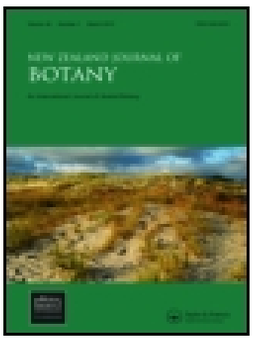

ISSN: 0028-825X (Print) 1175-8643 (Online) Journal homepage: https://www.tandfonline.com/loi/tnzb20

The karyotype of *Sophora tetraptera* (Fabaceae)

Laura B. Stiefkens , Gabriel Bernardello & Gregory J. Anderson

To cite this article: Laura B. Stiefkens , Gabriel Bernardello & Gregory J. Anderson (2003) The karyotype of *Sophora tetraptera* (Fabaceae), , 41:4, 731-735, DOI: 10.1080/0028825X.2003.9512885

To link to this article: https://doi.org/10.1080/0028825X.2003.9512885

New Zealand Journal of Botany, 2003, Vol. 41: 731–735
0028–825X/03/4104–0731 $7.00

Short communication

The karyotype of *Sophora tetraptera* (Fabaceae)

LAURA B. STIEFKENS
GABRIEL BERNARDELLO
Instituto Multidisciplinario de Biología Vegetal (CONICET-UNC)
C.C. 495
5000 Córdoba, Argentina
GREGORY J. ANDERSON
The University of Connecticut
Department of Ecology and Evolutionary Biology
Storrs, CT 06269-3043, USA

Abstract The karyotype of *Sophora tetraptera* (Fabaceae (Leguminosae), subfam. Papilionoideae) is reported for the first time. The chromosome number, $2n = 18$, coincides with former reports and is the most common number for the genus. The chromosomes are small (average length 1.65 μm). The karyotype is composed of 8 m (metacentric) + 1 sm (submetacentric) pairs. Microsatellites were observed on the short arm of the only sm pair. The intrachromosomal and interchromosomal asymmetry indices were $A_1 = 0.16$ and $A_2 = 0.12$, respectively. The nine species of *Sophora* analysed karyotypically so far in the literature suggest that karyotypic features are useful. The diversification of the group apparently has been accompanied by some chromosomal rearrangements yielding differences in karyotype formula and secondary constrictions. Thus, karyotypic data in *Sophora* are useful for elucidating its taxonomy and evolution.

Keywords chromosome numbers; karyotype; *Sophora tetraptera*; Fabaceae (Leguminosae); New Zealand flora

B02090; Online publication date 26 November 2003
Received 24 December 2002; accepted 20 June 2003

INTRODUCTION

Sophora (Fabaceae (Leguminosae), subfam. Papilionoideae) is included in Sophoreae, a tribe recently shown to be paraphyletic (Doyle et al. 2000). This genus is composed of 45–50 species of worldwide distribution with its centre of diversity in North America (Polhill 1981; Sousa S. & Rudd 1993; Peña et al. 2000). *Sophora* section *Edwardsia* (Salisb.) Taub. is considered monophyletic and well differentiated from other sections of the genus (Peña & Cassels 1996; Hurr et al. 1999; Peña et al. 2000). It includes 17 closely related, tree species of classical Antarctic-circumpolar distribution on islands across the Pacific, South Atlantic, and Indian Oceans and SW South America (Polhill 1981; Hurr et al. 1999; Peña et al. 2000).

In New Zealand, sect. *Edwardsia* is represented by eight species (Heenan et al. 2001). Among them, *S. tetraptera* J.S.Mill. (kowhai) is a tree up to 12 m tall with golden yellow flowers that inhabits the eastern North Island on terraces and hillslopes from East Cape (37°38′S) to 40°30′S (Allan 1961; Heenan et al. 2001).

About 30 species of *Sophora* have been counted worldwide with several chromosome numbers reported (e.g., Federov 1974; Goldblatt 1981a,b,c, 1984, 1988; Goldblatt & Johnson 1990, 1991, 1994, 1996, 1998). In New Zealand, all eight species have been examined for chromosome numbers (Atchison 1949; Rattenbury 1957; Hair & Beuzenberg 1966; de Lange & Murray 2002), including *S. tetraptera* ($2n = 18$; Atchinson 1949; Rattenbury 1957; Hair & Beuzenberg 1966; Yeh et al. 1986) but no detailed karyotype data are available. The karyotypes of only nine *Sophora* species worldwide have been critically analysed so far (Kawakami 1930; Hsu & Huang 1985; Bernal Gonzalez & Martínez Almeraya 1989; Kodama 1989; Kumari & Bir 1990; Palomino et al. 1993; Tian et al. 1993; Stiefkens et al. 2001), a small number considering the size of the genus and the significance of karyotypic data in understanding the diversification of flowering plants (Stebbins 1971). In this paper, we provide data on the somatic

chromosome number and the karyotype of *S. tetraptera* to determine the importance of chromosomal changes in the evolution of the group.

MATERIALS AND METHODS

Seeds were collected from a tree in Frasertown, Hawke's Bay (38°58′S, 177°24′E). A voucher specimen of the parent tree is deposited in CORD (Museo Botánico de Córdoba, Argentina).

Mitotic chromosomes in the somatic cells of root-tips were analysed from squashes of primary roots from germinating seeds. Seeds were soaked in tap water for 24 hours. They were then put in Petri dishes lined with filter paper moistened with gibberellic acid (GA3, 1000 ppm) and were regularly watered with the same solution. Petri dishes containing seeds were kept in the dark at 30°C. Root-tips were collected when the primary roots were 2–10 mm long and were pretreated at room temperature for 2 h in a saturated solution of p-dichlorobenzene in water. Root-tips were rinsed in distilled water and were fixed in freshly made ethanol:glacial acetic acid (3:1) at room temperature for 24 h. Following fixation, they were hydrolysed with 5N HCl for 40 min at room temperature and put in Feulgen solution (basic fuchsin) for 2 h at room temperature in the dark. Root-tip meristem cells were isolated on a slide and squashed. Slides were made permanent in Euparal by removing the cover slips through freezing with carbon dioxide.

Cells selected for measurements were photographed with phase contrast optics and Kodak T-Max film. Ten selected cells, each from a different individual, were photographed and the photographs were used to take measurements of the following for each chromosome pair: s (short arm), l (long arm), and c (total chromosome length). The centromeric index (I = 100s/c) and the arm ratio (r = l/s) were then calculated and used to classify the chromosomes, and determine homologues, as recognised by Levan et al. (1964). Karyograms were constructed by organising the chromosomes into groups according to their arm ratio (from m to sm), and ordering them by decreasing length within each category. The resulting idiogram was based on the mean values obtained. Karyotype asymmetry was estimated using the indices of Romero Zarco (1986) and Stebbins' (1971) classification.

RESULTS

The observation of 30 cells, from 10 individuals, confirmed that the somatic chromosome number of *S. tetraptera* is $2n = 18$ (Fig. 1). The chromosomes are small (Table 1) and quite homogeneous in size, ranging from 1.3 to 2 μm, with an average chromosome length of 1.65 μm. The total haploid chromosome length of the karyotype based on the mean chromosome length was 14.85 μm. The karyotype is symmetrical with 8 m (metacentric) + 1 sm (submetacentric) chromosome pairs (Fig. 2). The intrachromosomal and interchromosomal asymmetry indices were $A_1 = 0.16$ and $A_2 = 0.12$, respectively. According to Stebbins (1971) classification, the karyotype falls in the "1A" category. Small satellites were frequently observed on both of the short arms of the only submetacentric pair (Fig. 1).

Table 1 Measurements in μm (mean ± standard deviation) and chromosomic indices (r, arm ratio; i, centromeric index) of somatic chromosomes of *Sophora tetraptera*. Abbreviations after Levan et al. (1964): s, short arm; l, long arm; c, total chromosome length; r, arm ratio; i, centromeric index; m, metacentric; sm, submetacentric; *, indicates a microsatellite on the short arm.

Pair	s	l	c	r	i	Chromosome type
1	0.95 ± 0.14	1.05 ± 0.07	2.00 ± 0.20	1.13	46.85	m
2	0.89 ± 0.12	0.92 ± 0.08	1.81 ± 0.21	1.04	48.96	m
3	0.86 ± 0.12	0.92 ± 0.10	1.78 ± 0.21	1.06	48.42	m
4	0.79 ± 0.10	0.90 ± 0.11	1.69 ± 0.19	1.14	46.66	m
5	0.73 ± 0.08	0.89 ± 0.13	1.62 ± 0.17	1.21	45.17	m
6	0.74 ± 0.07	0.81 ± 0.10	1.55 ± 0.15	1.09	47.79	m
7	0.66 ± 0.06	0.79 ± 0.09	1.45 ± 0.12	1.20	45.25	m
8	0.60 ± 0.07	0.70 ± 0.09	1.30 ± 0.15	1.18	45.89	m
9	0.54 ± 0.05	1.14 ± 0.15	1.68 ± 0.19	2.12	32.08	sm*

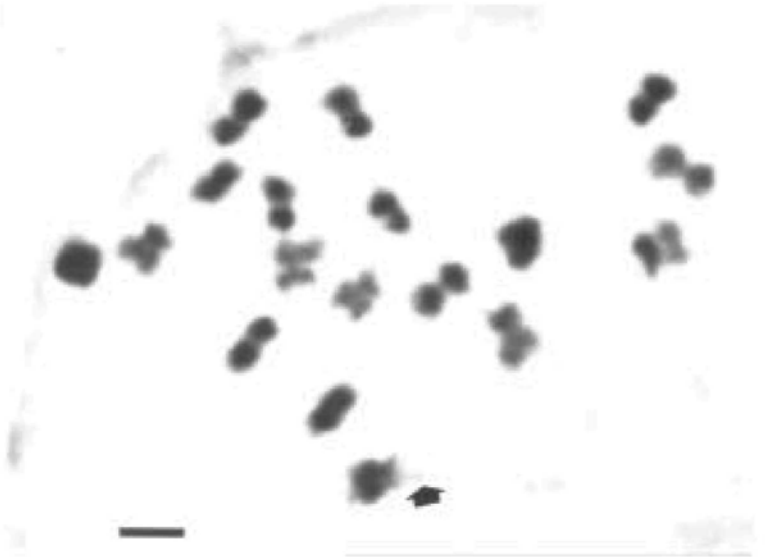

Fig. 1 Photomicrograph of a mitotic metaphase of *Sophora tetraptera*, $2n = 18$. Arrow points to a satellite. Scale bar = 2 μm.

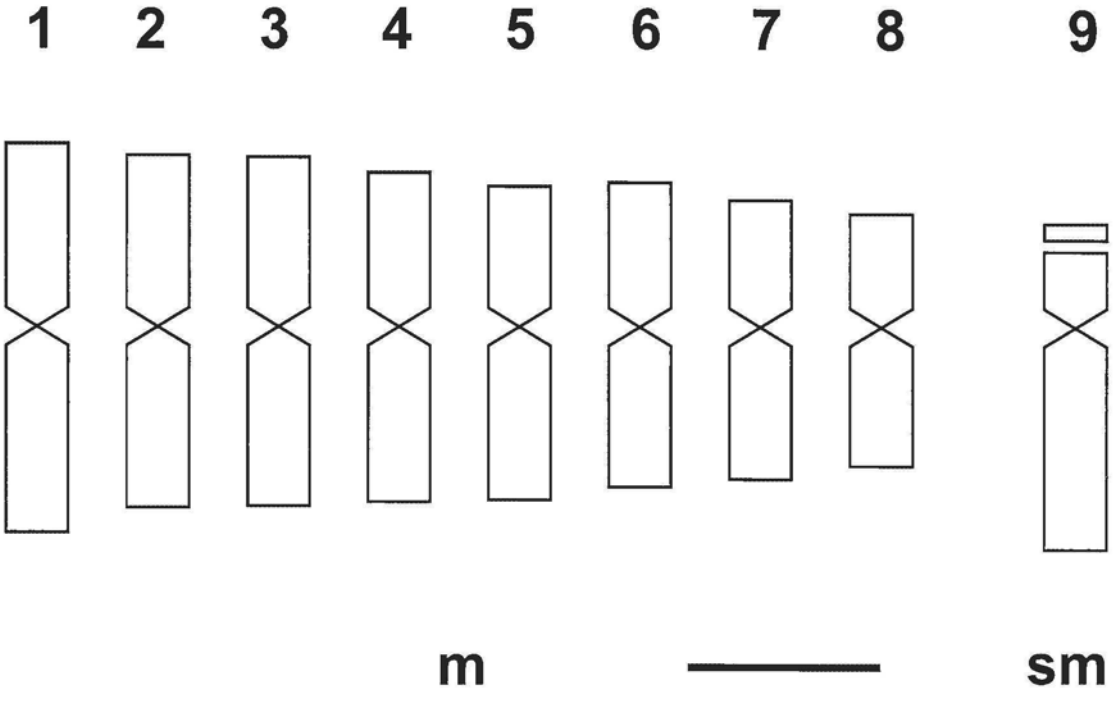

Fig. 2 Idiogram of *Sophora tetraptera* based on mean chromosome values (see Table 1). Scale bar = 1 μm.

DISCUSSION

The chromosome number of $2n = 18$ for *S. tetraptera* is diploid, and the most common number reported for *Sophora*. For this genus, several chromosome numbers have been reported: $n = 9$, 18, 11, and $2n =$ 18, 28, 36, 16, 32, 22, 54, in decreasing order of frequency in each case (e.g., Federov 1974; Goldblatt 1981a,b,c, 1984, 1988; Goldblatt & Johnson 1990, 1991, 1994, 1996, 1998). Goldblatt (1981a) suggested that tribe Sophoreae probably had a polyploid origin with the basic number $x = 14$, from whence a decreasing aneuploid series might have originated, resulting in $x = 8$, 9, and 11.

The karyotype of *S. tetraptera* is clearly symmetrical, a known trend in the family as a whole and in woody taxa from subfam. Papilionoideae in particular, where st chromosomes are rare and t chromosomes have never been detected (Bairiganjan & Patnaik 1989; Kumari & Bir 1990).

The nine species of *Sophora* karyotypically analysed so far are also mostly composed of m pairs with fewer sm pairs (Kawakami 1930; Hsu & Huang 1985; Bernal Gonzalez & Martínez Almeraya 1989; Kodama 1989; Kumari & Bir 1990; Palomino et al. 1993; Tian et al. 1993; Stiefkens et al. 2001). Karyotypic features seem to be taxonomically useful because data available indicate that there are differences in karyotype formula and in the presence and position of the secondary constrictions among them; for instance, *Sophora fernandeziana* (Phil.) Skottsb. was considered to be closely to *S. tetraptera* (Hemsley 1884; Johow 1896), but their karyotypes proved to be different (Stiefkens et al. 2001; this work): *S. fernandeziana* has two sm chromosome pairs and there are no satellited chromosomes. In addition, basic chromosome numbers were important in recognising the closely related genus *Styphnolobium* Schott (Palomino et al. 1993; Sousa S. & Rudd 1993). These data taken together suggest that the diversification of *Sophora* has been accompanied by chromosomal rearrangements and that cytological investigation is useful in elucidating its taxonomic and phylogenetic relationships.

ACKNOWLEDGMENTS

We thank the University of Connecticut Research Foundation and CONICET (Argentina) for support.

REFERENCES

Allan, H. H. 1961: Flora of New Zealand. Vol. I. Wellington, Government Printer.

Atchison, E. 1949: Studies in the Leguminosae. IV. Chromosome numbers and geographical relationships of Miscellaneous Leguminosae. *Journal of the Elisha Mitchell Scientific Society 65*: 118–122.

Bairiganjan, G. C.; Patnaik, S. N. 1989: Chromosomical evolution in Fabaceae. *Cytologia 54*: 51–64.

Bernal Gonzalez, M. C.; Martínez Almeraya, M. P. 1989: Determinación del cariótipo de algunas especies del género *Sophora* L. sens. lat. (Familia: Leguminosae). Unpublished MSc thesis, Universidad Nacional Autónoma de México, México.

de Lange, P. J.; Murray, B. G. 2002: Contributions to a chromosome atlas of the New Zealand flora—37. Miscellaneous families. *New Zealand Journal of Botany 40*: 1–23.

Doyle, J. J.; Chapill, J. A.; Bailey, C. D.; Kajita, T. 2000: Towards a comprehensive phylogeny of legumes: evidence from rbcL sequences and non-molecular data. *In*: Hendersen, P. S.; Bruneau, A. *ed.* Advances in legume systematics, Part 9. Kew, Royal Botanic Gardens. Pp. 1–20.

Federov, A. *ed.* 1974: Chromosome numbers of flowering plants. Koeningstein, Otto Koeltz Science Publishers.

Goldblatt, P. 1981a: Cytology and the phylogeny of Leguminosae. *In*: Polhill, R. M.; Raven, P. H. *ed.* Advances in legume systematics, Part 2. Kew, Royal Botanic Gardens. Pp. 427–463.

Goldblatt, P. 1981b: Index to plant chromosome numbers. 1975–1978. *Monographs in Systematic Botany, Missouri Botanical Garden 5*: 1–259.

Goldblatt, P. 1981c: Chromosome numbers in Legumes. II. *Annals of the Missouri Botanical Garden 68*: 551–557.

Goldblatt, P. 1984: Index to plant chromosome numbers. 1979–1981. *Monographs in Systematic Botany, Missouri Botanical Garden 8*: 1–194.

Goldblatt, P. 1988: Index to plant chromosome numbers. 1984–1985. *Monographs in Systematic Botany, Missouri Botanical Garden 23*: 1–122.

Goldblatt, P.; Johnson, D. E. 1990: Index to plant chromosome numbers. 1986–1987. *Monographs in Systematic Botany, Missouri Botanical Garden 30*: 1–243.

Goldblatt, P.; Johnson, D. E. 1991: Index to plant chromosome numbers. 1988–1989. *Monographs in Systematic Botany, Missouri Botanical Garden 40*: 1–238.

Goldblatt, P.; Johnson, D. E. 1994: Index to plant chromosome numbers. 1990–1991. *Monographs in Systematic Botany, Missouri Botanical Garden 51*: 1–267.

Goldblatt, P.; Johnson, D. E. 1996: Index to plant chromosome numbers. 1992–1993. *Monographs in Systematic Botany, Missouri Botanical Garden 58*: 1–276.

Goldblatt, P.; Johnson, D. E. 1998: Index to plant chromosome numbers. 1994–1995. *Monographs in Systematic Botany, Missouri Botanical Garden 69*: 1–208.

Hair, J. B.; Beuzenberg, E. J. 1966: Contributions to a chromosome atlas of the New Zealand flora. 7. *New Zealand Journal of Botany 4*: 255–266.

Heenan, P. B.; de Lange, P. J.; Wilton, A. D. 2001: *Sophora* (Fabaceae) in New Zealand: taxonomy, distribution, and biogeography. *New Zealand Journal of Botany 39*: 17–53.

Hemsley, W. B. 1884: Report on the Botany of Juan Fernandez, the south-eastern Moluccas, and the Admiralty islands. Report on the scientific results of the voyage of H.M.S. Challenger, Botany. Vol. 1. London, Macmillan. Pp. 1–275.

Hsu, P.; Huang, S. 1985: Karyotype of *Sophora flavescens* Ait. *Bulletin of Botanical Research from the NE Forestry College (Harbin) 5*: 123–126.

Hurr, K. A.; Lockhart, P. J.; Heenan, P. B.; Penny, D. 1999: Evidence for the recent dispersal of *Sophora* (Leguminosae) around the southern oceans: molecular data. *Journal of Biogeography 26*: 565–577.

Johow, F. 1896: Estudios sobre la flora de las Islas de Juan Fernández. Santiago (Chile), Imprenta Cervantes.

Kawakami, J. 1930: Chromosome numbers in Leguminosae. *Botanical Magazine, Tokyo 44*: 319–328.

Kodama, A. 1989: Karyotype analyses of chromosomes in eighteen species belonging to nine tribes in Leguminosae. *Bulletin of the Hiroshima Agricultural College 8*: 691–706.

Kumari, S.; Bir, S. S. 1990: Karyomorphological evolution in Papilionaceae. *Journal of Cytology and Genetics 25*: 173–219.

Levan, A.; Fredga, K.; Sandberg, A. 1964: Nomenclature for centromeric position on chromosomes. *Hereditas 52*: 201–220.

Palomino, G.; Martínez, P.; Bernal, C.; Sousa S., M. 1993: Diferencias cromosómicas entre algunas especies de los géneros *Sophora* L. y *Styphnolobium* SCHOTT. *Annals of the Missouri Botanical Garden 80*: 284–290.

Peña, R. C.; Cassels, B. K. 1996: Phylogenetic relationships between Chilean *Sophora* species (Papilionaceae). *Biochemical Ecology and Systematics 24*: 725–733.

Peña, R. C.; Iturriaga, L.; Montenegro, G.; Cassels, B. K. 2000: Phylogenetic and biogeographic aspects of *Sophora* Sect. *Edwardsia* (Papilionaceae). *Pacific Science 54*: 159–167.

Polhill, R. M. 1981: Sophoreae Sprengel (1818). *In*: Polhill, R. M.; Raven, P. H. *ed.* Advances in legume systematics, Part 1. Kew, Royal Botanic Gardens. Pp. 213–230.

Rattenbury, J. A. 1957: Chromosome numbers in New Zealand angiosperms. *Transactions of the Royal Society of New Zealand 84*: 936–938.

Romero Zarco, C. 1986: A new method for estimating karyotype asymmetry. *Taxon 35*: 526–530.

Sousa S., M.; Rudd, V. 1993: Revisión del género *Styphnolobium* (Leguminosae). *Annals of the Missouri Botanical Garden 80*: 270–283.

Stebbins, G. L. 1971: Chromosomal evolution in higher plants. London, E. Arnold.

Stiefkens, L. B.; Bernardello, G.; Anderson, G. J. 2001: The somatic chromosomes of *Sophora fernandeziana* (Fabaceae), an endemic tree from Robinson Crusoe Island. *Pacific Science 55*: 71–75.

Tian, X.; Zhu, B.; Xiao, Y.; Liu, Q.; Zhao, G. 1993: On the karyotypes of five species of Leguminosae. *Journal of the Shaanxi Normal University, Natural Sciences 21*: 62–65.

Yeh, M. S.; Yuasa, H.; Maekawa, F. 1986: Chromosome numbers in the leguminosae. *Science Reports of the Research Institute of Evolutionary Biology 3*: 57–71.