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The Somatic Chromosomes of *Sophora fernandeziana* (Fabaceae), an Endemic Tree from Robinson Crusoe Island¹

Laura B. Stiefkens,² Gabriel Bernardello,² and Gregory J. Anderson³

Abstract: The mitotic chromosome number and karyotype of *Sophora fernandeziana* (Phil.) Skotts. (subfamily Papilionoideae) are reported for the first time. The chromosome number, $2n = 18$, is the modal number reported for the genus. The chromosomes are small (average length $1.55 \pm 0.23 \mu\text{m}$) and bear no satellites. The intrachromosomal and interchromosomal asymmetry indices were $A_1 = 0.26$ and $A_2 = 0.18$, respectively. This symmetrical karyotype is composed of 7 metacentric + 2 submetacentric pairs. This species is related to *S. tetraptera* J. Mill. from New Zealand. Both share the same chromosome number; unfortunately comparative karyotype data are not available for *S. tetraptera*. Our data suggest that no changes in chromosome number have occurred during the speciation of *S. fernandeziana*, in accordance with previous studies of other endemic species in the Juan Fernández flora and for island endemics in general. However, only a small percentage of actual karyotypes of island endemics have been studied, so generalizations about chromosomal evolution for such species are not yet well founded.

ROBINSON CRUSOE ISLAND (Masatierra) is one of the three islands of the small Juan Fernández Archipelago in Chile. It is located in the Pacific Ocean, 667 km W of continental Chile at 33° S latitude, having an estimated age of ca. 4 million yr old (Stuessy et al. 1984). The archipelago is well known for the high level of endemism among vascular plants (ca. 63% of the flora [Marticorena et al. 1998]). Unfortunately, currently more than 62% of the flora is considered rare, and two species are already extinct (Stuessy et al. 1998). The flora is threatened by both

anthropogenic and natural phenomena, including fire, erosion, vegetation cutting, and continued introduction of animals and invasive plants (Stuessy et al. 1998).

There are the only two endemic legume species that inhabit the archipelago. Both are trees in the genus *Sophora* L. and each of the main islands supports one species: *S. masafuerana* (Phil.) Skotts. on Alexander Selkirk Island and *S. fernandeziana* (Phil.) Skotts. on Robinson Crusoe Island. *Sophora* includes ca. 43 species (Polhill 1981, Sousa S. and Rudd 1993) partitioned among three sections. *Sophora fernandeziana* belongs to *Sophora* sect. *Edwardsia* (Salisb.) Taub., characterized by having a calyx without an upper lip, stamens and style exerted, lower petals all basically similar, standard not strongly reflexed, and pods often four-winged. This section comprises about 10 species: one each on Réunion Island and Hawai'i, and the remainder on Lord Howe Island, New Zealand, and southwestern South America (Polhill 1981).

Chromosomal surveys based on haploid chromosome numbers (Sanders et al. 1983, Spooner et al. 1987, Sun et al. 1990) have been made for 38 of the 156 native and endemic species of the Juan Fernández Archipelago (Marticorena et al. 1998). However,

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none of these has included *Sophora*, and there are no published karyotype analyses. Worldwide, chromosomes have been counted for only 26 species of *Sophora*, with several chromosome numbers reported (cf. Federov 1974, Goldblatt 1981*a,b,c*, 1984, 1988, 1990, 1991, 1994, 1996, 1998). In this paper, we provide data on the somatic chromosome number and the karyotype of *S. fernandeziana*. These data are basic to determine the importance of chromosomal changes during evolution of species on this archipelago.

MATERIALS AND METHODS

The studied material was collected in Chile, Juan Fernández Archipelago, Robinson Crusoe Island, Corporación Nacional Forestal gardens, 27 January 1996 (G. J. Anderson 3064). A voucher specimen is deposited in Torrey Herbarium, University of Connecticut (CONN).

Mitotic chromosomes in somatic cells of root tips were analyzed from squashes of primary roots growing from germinating seeds. Seeds were soaked in tap water for 24 hr. 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 were kept in the dark in a warming oven at 30°C. Root tips were cut when the primary roots were 2–10 mm long and were pretreated at room temperature for 2 hr in a saturated solution of p-dichlorobenzene in water. Root tips were rinsed in distilled water and fixed in freshly made ethanol:glacial acetic acid (3:1) at room temperature for 24 hr. After fixation, they were hydrolyzed with 5N HCl for 40 min at room temperature and put in Feulgen solution (basic fuchsin) for 2 hr at room temperature in the dark. Root-tip meristem cells were isolated on a slide and squashed. Slides were made permanent in Euparal after removing the cover slips by freezing with liquid carbon dioxide.

Cells selected for measurements were photographed with phase-contrast optics and Kodak T-Max film. Ten cells from five individuals (two from each) were photographed and the photographs were used to determine

the length 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 according to Levan et al. (1964) and to determine homologous chromosomes. Karyograms were constructed by organizing the chromosomes into groups according to their arm ratio (from metacentric to submetacentric), 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' classification (1971).

RESULTS

The examination of 30 cells, from five individuals, showed that the somatic chromosome number of *S. fernandeziana* is $2n = 18$ (Figure 1). The chromosomes are small (Table 1), ranging from 1.0 to 2.3 μm , with an average mean chromosome length of 1.55 ± 0.23 . No satellites were observed. The total haploid chromosome length of the karyotype based on the mean chromosome length was 14 μm . The karyotype is symmetrical with 7 m (metacentric) + 2 sm (submetacentric) chromosome pairs (Figure 2). The intrachromosomal and interchromosomal asymmetry indices were $A_1 = 0.26$ and $A_2 = 0.18$, respectively. According to Steb-



FIGURE 1. Photomicrograph of mitotic metaphase of *Sophora fernandeziana*, $2n = 18$. Scale bar = 5 μm .

TABLE 1

Measurements in μm (Mean \pm SD) and Chromosomic Indices of Somatic Chromosomes of *Sophora fernandeziana*

Pair	s	l	c	r	i	Chromosome Type
1	0.88 \pm 0.11	1.09 \pm 0.18	1.96 \pm 0.24	1.24	44.58	m
2	0.78 \pm 0.12	0.98 \pm 0.11	1.75 \pm 0.22	1.26	44.28	m
3	0.71 \pm 0.11	0.94 \pm 0.14	1.65 \pm 0.23	1.33	42.88	m
4	0.71 \pm 0.14	0.84 \pm 0.15	1.55 \pm 0.25	1.17	46.07	m
5	0.65 \pm 0.10	0.80 \pm 0.13	1.45 \pm 0.22	1.22	44.94	m
6	0.63 \pm 0.10	0.75 \pm 0.10	1.37 \pm 0.16	1.19	45.55	m
7	0.54 \pm 0.15	0.65 \pm 0.09	1.19 \pm 0.15	1.20	45.54	m
8	0.57 \pm 0.10	1.16 \pm 0.15	1.73 \pm 0.24	2.04	32.85	sm
9	0.43 \pm 0.10	0.93 \pm 0.25	1.36 \pm 0.26	2.16	31.65	sm

Note: 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.

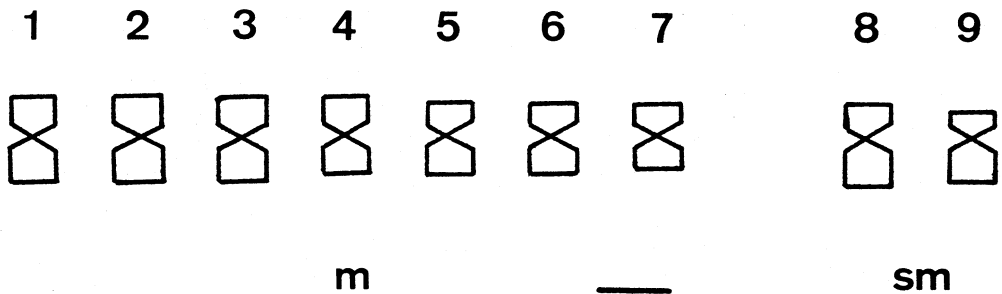


FIGURE 2. Idiogram of *Sophora fernandeziana* based on mean chromosome values (see Table 1). Scale bar = 2 μm .

bins' (1971) classification, the karyotype falls in the "2A" category.

DISCUSSION

The diploid chromosome number of *S. fernandeziana*, at $2n = 18$, is the modal number for *Sophora*. Several chromosome numbers have been reported for this genus: $n = 9, 18, 11$, and $2n = 18, 28, 36, 16, 32, 22, 54$, in decreasing order of frequency in each case (cf. Federov 1974, Goldblatt 1981*a,b,c*, 1984, 1988, 1990, 1991, 1994, 1996, 1998). The 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 (Goldblatt 1981*a*).

Based on both the Romero Zarco indices and Stebbins' classification, the karyotype

should be considered symmetrical, as commonly found in Fabaceae as a whole (Bairiganjan and Patnaik 1989, Kumari and Bir 1990). For instance, *st* chromosomes are rare in the Fabaceae, and *t* chromosomes have never been reported (Bairiganjan and Patnaik 1989, Kumari and Bir 1990). Our data agree with the general trends observed in karyotypes of tree taxa from Papilionoideae (Bairiganjan and Patnaik 1989, Kumari and Bir 1990): none of the studied trees exhibited chromosomes with secondary constrictions, and most karyotypes are symmetrical, falling in the "2A" category of Stebbins (1971).

The karyotypes of the eight other species of *Sophora* analyzed are also mostly composed of several m pairs with a few sm pairs (Kawakami 1930, Hsu and Huang 1985, Bernal Gonzalez and Martínez Almeraya 1989, Kodama 1989, Kumari and Bir 1990, Palo-

mino et al. 1993, Tian et al. 1993). Differences were found in the karyotype formula and in the presence of secondary constrictions among these examined species; karyotypic features seem to be taxonomically useful for *Sophora*. For instance, Sousa S. and Rudd (1993) recently recognized the genus *Styphnolobium* Schott as separate from *Sophora* based on the existence of postulated basic numbers ($x = 14$ for the former and $x = 9$ for the latter [Palomino et al. 1993]), as well as some other morphological features (Sousa S. and Rudd 1993).

Sophora fernandeziana is related to *S. tetraptera* J. Mill. from New Zealand and at one time was considered to be conspecific with this geographically distant species (Hemsley 1884, Johow 1896). Both share the same chromosome number (Yeh et al. 1986), but karyotype data are not available for *S. tetraptera*.

Previous studies of the species of the Juan Fernández Archipelago by Sanders et al. (1983), Spooner et al. (1987), and Sun et al. (1990) revealed relatively little chromosomal structural change associated with the endemic flora. In the paper by Sun et al. (1990) there is a report of one possible tetraploid and one possible aneuploid out of 38 species studied chromosomally. The data on *Sophora fernandeziana* add further strength to the conclusion presented above.

For the Hawaiian flora, Kyhos and Carr (1994) and Carr (1998) pointed out that most of the species are woody perennial forms and are thus in groups that have been evolving for some time. They argue that in such "older" groups there is more genomic/genic stability and thus less tolerance for chromosomal structural changes. The Bonin Island flora is interesting in this context and perhaps constitutes an exception because a substantial number of the species studied cytologically show chromosome differences (Ono 1991). These changes are mostly aneuploidy (about 18% of the total of 112 species of the endemic flora, and 48% of the 58 taxa cited in his paper). Ono (1991) noted that a few other species are presumably island polyploid derivatives or island diploid relicts (with polyploid continental presumed derivatives).

Few island endemics have been studied

cytologically. From the studies to date, the general pattern, if there is one, seems to be that speciation on islands is largely independent of major chromosomal changes—or at least chromosomal changes that are recognizable as number changes. This does not mean that structural changes (that do not result in changes in number) have not occurred. More data are required on chromosome number, and particularly on karyotypes, before more well-founded generalizations can be made.

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