A CYTOTAXONOMIC STUDY OF THE *DISPORUM* OF TAIWAN[1]

CHUAN-YING CHAO, TSAN-IANG CHUANG and
WILMA W. L. HU[2]

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Introduction

The genus *Disporum* contains about 32 species which are distributed from Himalaya, India, Malaysia, China, Japan and North America. The Eastern Asian species have large chromosomes while the North American species possess small ones.

Cytological work of the species of *Disporum* was initiated by Hasegawa (1932) who described the karyotypes of 3 Japanese species, namely *D. pullum*, *D. sessile* and *D. smilacinum*. Although all these 3 species have 8 pairs of chromosomes (4 large and 4 medium-sized), but their karyotypes are somewhat different. Therman (1956) worked out the cytology of 3 North American species: *D. smithii* (2n=16) has 3 SAT-chromosomes and 3 nucleoli, indicating that it may be a hybrid, and both *D. oreganum* and *D. hookeri* have chromosomes 2n=18. Meiosis of *D. hookeri* is irregular. In the study of the Western Himalayan Polygonateae, Mehra and Pathania (1960) found that the karyotype of *D. pullum* (=*D. cantoniense*) of Himalayan is different from that of the Japanese form, although both have 16 chromosomes.

So far the basic chromosome numbers reported in *Disporum* are x=6, 8, 9 and 11 (Darlington and Wylie, 1955). These numbers together with the findings stated above indicate that the genus consists of a heterogenous group of species. Further cytological studies on the species of this genus may provide more interesting information.

Cytological studies on two species, *D. Kawakamii* and *D. Shimadai*, both endemic on Taiwan Island, are reported in this paper. Analyses of two populations of the former indicate the hybrid nature of the species and the basic chromosome number x=7 of the latter species is first reported for this genus.

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(2) Professor, National Taiwan University, Assistant Research Fellow and Research Assistant, Institute of Botany, Academia Sinica, respectively.

In Hayata's (1911) revision of Taiwan *Disporum,* he listed 3 species, namely *D. pullum, D. Kawakamii,* and *D. Shimadai,* the latter two being described by him as new species. However, after careful examination of the herbarium specimens deposited in the Herberia of the National Taiwan University and the Taiwan Forest Research Institute, we found that the so-called specimens of *D. pullum* identified by Matsumura and Hayata should be named *D. Kawakamii.*

Taxonomic Treatment

The following brief taxonomic treatment will help to identify the two species of *Disporum* of Taiwan.

Key to species:

Leaves with short petioles; petals oblong, base slightly narrower, margin of apex papillate, texture rather thick; 2n=16..... *D. Kawakamii.*

Leaves sessile; petals pandurate, margin of apex not papillate, texture rather thin; 2n=14 .....*D. Shimadai.*

Disporum Kawakamii Hayata, Mat. Fl. Formosa 365, 1911. Masamune, Short Fl. Formosa 269, 1936 and List Vas. Pl. Taiwan 133, 1954.

Disporum pullum Matsumura et Hayata, in Enum. Pl. Formosa 443, 1906, non Forbes et Hemsl.

Distribution: Endemic in Taiwan.

Herbarium specimens examined:

Ilan Hsien: Shimada SH. 1309(TAI), SH. 1330(TAI); Suzuki 1165(TAI); Mori 5806(TAIF).

Taipei Hsien: Shimada SH. 1278(TAI); Suzuki 7633(TAI), 8392(TAI).

Hsien-chu Hsien: Shimada 606(TAI), 3983(TAI), SH 419(TAI).

Taichung Hsien: Liu *et al.* 18(AS).

Nantou Hsien: Liu *et al.* 18 (TAI); Huang 1937 (TAI); Kao K3981 (TAI), K3989 (TAI).

Chia-yi Hsien: Kao K4230 (TAI), Voucher specimen; Suzuki 498 (TAI); Hayata and Sasaki 5807(TAIF); Sasaki 5808(TAIF).

Tai-tung Hsien: Maeda SH 1389 (TAI); Matsuda June, 1917 (TAI); Chuang and Kao 1103(AS).

Hwalien Hsien: Kao K4146(TAI); Liu, Chen and Kao 162(TAI); Shimada SH 1305(TAI), SH 1301(TAI), SH 1307(TAI); Suzuki 10953(TAI), ST 17712 (TAI); Suzuki 10506(TAI), 9847(TAI).

Abbreviations used:

TAI—Herbarium of the National Taiwan University

TAIF—Herbarium of the Taiwan Forest Research Institute

AS—Herbarium of the Academia Sinica

Disporum Shimadai Hayata, Mat. Fl. Formosa 367. 1911. Mosamune, l. c.
Distribution: Endemic in Taiwan
Herbarium specimens examined:
Keelung: Kao K4500(TAI, AS); Chuang 4853(AS); Kawakami and Shimada March 6, 1907, 4811 Isotype.

Cytological Observations

The materials of *D. Kawakamii* used for this study were obtained from 2 collections. The collection *K4230* was obtained from Mt. Ali in southern Taiwan and the collection *S11643* from Hwalien in northeastern Taiwan. Since the karyotypes of these two populations are different, they will be treated separately. The materials of *D. Shimadai* were obtained from several collections (*K4500, TIC4853,* etc.) at seashore near Keelung. Same karyotype was identified in all materials.

Both the root tips and flower buds were fixed in Farmer's fluid. The root tips were treated with 1N HCl before squashing. Propiono-carmine smear technique was employed for both root tips and PMCs.

Disporum Kawakamii

Collection *K4230:*

Chromosome number and karyotype were determined from the root tips only. The somatic number is 2n=16. There are 4 pairs each of large and medium-sized chromosomes (Figs. 1 and 14). Figure 1 is the diagramatic representation of its karyotype. The chromosomes were alphabetically arranged by the descending order of their length. They range from 6.9μ to 21.0μ in length. The centromeres of chromosomes are either submedium or subterminal in position. There are 2 SAT-chromosomes—one c and one f. Thus, both c and f pairs are heteromorphic.

Collection *S11643:*

Somatic chromosomes—In this collection, only flower buds were available for study. However, abundant mitotic figures were observed in the tapetal cells of those anthers in which PMCs had not yet initiated their divisions. Thus, somatic chromosomes were also studied for this collection. As in *K4230,* plants of this population have 8 pairs of chromosomes (4 large and 4 medium-sized) which had been confirmed in PMCs. The chromosomes range from 8.5μ to 22.4μ in length. The centromeres are either submedium or subterminal (Figs. 2, 5, 6) in position. The karyotype of this population differs from that of the previous one in several aspects. There is no one chromosome of this population carries a satellite or secondary constriction. In addition, the two c chromosomes are heteromorphic—the long arm of one c having an extra segment, about 1/4 of the arm length. As a consequence, certain meiotic abnormalities will be expected.

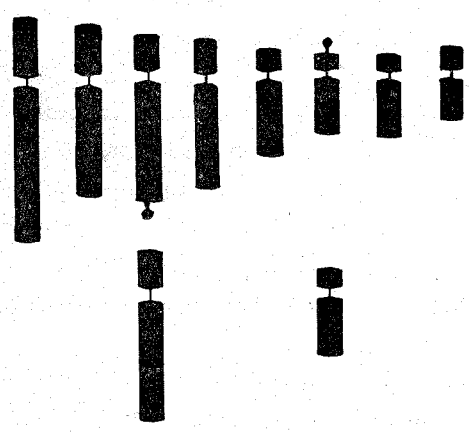


Fig. 1 Karyotype of *D. Kawakamii* (Collection *K4230*), 2n=16 (×1500). The c and f chromosome pairs are heteromorphic.

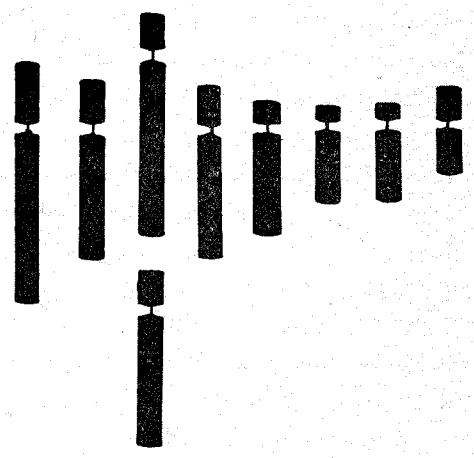


Fig. 2 Karyotype of *D. Kawakamii* (Collection *S11643*), 2n=16 (×1500). The c chromosomes are unequal in length.

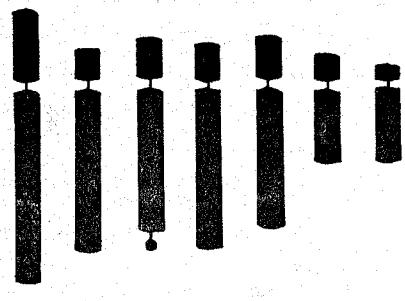


Fig. 3 Karyotype of *D. Shimadai*, 2n=14 (×1500).

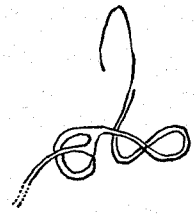


Fig. 4 Pachytene configuration of a portion of the c chromosome pair. Note the extra terminal segment of the one homologue and the inversion loop (×1100).

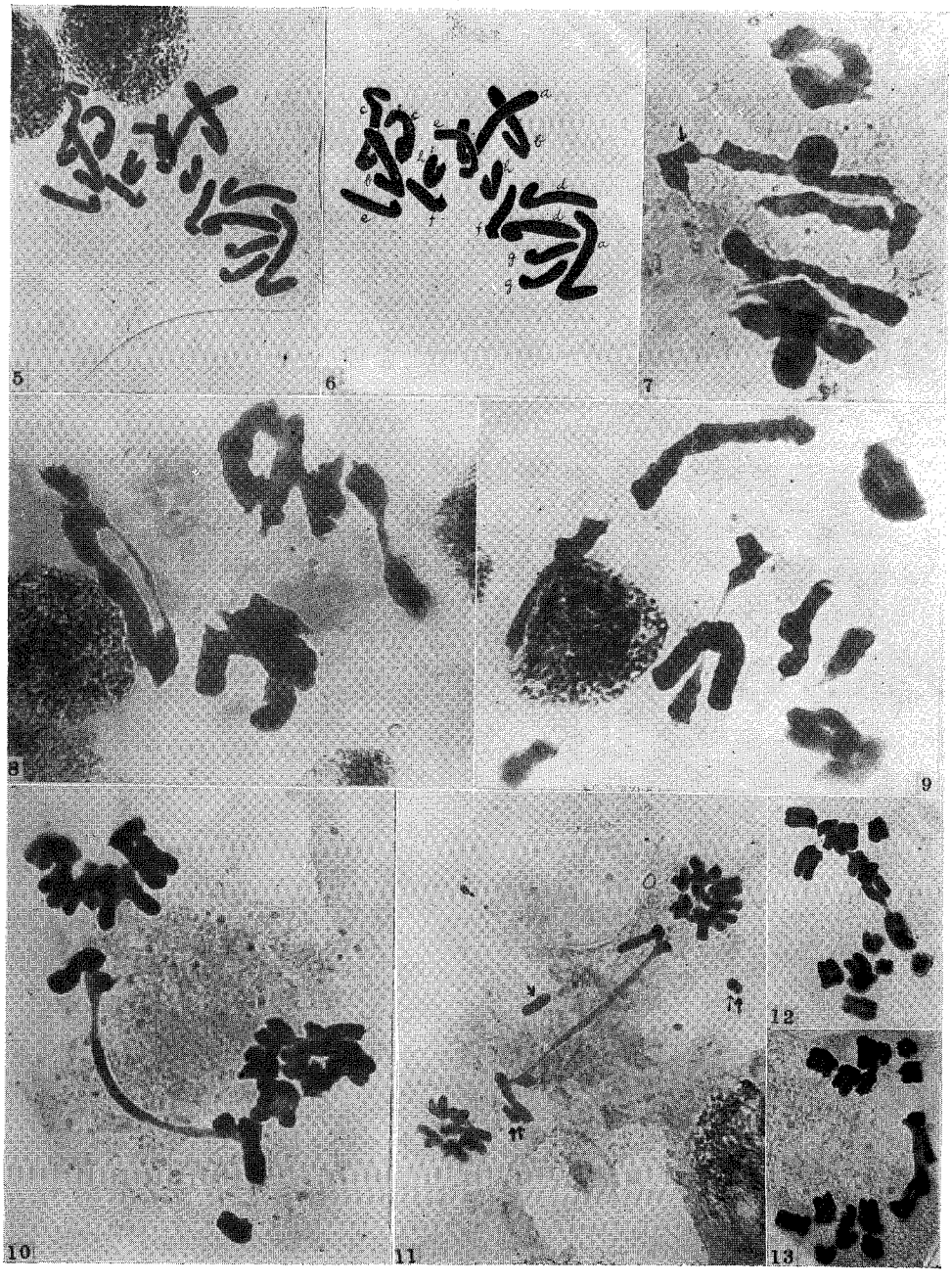
Meiotic behaviour—MI associations were observed in a great number of cells. However, only in a relatively small number of cells the various configurations could be analyzed without difficulty. In approximately 40% of these cells there are 8 distinct bivalents. The extra segment of one c chromosome was found to be either attached to or dissociated from the bivalent. Two members of one medium-sized bivalents were usually precociously separated. (Fig. 9).

In the remaining cells, there is a ring or chain of 4 chromosomes (Figs. 7 & 8)—2 large and 2 medium-sized. An extra chromosome segment is usually attached to one of the large chromosomes. Thus, the large chromosome pair involved in this tetravalent is the c pair. There is no way to trace which particular medium-sized chromosome pair is associated in this multivalent. However, it is interesting to note that, in many cases, there is a close association between the extra chromosome segment and one of the medium-sized chromosomes (Figs. 7 & 8).

Results obtained from the analysis of the AI configurations indicate that in approximately 6 percent of PMCs there is a dicentric bridge, either with or without an acentric fragment. Figure 10 shows a typical AI bridge as well as an acentric fragment in one PMC. The dicentric bridge is formed by two chromatic segments of the long arms of the two large chromosomes. The presence of an AI dicentric bridge and acentric fragment usually infers the presence of a paracentric inversion in heterozygous condition. In the present case, the inverted segment may be relatively short as shown by relatively low percentage of PMCs with AI bridge and acentric fragment.

From the AI configuration, one cannot tell which particular chromosome pair is heterozygous for an inversion. Due to overlapping of the most of the bivalents at pachynema, it is impossible to trace individual chromosome pairs of this species at this stage. However, after examining several hundred pachytene figures it was fortunate enough to find one interesting configuration in one PMC. In this particular cell one portion of a bivalent was stretched from the bivalent cluster so that its pairing condition could be traced and diagrammed as in Fig. 4. From the figure, it can be seen that one homologue of this portion of bivalent has an extra chromosomal segment at the terminal end.

Figs. 5-13. Photographs (except Fig. 6) of the somatic and meiotic chromosomes of *D. Kawakamii* (Collection *S11643*). Figs. 5-10. × ca. 600. Figs. 11-13 × ca. 350. 5, Somatic metaphase, showing 16 chromosomes. 6, Same as Fig. 5, camera lucida drawing. 7, MI, showing 6 bivalents and 1 chain of 4. The extra segment is indicated by the arrow. 8, MI, showing 6 bivalents and 1 ring of 4. Bivalents are not properly oriented. 9, MI, showing 8 bivalents which are not properly oriented. 10, Early telophase, showing the dicentric bridge and acentric fragment. 11, Early telophase, showing the dicentric bridge, acentrc fragment (indicated by single arrow) and 2 small chromosomes (or fragments ?) as indicated by double arrows, 1 being attached to a normal chromatid of a dyad from which the bridge originates. 12-13, AI, showing the laggard.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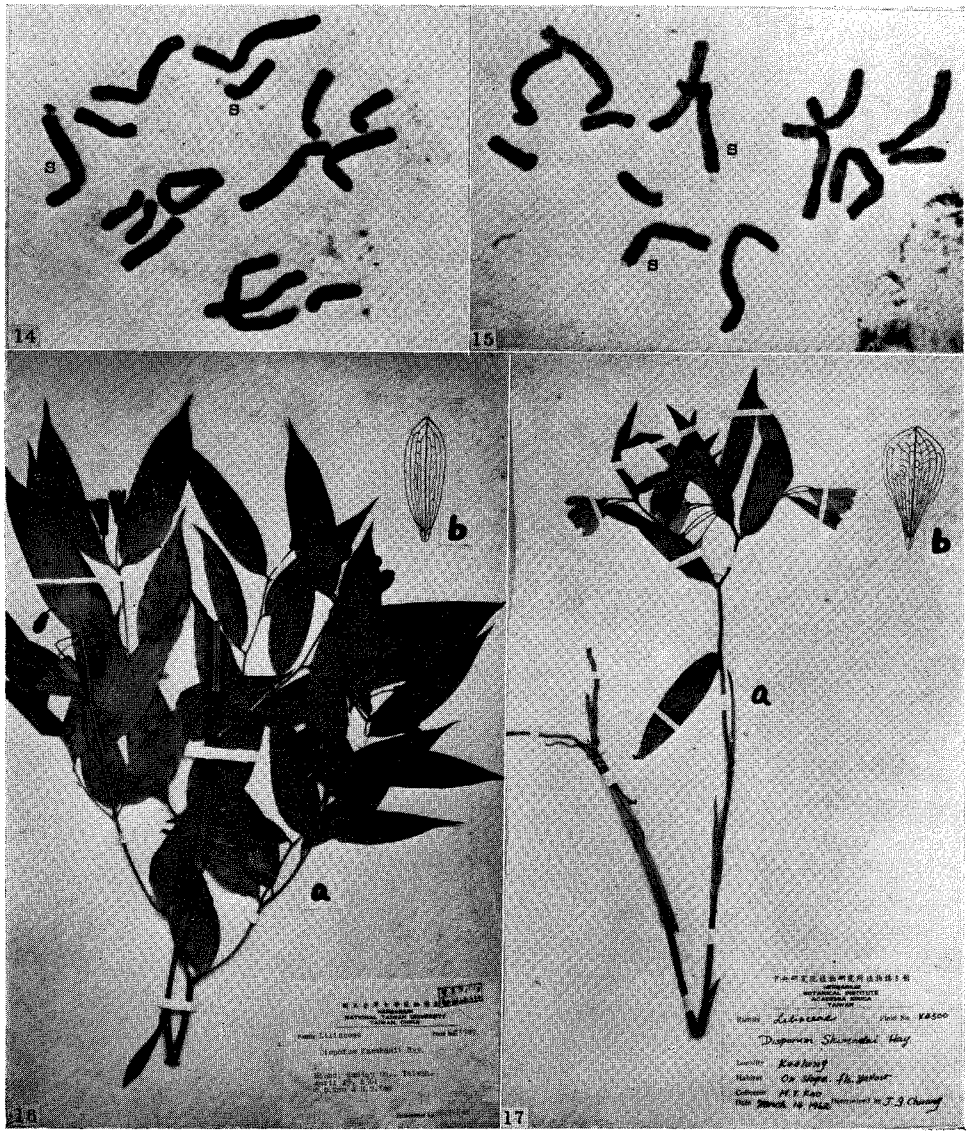

Figs. 14-17. Photographs of somatic chromosomes and flower branches of *D. Kawakamii* and *D. Shimadai*. s indicates the SAT-chromosome. 14, Somatic metaphase of *D. Kawakamii* (Collection *K4230*), showing 16 chromosomes. 15, Somatic metaphase of *D. Shimadai*, showing 14 chromosomes. 16, A flowering branch (a) and a petal (b) of *D. Kawakamii*. 17, A flowering branch (a) and a petal (b) of *D. Shimadai*.

The relative length of this extra segment to the whole chromosome could not be determined since the remaining part of the bivalent was in the cluster. Although there is no definite proof, it is most probably that this portion is part of the long arm of the c chromosome pair because no one chromosome in the somatic chromosome complement seems to have an extra segment except one of the two c chromosomes.

By close examination of this portion of bivalent, a small inversion loop was found in the interstitial regions (Fig. 4). If the assignment of this portion of bivalent to the c pair is correct, this chromosome pair will also be heterozygous for a paracentric inversion in the long arm.

Direct and indirect evidences thus obtained show the following abnormalities along the long arm of the c chromosome pair:

(1) Presence of an extra terminal chromosomal segment in one homologue.
(2) At least part of this extra segment being homologous with part of a particular medium-sized chromosome hitherto not yet determined.
(3) Heterozygous for a paracentric inversion in the long arm.

Figure 11 presents a more interesting configuration at early telophase. In this cell, there are 6 dyads at one pole, 7 at the other. Besides, there is a dicentric bridge formed by chromatids of the c chromosomes, an acentric fragment (indicated by single arrow) and 2 small chromosomes (or fragments? indicated by double arrows). One such small chromosome is associated with one of the normal chromatid of the c chromosome pair, and the other is free near the other pole. The normal chromatid of the c chromosome pair associated with the small chromosome is longer than the other normal one at other end of the bridge. This could be the result of the formation of a chiasma within the inversion loop and another one between the extra segment of one c chromosome and one medium-sized chromosome having homologous part with the extra segment. Since the nature of association could not be traced and determined, this is highly speculative but not wholly impossible.

D. Shimadai

In this species, the somatic chromosomes were studied in root tip cells. No flower buds were available for meiotic observations. Its haploid chromosome number n=7 (Figs. 3 and 15) reported here is the new basic number for the genus *Disporum*. The 5 pairs of large chromosomes and 2 pairs of medium-sized ones were diagramatically shown in Fig. 3. They range from 8.8μ to 25.5μ in length. No evidence of heteromorphism of any chromosome pair can be visualized. Two homologues of the c pair are SAT-chromosomes.

Briefly, the karyotype of this species differs from that of *D. Kawakamii* in having 1 additional pair of large chromosomes but 2 less pairs of medium-sized

chromosomes.

Discussion

All Asian species of *Disporum* which had been previously investigated cytologically have 4 pairs each of large and medium-sized chromosomes in spite of the fact that the karyotypes are somewhat different. In so far as the chromosome number and size are concerned, the species *D. Kawakamii* which is endemic in Taiwan follows the same course. However, analyses of two populations of this species collected from two distinct geographical localities on the island show that the karyotypes of these two populations are different and both indicate the hybrid nature of the populations. The presence of quadrivalent infers the chromosomal interchanges between two non-homologous chromosomes. Similarly, the presence of the AI bridge and acentric fragment infers the inversion of chromosomal segment within a single chromosome. These facts implicate that changes in chromosomal structures are still going on at the present level of this species. Since this species is widely distributed on the island it would be the valuable material for further detailed study.

The species *D. Shimadai* which is found only on the seashore near Keelung in northern Taiwan has 5 pairs of large chromosomes and 2 pairs of medium-sized chromosomes. Thus, it deviates from other Asian species of the same genus, whose karyotypes had been reported in having one more pair of large chromosomes and two less pairs of medium-sized chromosomes.

In this connection, it is worth to mention that in the evolution of certain species of *Drosophila* and *Crepis,* decrease in chromosome number through interchange of chromosomal segments between non-homologous chromosomes and eventually loss of small chromosomes resulted from interchange are well-established facts (Dobzhansky, 1951). At present, there is no evidence to show that the species *D. Shimadai* was originated from species with 4 pairs of large and 4 pairs of medium-sized chromosomes through chromosomal interchange between the non-homologous chromosomes and loss of resulting small chromosomes. Further cytogenetic study would be necessary before any definite conclusion can be made.

Summary

(1) According to our observation, specimens identified by Matsumura and Hayata as *D. Pullum* should be named *D. Kawakamii.*

Presumably only two species of *Disporum, D. Kawakamii* and *D. Shamadai* are present in Taiwan.

(2) *D. Kawakamii* has 16 somatic chromosomes. Cytological analyses of the karyotypes of 2 populations and meiosis of one population show the hybrid nature of the species.

(3) *D. Shimadai* has 7 pairs of chromosomes. It deviates from other Asian species of the same genus whose karyotypes had been analyzed in having 1 more large chromosome pair and 2 less medium-sized chromosome pairs.

臺灣百合科寶鐸草屬之細胞分類學的研究

趙傳纓 莊燦暘 胡慧琳

經作者等鑑定臺灣大學植物標本館及臺灣省林業試驗所標本館所藏植物標本之結果，臺灣之寶鐸草屬（*Disporum*）可分爲兩種，卽 *D. Kawakamii* 與 *D. Shimadai*.

D. Kawakamii 具有四對大型染色體及四對中型染色體 (2n＝16)。分析兩族羣核型及一族羣小孢子母細胞減數分裂之結果，發現有異型染色體對及減數分裂不正常之現象。因此推測其可能爲雜交種。

D. Shimadai 祇是分佈在基隆沿海一帶，具有五對大型染色體及二對中型染色體 (2n＝14)。未發現有異型染色體對。(摘要)

Literature Cited

DARLINGTON, D. C., and A. P. WYLIE. Chromosome Atlas of Flowering Plants, 2rd. ed. Allen & Unwin, London, pp. 519, 1955.

DOBZHANSKY, TH. G. Genetics and the Origin of Species. 3rd. ed. Columbia Univ. Press. New York, pp. 364, 1951.

HASEGAWA, N. Comparison of chromosome types in *Disporum*. Cytologia **3**:350-368, 1932.

HAYATA, B. Materials for a Flora of Formosa 365-367, 1911.

MEHRA P. N., and R. S. PATHANIA. A cytotaxonomic study of the West Himalayan Polygonatae. Cytologia. **25**:179-194, 1960.

THERMAN, EEVA. Cytotaxonomy of the tribe Polygonateae. Amer. Journ. Bot. **43**:134-142, 1956.