R.L.C. Bortoluzzi & A. Reis, 1131 / a
	SC, Joinville	R.L.C. Bortoluzzi *et al.*, 1083 / a
	SC, Garuva	R.L.C. Bortoluzzi *et al.*, 1086 / a
	SC, Joinville	R.L.C. Bortoluzzi *et al.*, 1052 / a,b
	SC, Ilhota	E.Biondo, 273 / a,b
	SC, Joinville	R.L.C. Bortoluzzi & R.A.Camargo, 1290 /c

* no voucher; ** sub-spontaneous species
a – meiotic behaviour; b – meiotic index; c – pollen viability
PR – Paraná State; RS – Rio Grande do Sul State; SC-Santa Catarina State

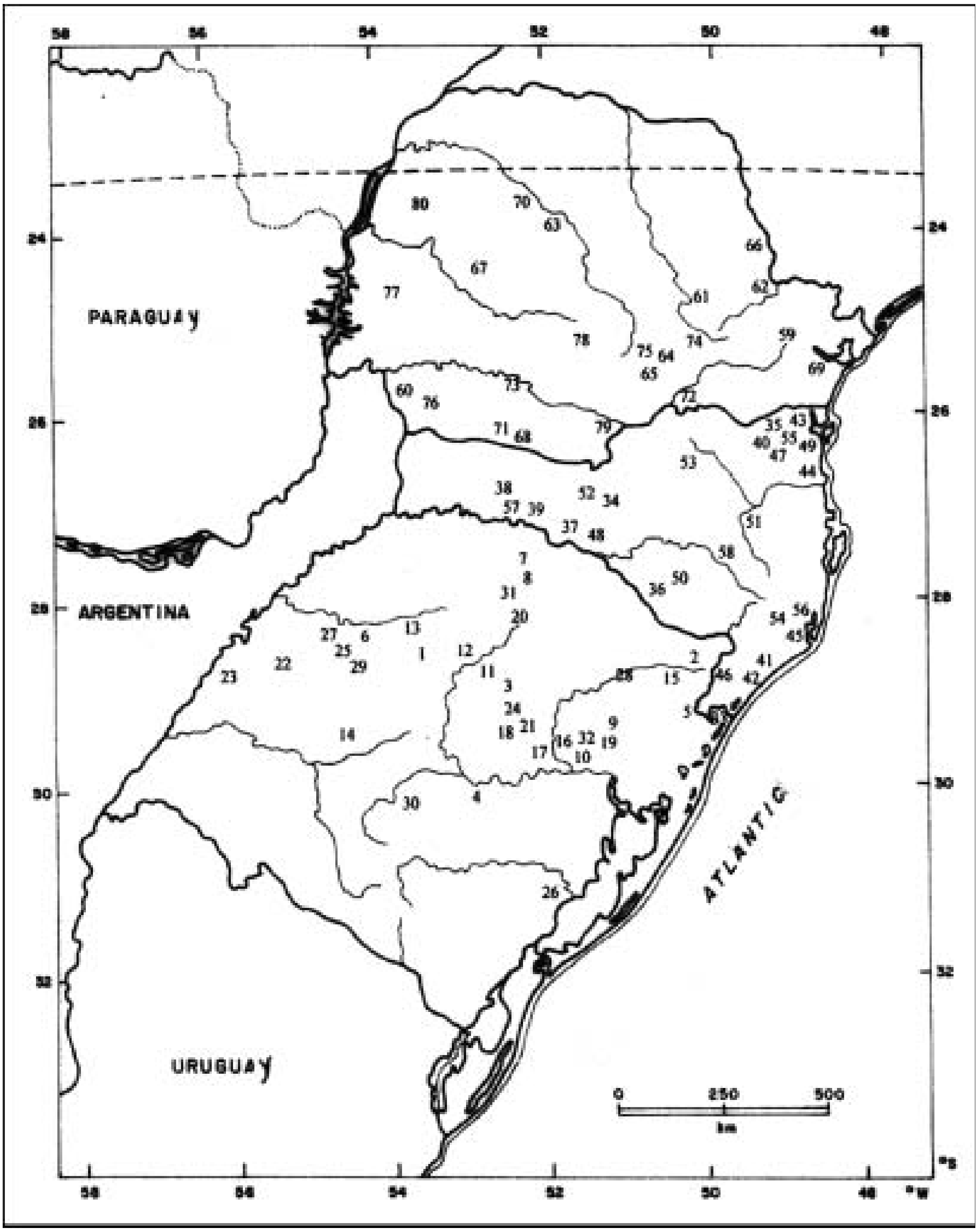

Figure 1 — Map of the places of collection listed in Table 1 - **Rio Grande do Sul:** 1. Boa Vista do Cadeado; 2. Bom Jesus; 3. Boqueirão do Butiá; 4. Cachoeira do Sul; 5. Cambará do Sul; 6. Entre Ijuís; 7. Erechim; 8. Espumoso; 9. Galópolis; 10. General Câmara; 11. Getúlio Vargas; 12. Ibirubá; 13. Ijuí; 14. Jaguari; 15. Jaquirana; 16. Lajeado; 17. Mariante; 18. Marques de Souza; 19. Montenegro; 20. Passo Fundo; 21. Pouso Novo; 22. Santo Antônio das Missões; 23. São Borja; 24. São José do Herval; 25. SãoLourenço das Missões; 26. São Lourenço do Sul; 27. São Luís Gonzaga; 28. São Marcos; 29. São Miguel das Missões; 30. São Sepé; 31. Sertão; 32. Tabaí; 33. Torres. **Santa Catarina:** 34. Brunópolis; 35. Campo Alegre; 36. Capão Alto; 37. Catanduvas; 38. Chapecó; 39. Concórdia; 40. Corupá; 41. Criciúma; 42. Ermo; 43. Garuva; 44. Ilhota; 45. Imbituba; 46. Jacinto Machado; 47. Jaraguá do Sul; 48. Joaçaba; 49. Joinville; 50. Lages; 51. Lontras; 52. Luzerna; 53. Monte Castelo; 54. Orleans; 55. São Bento do Sul; 56. São José; 57. Seara; 58. Trombudo. **Paraná:** 59. Campina Grande do Sul; 60. Capanema; 61. Castro; 62. Doutor Ulisses; 63. Fênix; 64. Imbituva; 65. Irati; 66. Jaguariaíva; 67. Juranda; 68. Marióplis; 69. Morretes; 70. Nova Esperança; 71. Pato Branco; 72. Paulo Frontin; 73. Paz; 74. Ponta Grossa; 75. Prudentópolis; 76. Realeza; 77. Toledo; 78. Turvo; 79. União da Vitória; 80. Xambrê.

Slides were prepared by squashing the anthers in 2% propionic carmine. All available meiotic phases were analysed. Meiotic indexes were calculated following Löve (1949), from 500 pollen tetrads per plant. Pollen viability was estimated by the staining reaction in 1500 mature pollen grains from three different slide per each plant. Pollen grain size was determined by measuring the large (P) and small axis (E) of 20 grains per plant, with the aid of a micrometer eyepiece at 400 x magnification. The grains were classified according to the P/E ratio, following Saenz (1978).

Data was recorded with the aid of a digital imaging capture system and photomicrographs.

RESULTS AND DISCUSSION

All the accessions of the 17 *Senna* species analysed are diploid, with n = 11, 12, 13 and 14 chromosomes, except the *S. rugosa* accession that had n=28 chromosomes, therefore considered as tetraploid (Table 2, Figures 2 a, b, c, d, e and f). The haploid chromosome number of n=14 was found in ten of the 17 species, n=13 in four taxa, n=12 and n=11 in one species each, suggesting a disploid series. No intraspecific variability for chromosome number was verified. Interspecific variation in chromosome number for *Senna* species have been reported in the literature (Irwin and Turner 1960; Bandel 1974; Coleman and Demenezes 1980; Goldblatt 1981; Biondo *et al.* 2002; Souza and Benko-Iseppon 2004). Regarding *S. rugosa*, the only chromosome count known for the species (Coleman and Demenezes 1980) is n=14.

In all the ten species analysed of series Basiglandulosae and Coluteoideae (section Chamaefistula, Table 1), which have some distinct morphological characters such as glands bewteen pulvinoles pairs in Coluteoideae, and glands within the pecioles near the leaflets in Basiglandulosae (Irwin and Barneby 1982), n = 14 is the predominant number. For the species of series Baccilares n = 13 was found in *S. angulata*, *S. macranthera* and *S. splendida* (Figure 2 e), and n=28 in the poly-

Table 2 — Haploid chromosome number (n), meiotic behaviour, meiotic indexes and pollen fertility in 17 *Senna* species occurring in Southern Brazil

Species	n	Meiosis I: Chromosome associations (diakinesis and metaphase I)[1]	Meiosis II: Chromosome disjunction (anaphase I)	Meiosis II: Chromosome disjunction (anaphase II)	Meiotic index (%)
S. angulata	13 [1]	13II (29)	Regular (7)	-	100.00 [1]
S. macranthera	13 [2]	13II (51)	Regular (8)	Regular (4)	99.02 [1]
S. rugosa	28 [1]	28II (11); 24II + 2IV (2) 22II + 2IV + 4I (3); 22II + M (6)	Regular (1)	-	-
S. splendida	13 [1]	13II (48); 11II + 1IV (1)	Regular (8) a	Regular (5)	-
S. cernua	14 [1]	14II (13)	-	Regular (10)	99.93 [6]
S. hirsuta	14 [8]	14II (169); 12II + 1IV (1)	Regular (6)	Regular (33)	99.53 [7]
S. neglecta	14 [12]	14II (345)	Regular (14)	Regular (37)	98.41 [14]
S. occidentalis	14 [7]	14II (80)	Regular (26) b	Regular (60) d	95.35 [9]
S. araucarietorum	14 [6]	14II (74)	Regular (8)	Regular (32)	99.80 [8]
S. corymbosa	14 [3]	14II (76); 13II +2I (1); 12II + 1IV (4); 11II + 2III (2); 10II + 2IV (3); 9II + 2IV + 2I (1); 8II + M (2); 6II + M (1)	Regular (3)	-	99.85 [4]
S. hilariana	14 [3]	14II (82)	Regular (2)	Regular (18)	99,73 [3]
S. oblongifolia	14 [2]	14II (25)	-	-	100 [2]
S. pendula	14 [7]	14II (141)	Regular (8)	Regular (25)	100 [10]
S. septemtrionalis	14 [4]	14II (142)	Regular (17)	Regular (19)	99,90 [4]
S. obtusifolia	13 [3]	13II (97)	Regular (5)	Regular (35)	99,94 [3]
S. pilifera	11 [3]	11II (67)	Regular (11)	Regular (16)	99,41 [1]
S. multijuga	12 [8]	12II (108); 10II + A (2); 10II + 1IV (7)	Regular (14) c	Regular (82)	99,89 [7]

[ ] number of accessions analysed; () number of cells analysed; [1] I – univalents, II – bivalents, III – trivalents, IV – quadrivalents, M – multiple associations, A – adherences;
a – one cell with 13/12 and one laggard; b – one cell with 19/9 segregation; c – two cells with bridges; d – one cell with 13/15 segregation

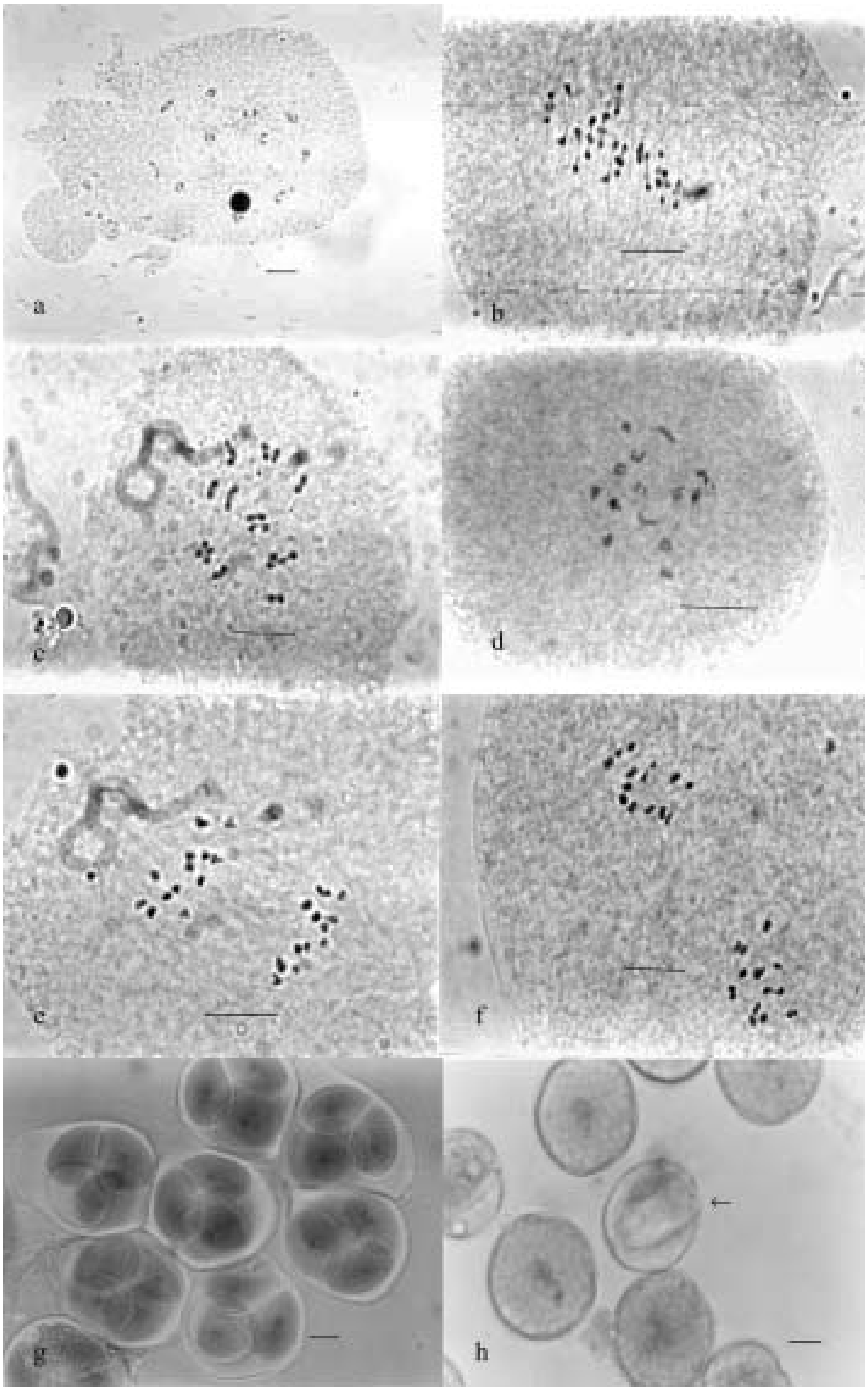

Figure 2 — (a) diakinesis in *Senna pilifera* (n = 11); (b) metaphase I in *S. macranthera* (n = 13); (c) metaphase I in *S. septemtrionalis* (n = 14); (d) metaphase I, polar view, in *S. hirsuta* (n = 14); (e) anaphase I in *S. angulata* (n = 13); (f) metaphase II in *S. occidentalis* (n = 14); (g) normal pollen tetrads in *S. occidentalis*; (h) viable and inviable (arrow) pollen grains in *S. pendula* (n = 14). Scale bar = 10 μm.

ploid *S. rugosa* (n = 28). The two species analysed of series Trigonella presented different chromosome numbers, n=13 in *S. obtusifolia* and n = 11 in *S. pilifera* (Figure 2 a; Table 1 and 2), the last one probably representing the end of the disploid series among the species studied. Series Baccilares and Trigonella are morphologically separated by the number of leaflets per leaf. The only one species of series Interglandulosae examined, *S. multijuga*, had n = 12.

Our data support what has been previously suggested by other authors (Irwin and Turner 1960; Goldblatt 1981; Souza and Benko-Iseppon 2004), that x=14 is the basic number, probably a derived basic number, for *Senna*, and that the other numbers would reflect disploidy as an evolutionary tendence in the group. However, it should be considered that only ca. 10% of the 260 *Senna* species have been analysed. Therefore, a wider survey of chromosome number in the genus should be undertaken in order to allow more comprehensive conclusions about chromosome evolutionary trends.

Meiotic behaviour was analysed in 72 accessions (Table 2), and meiotic indexes and pollen fertility in 80 and 118, respectively (Table 3). Meiotic behaviour was generally regular with predominance of bivalent pairing in diakinesis and metaphase I, and normal chromosome segregation at anaphases I and II (Table 2, Figure 2 a - f).

Some irregularities, such as quadrivalens (Figure 3 l), multivalents (Figure 3 j) and univalents (Figure 3 k) at diakinesis and metaphase I, and bridges and unequal segregation (Figure 3 m) at anaphases were observed in some accessions of some species, such as in *S. splendida* (Figure 3 k), *S. multijuga*, *S. corymbosa* and *S. occidentalis* (Figure 3 m). Secondary associations of bivalents were also verified (Figure 3 i). Quadrivalents and other multivalents were found in ca. 50% of the examined cells of the tetraploid *S. rugosa* (Table 2).

Meiotic indexes were high, ranging from 95.35% to 100% among species (Table 2, Figure 2 g), indicating, according to Löve (1949), that the *Senna* species and accessions analysed may be considered as meiotically stable. Few abnormalities were seen at this stage (Figure 3 n - p). Pollen viability averages ranged from 81.82% to 97.73% among species (Table 3, Figure 2 h), but in some cases there was a wide variation in pollen fertility within species, such as in *S. occidentalis*, *S. corymbosa*, *S. hilariana* and *S. oblongifolia* (Table 3). *S. corymbosa* was the species in which most accessions showed irregularities at diakinesis and metaphase I (Table 2), even taking into account the small number of cells examined for most of them, and the species in which pollen fertility ranged from 47.02 to 97.60% (Table 3). However, generally, no clear relations between meiotic behavior, meiotic indexes and low pollen fertility could be established.

Comparing our data and that of Coleman and Demenezes (1980), for *S. rugosa*, it can be seen that diploid and polyploid races occurr in this taxon, a fact already described for many other species such as *Caesalpinia ferrea* Mart. ex Tul. (Beltrao and Guerra 1990), *Adesmia incana* (Tedesco *et al.* 2002) and *Leucaena trichandra* (Schifino-Wittmann *et al.* 2000), among many others. The extent and importance of polyploidy

Table 3 — Pollen fertility and pollen grain size in 15 *Senna* species occurring in Southern Brazil

Species	No of acessions analysed	Viability (range of variation) %	Grain size - mean values (μm)		P/E
			Larger axis (P)	Smaller axis (E)	
S. neglecta	21	95.88 (69.40 - 99.93)	50.24	46.36	1.08
S. hirsuta	14	89,.3 (74.46 - 99.86)	47.80	45.07	1.06
S. occidentalis	14	89.99 (17.47 - 99.60)	52.43	49.22	1.06
S. cernua	6	97.73 (96.40 - 98.60)	52.20	48.04	1.08
S. septemtrionalis	6	94.77 (82.73 - 98.93)	39.27	36.53	107
S. araucarietorum	9	95.32 (79.86 - 98.93)	43.30	40.66	1.06
S. pendula	17	96.82 (94.73 - 99.73)	41.32	38.72	1.06
S. corymbosa	5	84.60 (47.02 - 97.60)	37.89	35.36	1.07
S. hilariana	3	81.82 (50.13 - 98.33)	37.07	33.93	1.09
S. oblongifolia	6	85.01 (58.86 - 97.46)	36.06	32.91	1.09
S. splendida	2	93.63 (88.46 - 98.80)	42.78	38.94	1.09
S. rugosa	2	98.23 (98.00 - 98.46)	49.16	46.49	1.05
S. pilifera	5	94.75 (92.06 - 96.53)	45.00	42.26	1.06
S. obtusifolia	5	95.01 (92.06 - 98.40)	46.77	43.74	1.06
S. multijuga	3	94.79 (88.93 - 98.20)	37.43	34.75	1.07

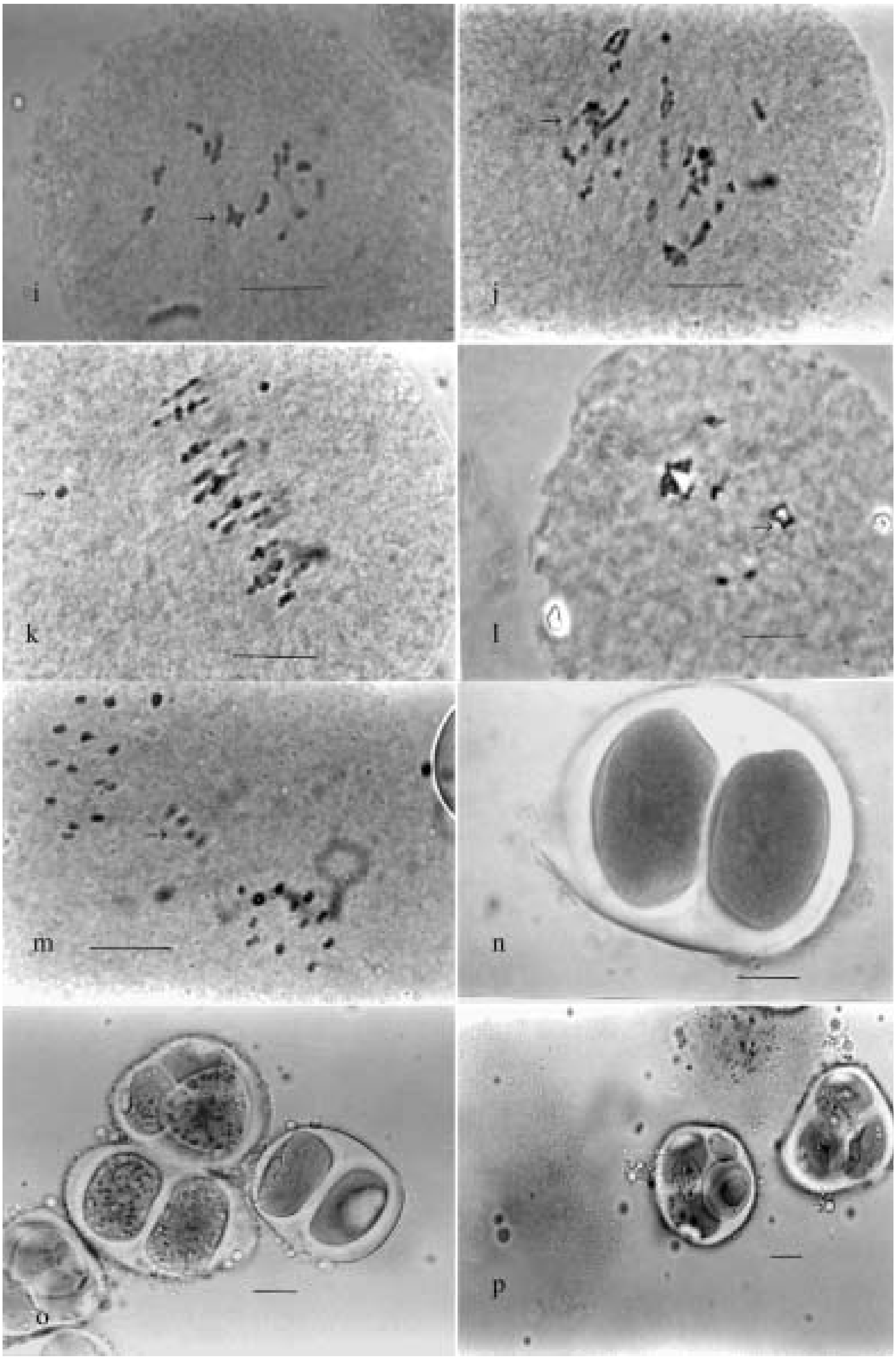

Figure 3 — (i) metaphase I with secondary association of bivalents (arrow) in *S. corymbosa* (n = 14); (j) metaphase I with multivalent (arrow) and in (k) metaphase I with univalent (arrow) in *S. rugosa* (n = 28); (l) metaphase I with quadrivalent (arrow) in *S. splendida* (n=13); (m) irregular segregation at anaphase II in *S. occidentalis* (n = 14); (n) dyad in *Senna neglecta* (n = 14); (o) dyad and (p) unequal cell sized tetrad (arrow) in *S. occidentalis*. Scale bar = 10 μm.

in plant evolution has been thoroughly discussed in the literature (STEBBINS 1971; HARLAN and DE WET 1975; LEICHT and BENNETT 1997; SOLTIS and SOLTIS 1999; RAMSEY and SCHEMSKE 1998; 2002, among many others). The rather consistent formation of quadrivalents in the accession of *S. rugosa* we analysed would support an autopolyploid origin, as would be expected considering intraspecific polyploidy. However, due to the limited available cytogenetic data on this species, and to the different point of views on how chromosome pairing reflects the type of polyploidy (STEBBINS 1971; GUERRA 1988; RAMSEY and SCHEMSKE 2002) any more direct conclusion is premature.

Interesting observations were made at the pollen tetrad stage in one accession each of *S. neglecta* and *S. occidentalis*, which formed, respectively 17.31% and 19.78% of dyads (Figure 3n). Dyads are one of the cytological indications of unreduced gametes production. This fact, together with the verification of bigger pollen grains (P =59.44 μm and E=56,07 μm) in this specific *S. neglecta* accession when compared to the average for the species (Table 3), supports the conclusion that these two plants are unreduced gametes producers. Unreduced gametes are accepetd to be the commonest way of polyploid formation in nature (HARLAN and DE WET 1975; RAMSEY and SCHEMSKE 1998).

There are many references of detailed meiotic behaviour analysis of natural legume species populations, most displaying generally regular behaviour and high meiotic indexes and pollen fertility, such as *Lathyrus* L. and *Vicia* L. (SCHIFINO-WITTMANN *et al*. 1994), *Adesmia* DC. (TEDESCO *et al*. 2002), *Caesalpinia* L. (BELTRAO and GUERRA 1990), *Macroptilium* (Benth.) Urban. (GARCIA *et al*. 2000), *Dalsthedtia* Malme (TEIXEIRA *et al*. 2002), among others. On the other hand, only two references on meiosis in *Senna* species have been found. IRWIN and TURNER (1960), still using the *Cassia* (*s.l.*) classification, simply reported regular chromosome pairing in some American species. The same kind of comment was made for some Nigerian Caesalpinioideae species by GILL and HUSAINI (1982), who also reported a 95% pollen fertility for the two *Cassia* (*s.l.*) species they analysed. No detailed meiotic analysis were found for these species.

All the 17 *Senna* species studied by us had pollen grains of the prolate/spheirodal type, P/E ranging from 1,00 t 1,14 according to SAENZ (1978) (Table 3, Figure 2 h). *S. occidentalis* presented the biggest pollen grains (P=52.43 μm and E=49.22 μm) and *S. oblongifolia* the smallest ones (P=36.03 μm and E=32.91 μm). MIRANDA and ANDRADE (1984) analysed 35 *Cassia* (*s.l.*) species and verified predominance of prolate/spheirodal and sub-prolate types of pollen grains and a range of pollen grain size from 25 to 50 μm.

Pollen grain size may be a good taxonomic character (DAJOZ *et al*. 1995), but not in the present case, as no relation between taxonomic section and pollen sizes, nor between chromosome number and pollen grain size could be established.

Concluding, in order to draw more general conclusions on the cytogenetic patterns of *Senna* species, a broader survey of a more representative number of species along the geographic distribution of the genus has to be undertaken. Issues that should be especially addressed are intra and interspecific variability in chromosome number, extent of polyploidy and the role of disploidy in *Senna* evolution.

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