KARYOMORPHOLOGICAL STUDIES OF THE *TAXODIACAE*

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ABSTRACT

Taxodiaceae species were classified into the following 4 groups with regard to chromosome structure. Group I (Köpfchen type): The trees of this group have a pair of chromosomes carrying a secondary constriction consisting of a nipple-shaped short arm, connecting fibres and a large-sized trabant. *Cryptomeria japonica, C. fortunei, Glyptostrobus pensilis, Taxodium distichum, T. ascendens, Sequoiadendron giganteum, Taiwania cryptomerioides and Metasequoia glyptostroboides* are included in this group. Group II (Telomere type): The trees of this group contain some chromosomes with a secondary constriction connecting telomeres. This group includes *Cunninghamia lanceolata* and *Sequoia sempervirens*. Group III (Köpfchen and Telomere type): The trees have pairs of chromosomes carrying the secondary constriction characteristic of group I and group II respectively. *Taxodium mucronatum* is classified in this group. Group IV: The trees of this group have pairs of chromosomes with a secondary constriction that cannot be observed in either group I, II or III. This group includes *Athrotaxis cupressoides, A. laxifolia* and *A. selaginoides*. There tends to be a coincidence between the number of chromosomes with secondary constriction and the maximum number of nucleoli per cell for each group.

Key words: cytogenetics, chromosome morphology, idiogram

INTRODUCTION

Comparative studies of chromosome structural similarities and differences among related species and genera are considered important for the advance of forest tree breeding. The present study was a karyomorphological analysis of 14 species in 9 genera covering all of the *Taxodiaceae*, which are widely utilized commercially. Other realated cytogenetic research has been previously published by TODA (1978, 1980, 1983, 1987, 1989, 1992, 1993a, 1993b.

MATERIALS AND METHODS

The plant material used for this study were obtained from various domestic research institutes such as the Kyushu Forest Tree Breeding Institute. Root tip meristem cells were used. All operations, including the pretreatment of material for karyotype analysis (cold and warm treatment with 8-oxyquinoline), making of preparations, measurement of chromosomes, determination of homologous chromosomes and characterization of karyotype were performed by the method of TODA (1985). For the observation of nucleoli, the cover glass was taken off after making the preparation, while at the same time maintaining the saturated state of humidity; staining was by treatment with 50 % silver nitrate solution for 2–6 hours (Ag–I method).

RESULTS AND DISCUSSION

The karyotype of the Taxodiaceae trees showed a gradual transition from long to short chromosomes, including both metacentric and submetacentric types.

The karyotype of *Taxodiaceae* (Figure 9) is classified into the following four groups by the presence of chromosomes of distinctive shape: Group I, Köpfchen type, with the short arm having a "nipple" form and the connecting fibre and trabant of large size; Group II, possessing a telomere; Group III, middle type, both the Köpfchen type and the Telomere type; Group IV, the genus *Athrotaxis*, having chromosomes with a secondary constriction not found in Group I, II or III.

Species Group I

This group has J chromosomes with a secondary constriction that are characteristic of the karyotype of *Cryptomeria* (Figure 1). Types 0, I and II, characterized as having the F chromosome, were observed in *Cryptomeria japonica* (L. F.) D. Don and *C. fortunei* Hooib., two species of genus *Cryptomeria*. Intraspecific variation in the karyotype was as follows:

Type 0 (Figure 1a): F chromosome consisting of one pair of metacentric chromosomes.

Type I (Figure 1b): F chromosome consisting of one chromosome with a secondary constriction and one metacentric chromosome.

Table 1 Number of nucleoli and secondary constrictions in *Taxodiaceae*

Species		Chromosome number ($2n$)	No of secondary constrictions	No. of nucleoli						
				1	2	3	4	5	6	7
				Appearance ratio (%)						
Cryptomeria japonica	I	22	2	24.2	75.8	–	–	–	–	–
	II	22	3	10.2	39.1	50.7	–	–	–	–
	III	22	4	1.1	14.0	41.0	43.7	–	–	–
Glyptostrobus pensilis		22	2	17.9	82.1	–	–	–	–	–
Sequoiadendron giganteum		22	2	14.2	85.8	–	–	–	–	–
Taxodium distichum		22	2	24.9	70.5	4.3	0.3	–	–	–
Taxodium ascendens		22	2	–	–	–	–	–	–	–
Taxodium mucronatum		22	4	43.3	56.7	–	–	–	–	–
Taiwania cryptomeroides		22	2	92.7	7.3	–	–	–	–	–
Metasequoia glyptostroboides		22	6	0.1	4.2	11.7	21.5	26.9	35.1	0.5
Cunninghamia lanceolata		22	2	0.4	99.6	–	–	–	–	–
Sequoia sempervirens		66	6	0.4	5.4	9.9	17.5	25.3	40.5	1.1
Athrotaxis selaginoides		22	–	40.9	59.1	–	–	–	–	–
Athrotaxis laxifolia		22	–	22.6	55.3	22.1	–	–	–	–
Athrotaxis cupressoides		22	–	4.2	31.0	52.7	12.0	–	–	–

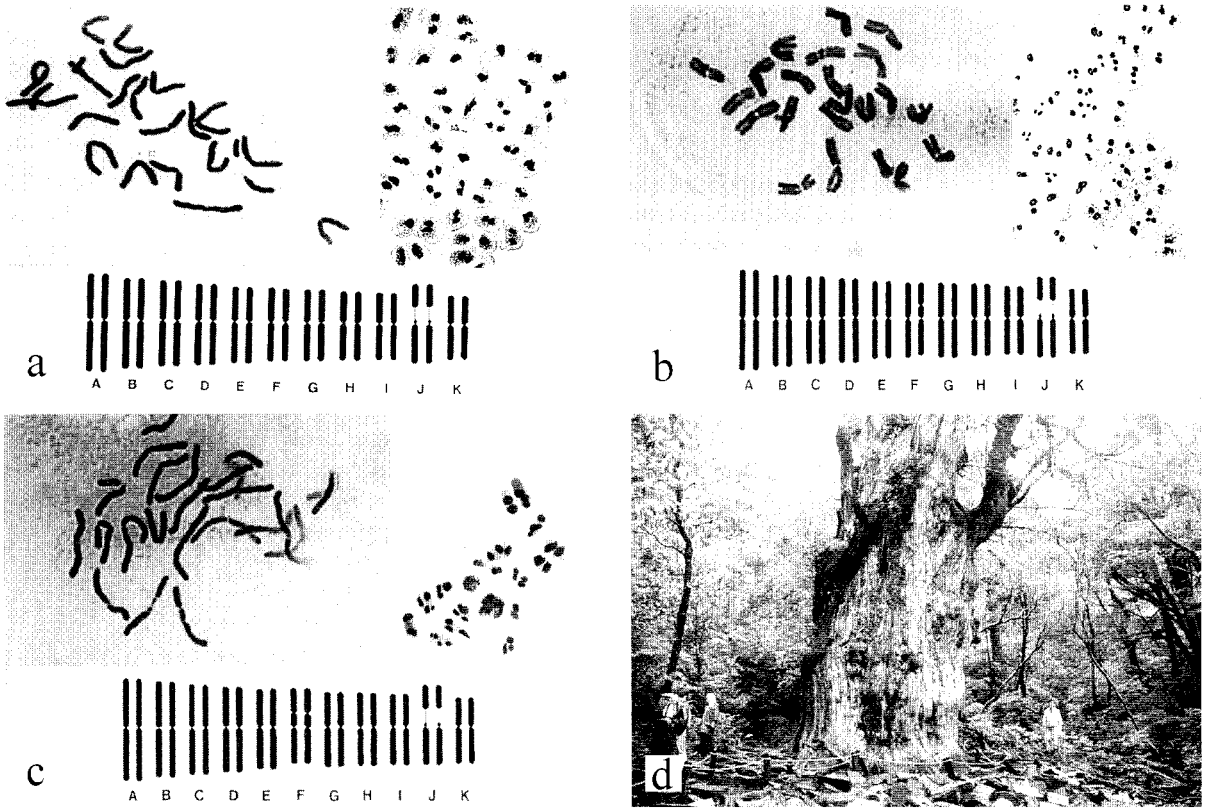

Figure 1 Karyomorphology of *Cryptomeria japonica* (Group I): **a** – somatic chromosomes, nucleoli and idiogram of type 0; **b** – somatic chromosomes, nucleoli and idiogram of type I; **c** – somatic chromosomes, nucleoli and idiogram of type II; **d** – a gigantic tree.

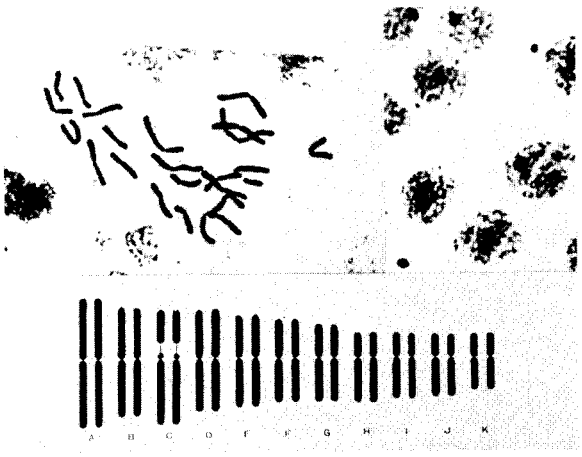

Figure 2 Karyomorphology of *Taiwania cryptomerioides* (Group I) – somatic chromosomes, nucleoli of horseshoe shape and idiogram of karyotype. K(22) = $2A^{m}+2B^{m}+2^{sc}C^{sm}+2D^{m}+2E^{m}+2F^{m}+2G^{m}+2H^{sm}+2I^{sm}+2J^{sm}+2K^{sm}$

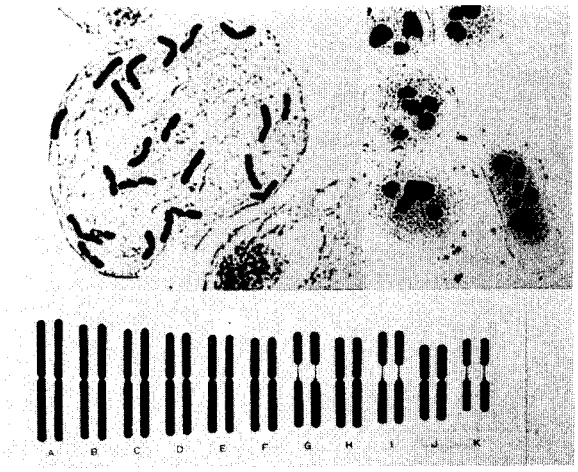

Figure 3 Karyomorphology of *Metasequoia glyptostroboides* (Group I) – somatic chromosomes, 6 nucleoli and idiogram of karyotype. K(22)=$2A^{m}+2B^{m}+2C^{m}+2D^{m}+2E^{m}+2F^{m}+2^{sc}G^{m}+2H^{m}+2^{sc}I^{m}+2J^{m}+2^{sc}K^{m}$

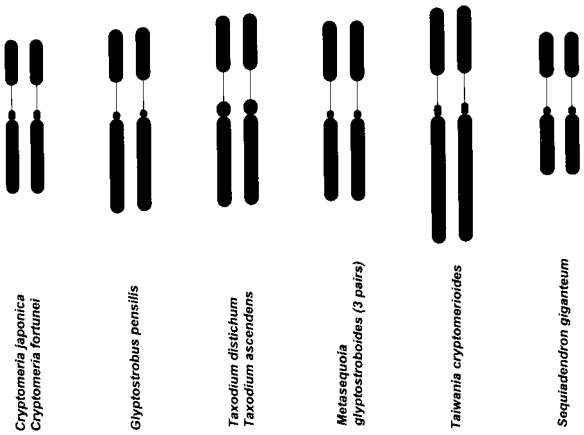

Figure 4 The chromosomes of distinctive shape in Group I (Köpfchen type).

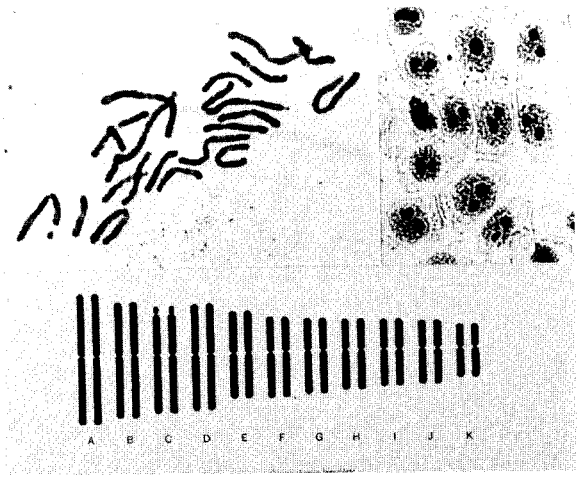

Figure 5 Karyomorphology of *Cunninghamia lanceolata* (Group II), somatic chromosomes, nucleoli and idiogram of karyotype. K(22)=$2A^{m}+2B^{m}+2^{sc}C^{sm}+2D^{m}+2E^{m}+2F^{m}+2G^{m}+2H^{m}+2I^{m}+2J^{m}+2K^{m}$.

Type II (Figure 1c): F chromosome consisting of one pair of chromosomes with secondary constriction.

In *C. japonica,* Type I can be obtained by the crossing of Types 0 and II; Intraspecific variation in the karyotype could be clarified by the observation of meiosis in each type.

In *Glyptostrobus pensilis* D. Don, *Taxodium distichum* (L.) L. C. Rich., *Taxodium ascendens* Brongn., and *Sequoiadendron giganteum* (Lindl.) Buchholz, a pair of chromosomes was observed that had the secondary constriction, consisting of the short arm of nipple form; the maximum number of nucleoli per cell was two.

In *Taiwania cryptomerioides* Hayata (Figure 2), two chromosomes in a pair possessing the secondary constriction were observed, but the nucleoli had a "horseshoe" shape; it is presumed that two nucleoli were fused.

The $2n$ chromosome number of *Metasequoia glyptostroboides* Hu et Cheng is 22 (Figure 3); 6 chromosomes in 3 pairs having the secondary constriction are present, and there were 6 nucleoli per cell. Figure 4 shows the chromosome of distinctive shape of each species in group I.

Species Group II

The karyotype of this group is characterized by possession of the telomere chromosome.

In *Cunninghamia lanceolata* (Lamb.) Hook. (Figure

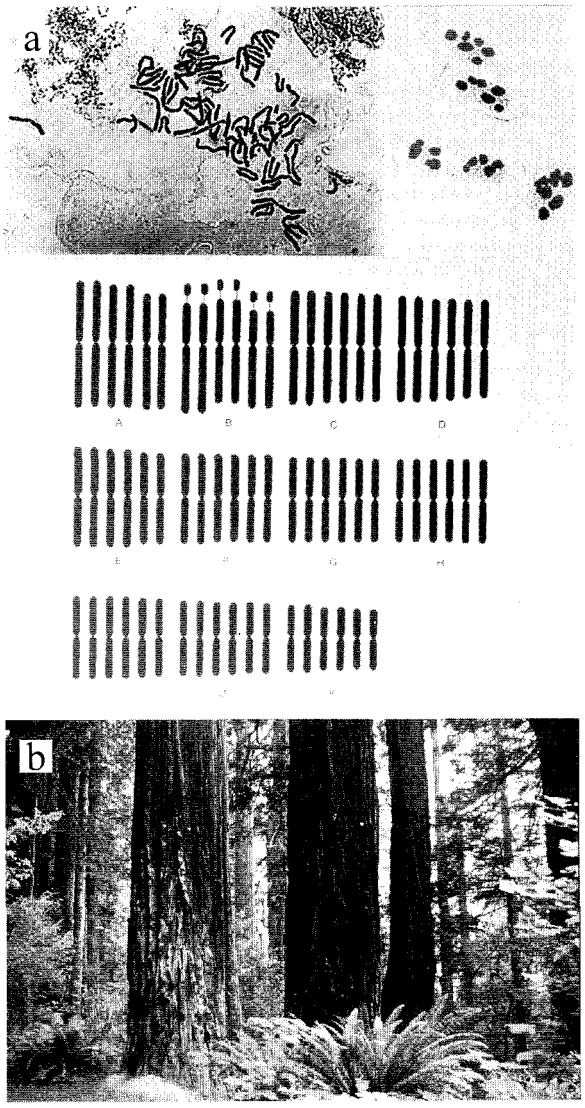

Figure 6 Karyomorphology of *Sequoia sempervirens* (Group II): **a** – somatic chromosomes ($2n$ = 66), 6 nucleoli and idiogram of karyotype; **b** – stand of *Sequoia sempervirens* in California.

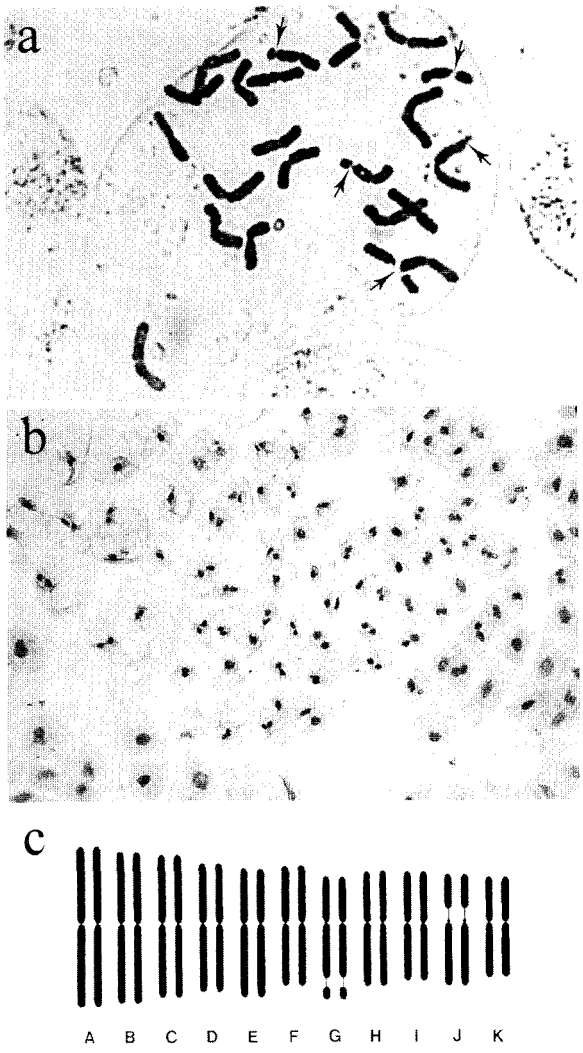

Figure 7 Karyomorphology of *Taxodium mucronatum* (Group III): **a** – somatic chromosomes; **b** – nucleoli; **c** – idiogram of karyotype. K(22) = $2A^{m}+2B^{m}+2C^{sm}+2D^{m}+2E^{sm}+2F^{m}+2^{sc}G^{m}+2H^{m}+2I^{m}+2^{sc}J^{sm}+2K^{m}$.

5), a pair of C chromosomes was observed with the secondary constriction consisting of the telomere, and the maximum number of nucleoli per cell was two.

Sequoia sempervirens (D. Don) Endl. (Figure 6) had the $2n$ chromosome number 66; 3 pairs of B chromosomes of distinctive shape did not all have the same form. Although 6 nucleoli per cell were observed, this species is considered to be allo-hexaploid because of the size differences.

Species Group III

The diploid chromosome number of *Taxodium mucronatum* Ten. is 22; 2 pairs of chromosomes had secondary constrictions (Figure 7). A maximum of 4 nucleoli per cell was observed.

A study of *Taxodium mucronatum* is still under way. Previously the karyotype would have been classified as being in either Group I or Group II, judging by the structure of the trabant chromosome. However, I would place it in Group III, because it has one pair of chromosomes of Group I type (Köpfchen type) and one of Group II type (Telomere type).

Species Group IV

Three species in the genus *Athrotaxis* occur in Tasmania, visited by the author in February and March of 1990, 1991 and 1993. *Athrotaxis cupressoides* D. Don is often found at the water's edge, *Athrotaxis selagi-*

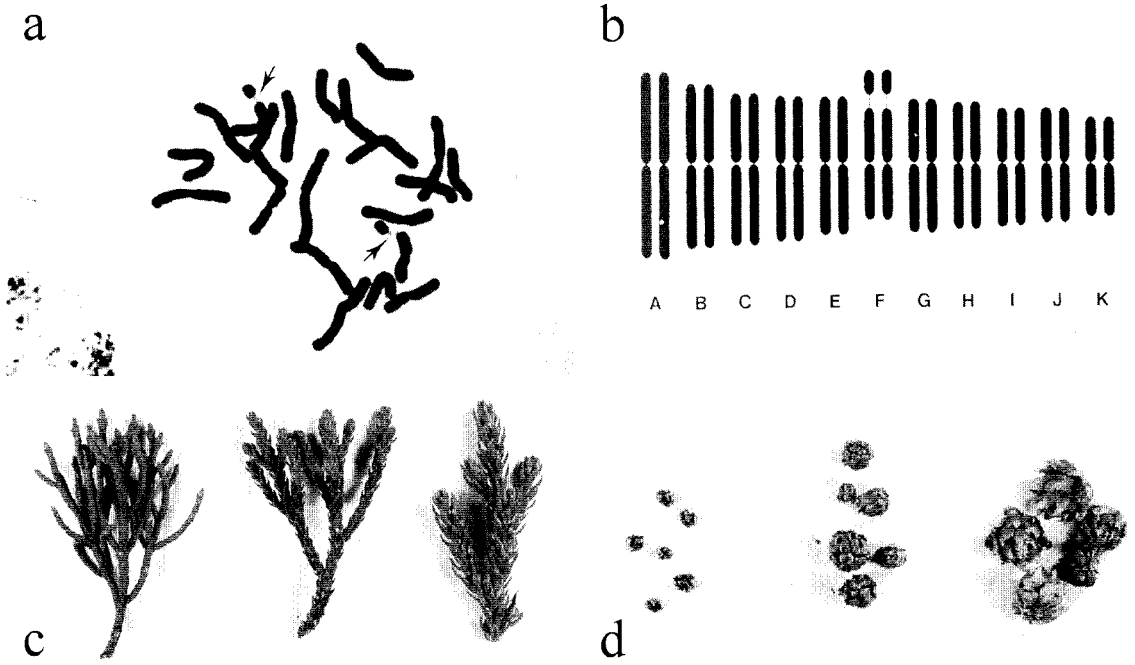

Figure 8 Karyomorphology of *Athrotaxis* (Group IV): **a** – somatic chromosomes of *A. selaginoides*; **b** – idiogram of karyotype in *A. selaginoides*; **c** – leaves (1 – *A. cupressoides*, 2 – *A. laxifolia*, 3 – *A. selaginoides*; **d** – cones (1, 2, 3 – as in **c**).

noides D. Don occurs in rocky areas, and *Athrotaxis laxifolia* Hook. is distributed between them. *A. laxifolia* is considered to be a natural hybrid of *A. cupressoides* and *A. selaginoides*. Leaf shape and fruit size of *A. laxifolia* are intermediate between *cupressoides* and *selaginoides*, shown in Figures 8c and 8d. Its fruit weight was also intermediate. The 3 species of *Athrotaxis* had the chromosome number $2n = 22$; one pair was found to have the typical secondary constriction (Figures 8a, 8b). The karyotype of these 3 species of *Athrotaxis* belongs to Group IV of *the Taxodiaceae*, because they have pairs of chromosomes with the secondary constrictions absent from Groups I, II and III. It is noteworthy that in *A. selaginoides*, the maximum number of nucleoli per cell was 2, in A. *laxifolia* 3, and in *A. cupressoides* 4. Study of this trait is continuing. The intermediate maximum of nucleoli found in *A. laxifolia* provides additional evidence that this species is a hybrid between *A. cupressoides* and *A. selaginoides*.

Table 1 shows the total number of chromosomes, the number of chromosomes with a secondary constriction, and the frequency of occurrence of various numbers of nucleoli per cell. There are indications of a positive relationship between the number of chromosomes with a secondary constriction and the maximum number of nucleoli per cell, except in *Taxodium mucronatum* and *Athrotaxis* spp.

The author suggests that the comparative study of species and genera would be advanced further by differential staining of chromosomes.

REFERENCES

TODA, Y., 1978: On the karyotype of *Cryptomeria japonica* D. Don. *Bull. of Kyushu Branch of Jap. For. Society* **32**:151-152.

TODA, Y., 1980: On the karyotype of *Cryptomeria japonica* D. Don. *Journal of the Jap. For. Society* **62**(7):264-269.

TODA, Y., 1983: On the karyotype of *Cryptomeria japonica* D. Don. *Proc. of the Fac. of Agr. Kyushu Tokai Univ.* **2**:19-24.

TODA, Y., 1985: Fundamental studies on cross breeding in *Cryptomeria japonica* D. DON by the karyotype analysis, *Japanese For. Breed. Association*, Tokyo, 140 pp.

TODA, Y., 1987: Intraspecific variation of the karyotype in *Cryptomeria japonica* D. Don. *Proc. Sino-Japanese Symposium on Plant Chromosome Research* 85-93.

TODA, Y., 1989: Karyological studies on *Taxodiaceae* trees. *Proc. of the 6th International Congress of SABRAO*, 857-860.

TODA, Y., 1992: Karyomorphology of Gymnosperms, Proc. 2nd Sino-Japanese Symposium on Plant Chromosomes, *Plant Chromosome Research* 233–239.

TODA, Y., 1993a: Studies on the intraspecific variation of karyotype in conifers. *Proc. of the XV. International Botanical Congress*, 511 p.

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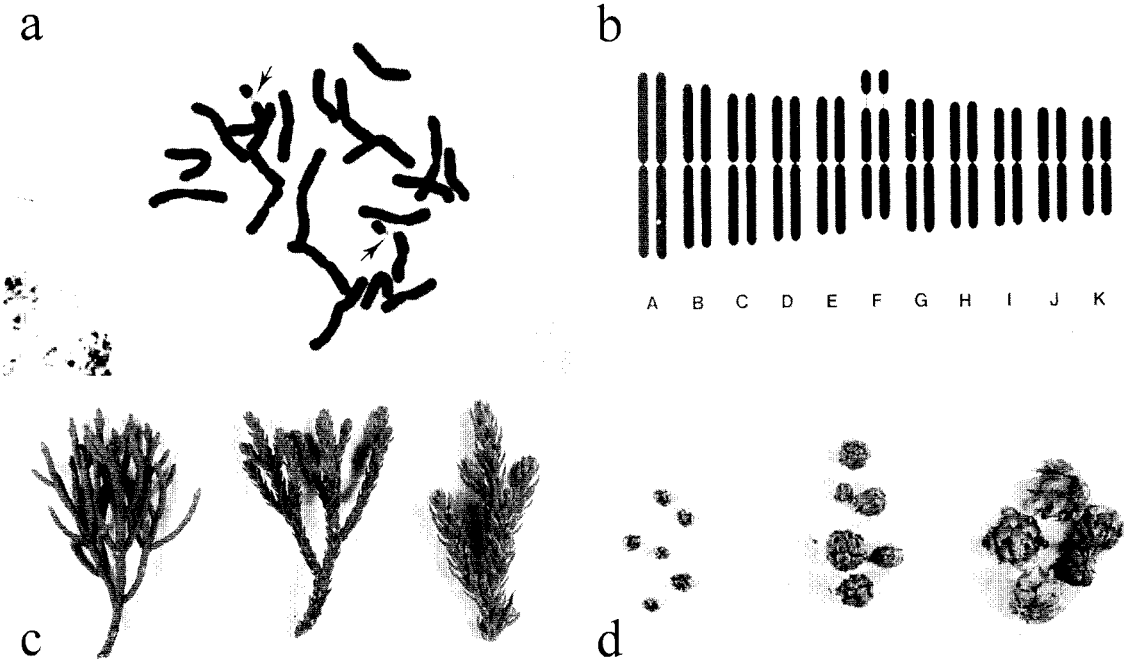

Figure 8 Karyomorphology of *Athrotaxis* (Group IV): **a** – somatic chromosomes of *A. selaginoides*; **b** – idiogram of karyotype in *A. selaginoides*; **c** – leaves (1 – *A. cupressoides*, 2 – *A. laxifolia*, 3 – *A. selaginoides*; **d** – cones (1, 2, 3 – as in **c**).

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REFERENCES

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TODA, Y., 1980: On the karyotype of *Cryptomeria japonica* D. Don. *Journal of the Jap. For. Society* **62**(7):264-269.

TODA, Y., 1983: On the karyotype of *Cryptomeria japonica* D. Don. *Proc. of the Fac. of Agr. Kyushu Tokai Univ.* **2**:19-24.

TODA, Y., 1985: Fundamental studies on cross breeding in *Cryptomeria japonica* D. DON by the karyotype analysis, *Japanese For. Breed. Association*, Tokyo, 140 pp.

TODA, Y., 1987: Intraspecific variation of the karyotype in *Cryptomeria japonica* D. Don. *Proc. Sino-Japanese Symposium on Plant Chromosome Research* 85-93.

TODA, Y., 1989: Karyological studies on *Taxodiaceae* trees. *Proc. of the 6th International Congress of SABRAO*, 857-860.

TODA, Y., 1992: Karyomorphology of Gymnosperms, Proc. 2nd Sino-Japanese Symposium on Plant Chromosomes, *Plant Chromosome Research* 233–239.

TODA, Y., 1993a: Studies on the intraspecific variation of karyotype in conifers. *Proc. of the XV. International Botanical Congress*, 511 p.

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