Fluorescent Chromosome Banding in the Genus *Epimedium*

TAKAHASHI Chigusa*

Introduction

The genus *Epimedium*, a member of the family Berberidaceae, which is composed of about twenty species, occurs from eastern Asia to the Mediterranean regions (Stearn 1938, 1990, 1993, 1995). Taxonomical and phylogenetical studies in *Epimedium* have been made by various researchers (Makino 1909, 1931, Koidzumi 1932, 1936, 1938, 1939, Maekawa 1932, 1955, Nakai 1944, 1953, Ohwi 1953, 1965, Yamanaka 1953, Shimizu 1960, Kitamura and Murata 1961, Ying 1975, Suzuki 1978, 1982, 1986, Ohwi and Kitagawa 1983, Wu and Qian 1985). However, these species are highly polymorphic in external morphology as interspecific sterility is very weak, which causes taxonomic complications for researchers.

In contrast to these variations of external morphology, a number of researchers found clear similarities in number, size and shape of chromosomes in all of the species reported (Langlet 1928, Miyaji 1930, Maude 1939, Suzuka 1953, Kurita 1956, Koyama 1965, Kuroki 1967, 1970, Ackerman 1976, Loon and Oudemans 1976, Kosenko 1979, Tören 1979, Loon 1980, Loon and Kieft 1980). Owing to a lack of karyological markers, it has also been impossible to clarify their taxonomical relationships from the cytological information.

Following earlier work, Tanaka and Takahashi (1981) and Takahashi (1989) produced a detailed karyotype of 28 taxa in *Epimedium* by the C-banding method. This method served as a useful aid to the understanding of interspecific relationships in this genus. The karyological groupings by C-bands of *Epimedium* studied correlated with differences in geographical occurrences.

It is important to know the base composition of heterochromatic regions revealed by the C-banding method. The use of fluorochromes for chromosome banding has been most useful for the information it provides about chromosome organization. The banding patterns produced by fluorochrome have been interpreted in terms of DNA base composition. In this paper, I applied the fluorescent banding method using base-specific fluorochromes 4'-6-diamidino-2-phenylindole (DAPI) and chromomycin A_3 (CMA) to demonstrate the base composition of the chromosomes of *Epimedium*. In addition, a comparison was made between the C-banding pattern and the fluorescent banding pattern.

*Department of Curriculum and Instruction, Course of School Education

Table 1. Localities and chromosome number of taxa in *Epimedium* studied

Taxa	Localities or Sources	No. of clones studied	Chromosome number (2n)
E. diphyllum (Morr. et Decne.) Lodd.			
	Shunda-Cho, Hiroshima Prefecture	6	12
	Sagawa-Cho, Kochi Prefecture	10	12
E. grandiflorum Morr. var. *thunbergianum* (Miq.) Nakai			
	Mt. Kushigata, Yamanashi Prefecture	1	12
	Shijyonawate City, Osaka Prefecture	10	12
E. sempervirens Nakai var. *hypoglaucum* (Makino) Ohwi			
	Echizen-misaki, Fukui Prefecture	6	12
	Mt. Hiei, Shiga Prefecture	10	12
	Mt. Naka-hiruzen, Okayama Prefecture	8	12
	Hiwa-Cho, Hiroshima Prefecture	5	12
	Kuchiwa-Cho, Hiroshima Prefecture	4	12
	Takano-Cho, Hiroshima Prefecture	4	12
E. trifoliatobinatum (Koidz.) Koidz.			
	Kochi-City, Kochi Prefecture	10	12
	Uwa-Cho, Ehime Prefecture	5	12
	Oshima-Cho, Yamaguchi Prefecture	10	12
E. acuminatum Franch.			
	Mt. Omei, Szechwan, China	1	12
	Shunso Garden	2	12
	Mori Alpines Trinity Garden	1	12
E. pubescens Maxim.			
	Mt. Ching cheng, Szechwan, China	1	12
E. sagittatum (Sieb. et Zucc.) Maxim.			
	Makino Herbarium, Tokyo Metropolitan Univ.	1	12
	Tsumura Laboratory	1	12
E. pinnatum Fisch. subsp. *ß colchicum* Boiss.			
	Arnold Arboretum, USA	2	12
	Kuroishi Botanical Garden	1	12
	Chugai Botanical Garden	1	12
E. perralderianum Coss.			
	Arnold Arboretum, USA	1	12
	Kuroishi Botanical Garden	1	12
E. alpinum Linn.			
	Kuroishi Botanical Garden	1	12
	Chuo Botanical Garden	2	12
	Chugai Botanical Garden	1	12
	Mori Alpines Trinity Garden	1	12

Materials and Methods

Plant materials

Ten taxa of *Epimedium* were investigated karyomorphologically. The localities and sources of the materials are listed in Table 1. Taxonomic treatment followed Shimizu (1960), Stearn (1938, 1990, 1993, 1995), Suzuki (1982, 1986) and Ying (1975).

Chromosomal preparations

Root tips were treated with 0.002 M 8-hydroxyquinoline at 18-20℃ for 2.5-3 hours and fixed in ethanol : glacial acetic acid (3 : 1 v/v) at 5℃ overnight. The fixed root tips were macerated in 45% acetic acid at 60℃ for 10 minutes. After washing in distilled water, meristematic tissues were squashed in 45% acetic acid on clean slides. The slides were air-dried after removing the cover slips by the dry-ice technique.

Sequential Fluorochrome Staining

Staining procedures followed Schweizer (1976, 1983) and Hizume *et al.* (1992) with some minor modifications. Chromomycin A_3 (CMA, 0.1mg/ml) was used for fluorochrome showing base specificity for A+T-rich DNA and 4'-6-diamidino-2-phenylindole (DAPI, 0.1 μg/ml) for G+C-rich DNA. Prior to fluorochrome staining, the slides were treated with counterstain reagents binding for opposite bases to increase contrast, distamycin A (0.1 mg/ml) for CMA and actinomycin D (0.25mg/ml) for DAPI.

Fluorescence was viewed with an epifluorescent microscope equipped with the B filter for CMA and UV filter for DAPI, respectively.

Results

1. *Epimedium diphyllum* (Morr. et Decne.) Lodd.

Sixteen plants of this species were examined from two localities (Table1). These plants were morphologically placed within the species ranges. The chromosome number in the 12 plants was 2n=12. The 12 chromosomes varied in length from 7.0 μm to 5.0 μm and were classified into six pairs which were grouped into three pairs with median centromere (chromosome pair 1, 2and 3) and three pairs with submedian centromere (chromosome pair 4, 5 and 6). One of the metacentric pairs (chromosome pair 1) has a secondary constriction in the proximal side of the short arm. Thus, the karyotype obtained for this species by aceto-orcein staining was similar to that previously reported by Takahashi (1989).

The CMA-band was placed only at the secondary constriction of chromosome pair 1 (Figs. 1A and 11A-1). DAPI-bands were present in all of the somatic chromosomes (Figs. 1B and 11A). Their position and number are as follows. In chromosome pair 1, two DAPI-bands were located respectively in the interstitial region and in the terminal region of the long arm. The interstitial band was thick. In

chromosome pair 2, two DAPI-bands were located respectively in the terminal region of the both arms. In chromosome pair 3, two DAPI-bands were located respectively in the interstitial region and in the terminal region of the long arm. In chromosome pair 4, DAPI-bands were located in the interstitial and terminal regions of the long arm. One chromosome showed three interstitial bands, while the other chromosome showed two. In chromosome pair 5, DAPI-bands were observed in the interstitial region and in the terminal region of the long arm. In the interstitial region, one chromosome showed two bands, while the other chromosome showed one. In chromosome pair 6, two DAPI-bands were located in the interstitial region and one in the terminal region of the long arm.

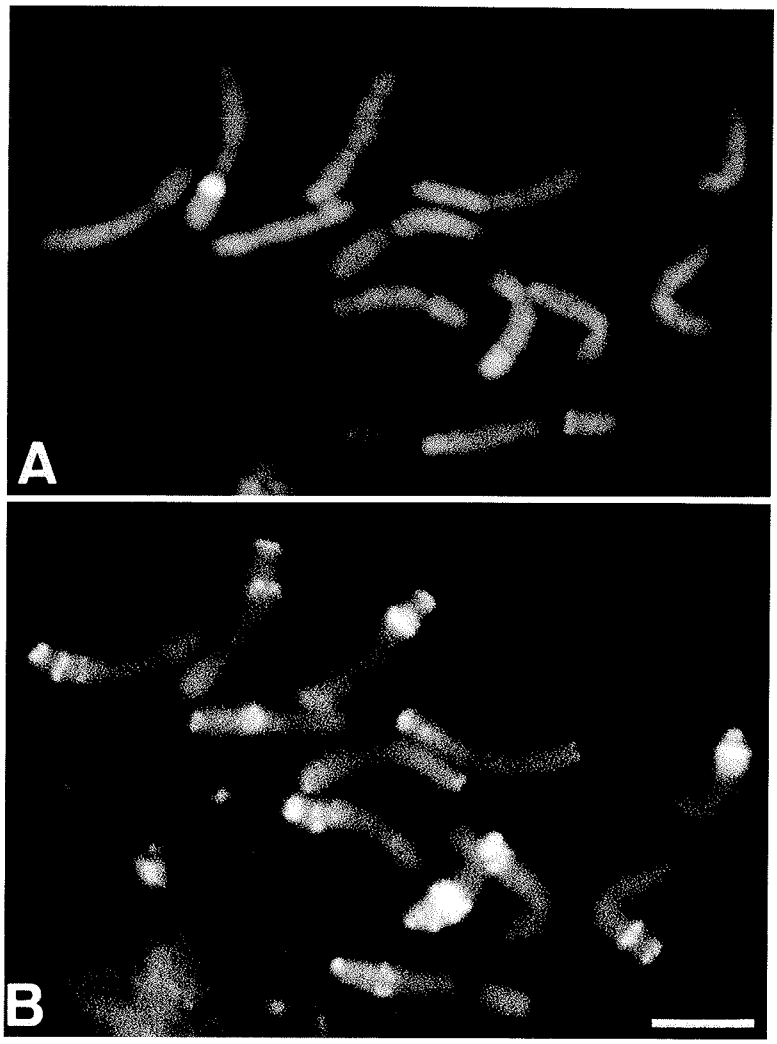

Fig. 1. Root tip metaphase chromosomes from *Epimedium diphyllum* after fluorochrome staining. A : CMA staining, B : DAPI staining. Scale bar represents 5 μm.

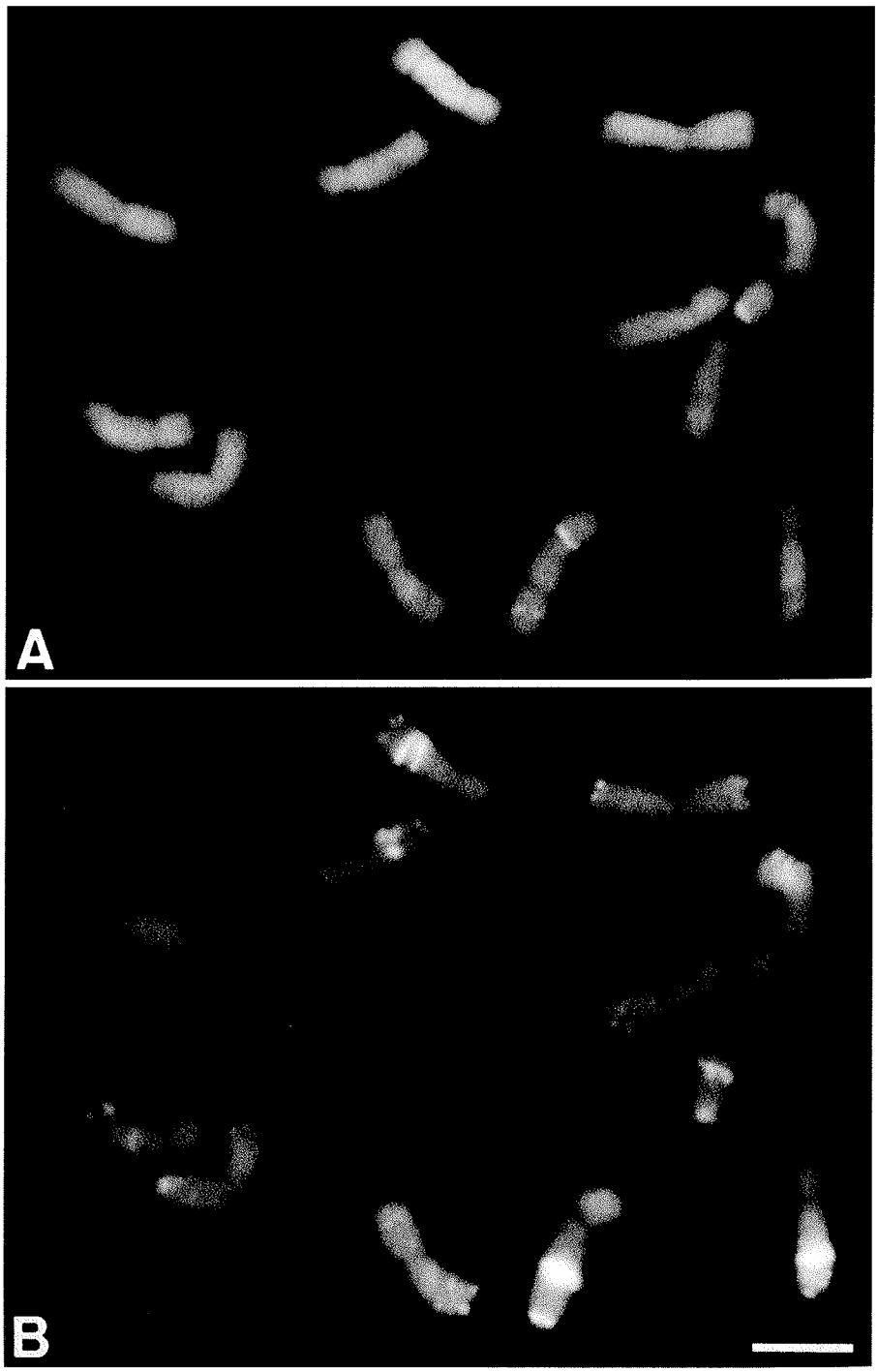

Fig. 2. Root tip metaphase chromosomes from *E. grandiflorum* var. *thunbergianum* after fluorochrome staining. A： CMA staining, B： DAPI staining. Scale bar represents 5 μm.

2. *E. grandiflorum* Morr. var. *thunbergianum*(Miq.)Nakai

Eleven plants of this taxon were examined from two localities(Table1). Morphological features of these plants were found to be typical. The chromosome number in all of the plants examined was 2n=12, and the shape and size of somatic chromosomes at the mitotic stage were similar to those of *E. diphyllum*.

The CMA-band was placed only at the secondary constriction of chromosome pair 1(Figs. 2A and 11B-1). DAPI-bands were present in all of the somatic chromosomes(Figs. 2B and 11B). Their position and number are as follows. In chromosome pair 1, two DAPI-bands were located respectively in the interstitial region and in the terminal region of the long arm. The interstitial band was thick. In chromosome pair 2, two DAPI-bands were located respectively in the terminal region of the both arms. In chromosome pair 3, each homologue showed a DAPI-band in the terminal region and a very thin band in the interstitial region of the long arm. Furthermore, one chromosome showed a DAPI-band in the terminal region of the short arm. In chromosome pair 4, two DAPI-bands were located in the interstitial region and one in the terminal region of the long arm. In chromosome pair 5, each homologue showed a DAPI-band in the terminal region of the long arm. One chromosome showed a very thin DAPI-band in the interstitial region of the long arm. For chromosome pair 6, two DAPI-bands were located in the interstitial region and one in the terminal region of the long arm.

3. *E. sempervirens* Nakai var. *hypoglaucum*(Makino)Ohwi

Thirty seven plants of this species were examined from six localities(Table 1). Morphological features of these plants are within the variety range. The chromosome number in all of the plants was 2n=12. The karyotype of this species was similar to that of the previous *E. diphyllum*.

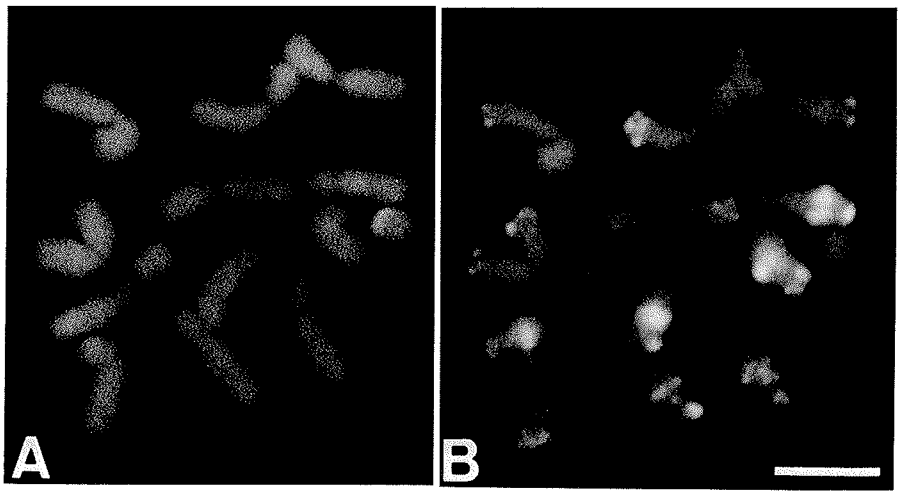

Fig. 3. Root tip metaphase chromosomes from *E. sempervirens* var. *hypoglaucum* after fluorochrome staining. A : CMA staining, B : DAPI staining. Scale bar represents 5 μm.

The CMA-band was placed only at the secondary constriction of chromosome pair 1 (Figs. 3A and 11C-1). DAPI-bands were present in all of the somatic chromosomes (Figs. 3B and 11C). Their position and number are as follows. In chromosome pair 1, two DAPI-bands were located in the interstitial region and in the terminal region of the long arm, respectively. The interstitial band was thick. In chromosome pair 2, two DAPI-bands were located in the terminal region of the both arms. In chromosome pair 3, each homologue showed a DAPI-band in the terminal region of the long arm. Furthermore, one chromosome showed a thin DAPI-band in the interstitial region of the long arm. In chromosome pair 4, DAPI-bands were observed in the interstitial region and in the terminal region of the long arm. In the interstitial region, one chromosome showed three bands, while the other chromosome showed two bands. In chromosome pair 5, one DAPI-band was located in the interstitial region near by the distal end of the long arm and one in the terminal region of the long arm. For chromosome pair 6, two DAPI-bands were situated close together in the interstitial region and one in the terminal region of the long arm.

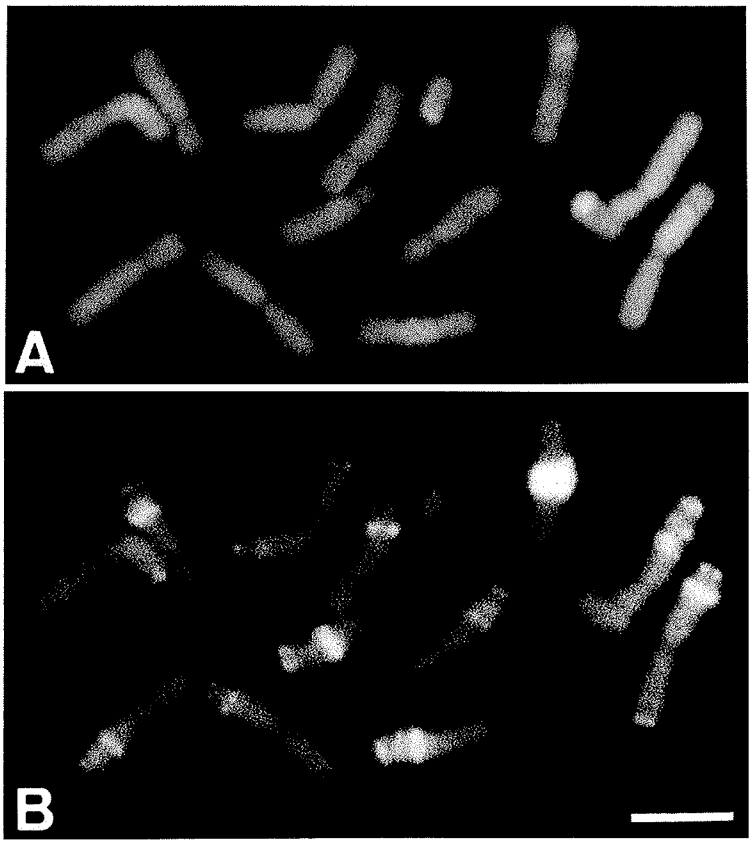

Fig. 4. Root tip metaphase chromosomes from *E. trifoliatobinatum* after fluorochrome staining. A : CMA staining, B : DAPI staining. Scale bar represents 5 μm.

4. *E. trifoliatobinatum*(Koidz.)Koidz.

Twenty five plants of this species were examined from three localities(Table 1). All of the plants had standard morphological characters within the species range. The chromosome number was 2n=12 and the somatic chromosomes at the mitotic stage were similar to those of *E. diphyllum*.

The CMA-band was placed only at the secondary constriction of chromosome pair 1(Figs. 4A and 11D-1). DAPI-bands were present in all of the somatic chromosomes(Figs. 4B and 11D). Their position and number are as follows. In chromosome pair 1, two DAPI-bands were located respectively in the interstitial region and in the terminal region of the long arm. The interstitial band was thick. In chromosome pair 2, three DAPI-bands were located respectively in the interstitial region of the long arm and in the terminal region of both arms. In chromosome pair 3, each homologue showed a DAPI-band in the terminal region of the long arm. One chromosome showed a DAPI-band in the interstitial region of the long arm. In chromosome pair 4, two DAPI-bands were located in the interstitial region and one in the terminal region of the long arm. In chromosome pair 5, two DAPI-bands were located respectively in the interstitial region and in the terminal region of the long arm. In chromosome pair 6, two DAPI-bands were located in the interstitial region and one in the terminal region of the long arm.

5. *E. acuminatum* Franch.

Four plants of this species were examined from three sources(Table 1). They showed typical external morphology such as leaves and flowers within the species ranges. The chromosome number was 2n=12. The orcein-stained somatic chromosomes at the mitotic stage were similar to those of *E. diphyllum*.

The CMA-band was placed only at the secondary constriction of chromosome pair 1(Figs. 5A and 12A-1). DAPI-bands were present in all of the somatic chromosomes(Figs. 5B and 12A). Their position and number are as follows. In chromosome pair 1, a DAPI-band was located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was widely distributed in the interstitial region of the long arm, about 1.5 μm in length. In chromosome pair 2, a cluster of pale DAPI-bands resembling very small dots was widely distributed about 1.5 μm in length in the short arm and about 2.0 μm in length in the long arm. In one chromosome, two weak DAPI-bands were located respectively in the terminal region of both arms. In chromosome pair 3, a DAPI-band was located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands was widely distributed in both arms about 1.5 μm in length. In one chromosome, a weak DAPI-band was found in the terminal region of the long arm. In chromosome pair 4, two DAPI-bands were located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was scattered in the short arm, about 0.5 μm in length. In chromosome pair 5, a DAPI-band was located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was scattered in the short arm, about 0.5 μm in length. In one chromosome, a weak DAPI-band was found in the terminal region of the long arm. In chromosome pair 6, two DAPI-bands were located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very

small dots was widely scattered in the short arm, about 1.0 μm in length.

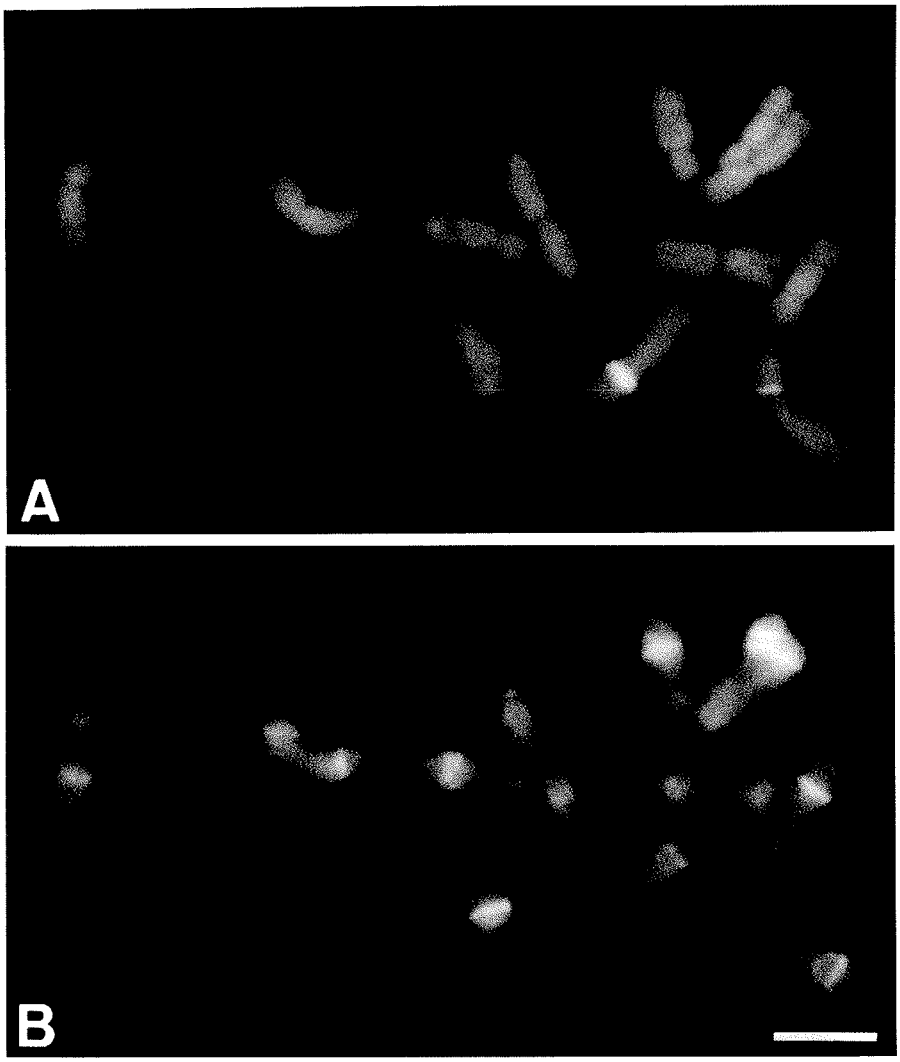

Fig. 5. Root tip metaphase chromosomes from *E. acuminatum* after fluorochrome staining. A： CMA staining, B： DAPI staining. Scale bar represents 5 μm.

6. *E. pubescens* Maxim.

One plant of this species was examined from one locality (Table 1). Morphological characters of the plant were found to be typical. The chromosome number was 2n=12 and orcein-stained somatic chromosomes at the mitotic stage were similar to those of *E. diphyllum*.

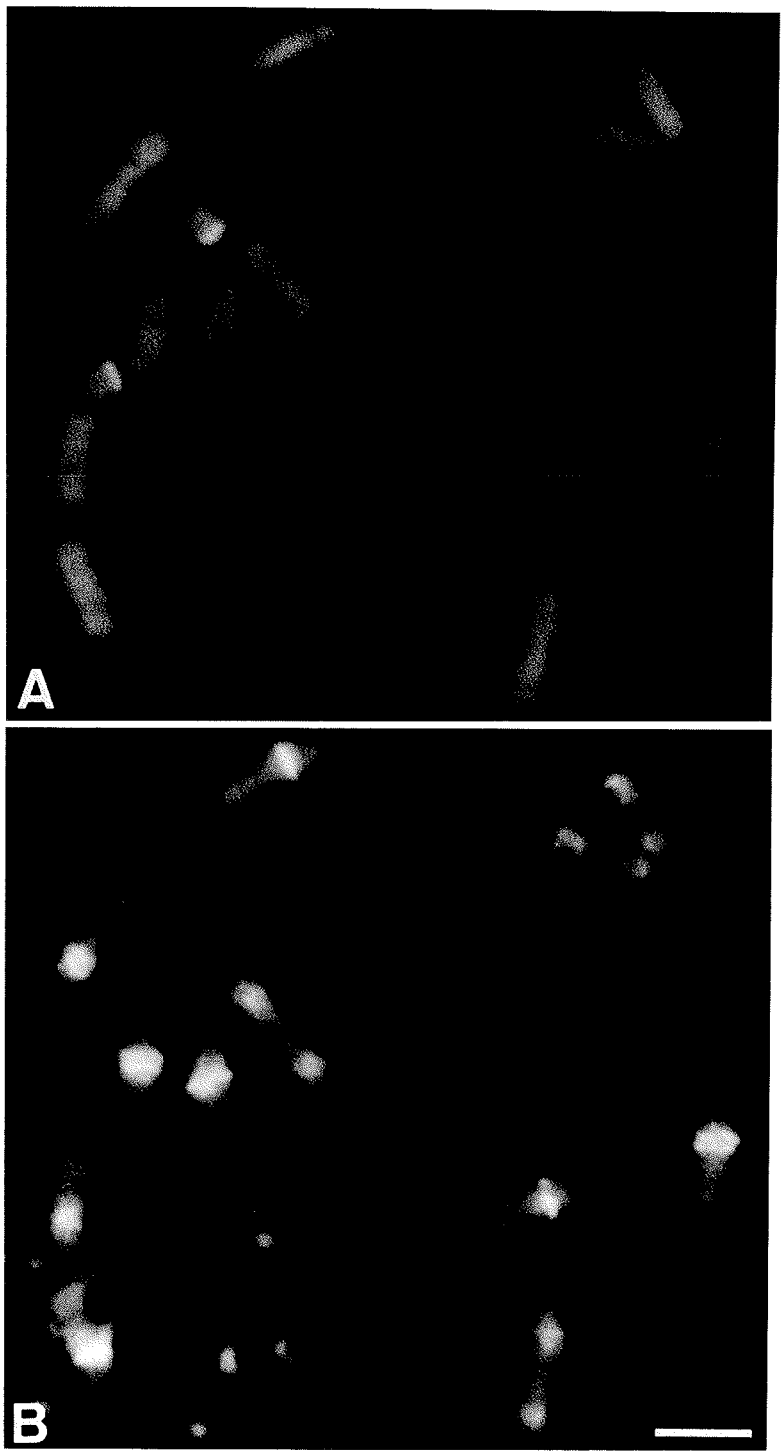

Fig. 6. Root tip metaphase chromosomes from *E. pubescens* after fluorochrome staining. A : CMA staining, B : DAPI staining. Scale bar represents 5 μm.

The CMA-band was placed only at the secondary constriction of chromosome pair 1 (Figs. 6A and 12B-1). DAPI-bands were present in all of the somatic chromosomes (Figs. 6B and 12B). Their position and number are as follows. In chromosome pair 1, two DAPI-bands were located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was widely dispersed in the long arm, 1.0 μm in length. In one chromosome, a weak DAPI-band was located in the terminal region of the long arm. In chromosome pair 2, a weak DAPI-band was located in the terminal region of the long arm. A cluster of pale DAPI-bands resembling very small dots was widely scattered in the short arm, 1.5 μm in length and in the long arm, 2.0 μm in length. In chromosome pair 3, a chromosome exhibited two DAPI-bands in the interstitial region of the long arm and a cluster of pale DAPI-bands was scattered in the interstitial region of the short arm, less than 0.5 μm in length. The other chromosome exhibited only one DAPI-band in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was widely scattered in both arms about 1.5 μm in length. In chromosome pair 4, two DAPI-bands were located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was scattered in the short arm, less than 0.5 μm in length and in the long arm, 0.5 μm in length. In chromosome pair 5, a DAPI-band was located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small-sized dots was scattered in both arms about 0.5 μm in length. In one chromosome, a weak DAPI-band was located in the terminal region of the long arm. In chromosome pair 6, two DAPI-bands were located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was scattered in the short arm, 1.0 μm in length. In one chromosome, a weak DAPI-band was located in the terminal region of the long arm.

7. *E. sagittatum* (Sieb. et Zucc.) Maxim.

Two plants of this species were examined from two sources (Table 1). They were morphologically placed with the typical species ranges. The chromosome number in all of the plants examined was 2n=12. The karyotype of this species by aceto-orcein staining was similar to that of the previous *E. diphyllum*.

The CMA-band was placed only at the secondary constriction of chromosome pair 1 (Figs. 7A and 12C-1). DAPI-bands were present in all of the somatic chromosomes (Figs. 7B and 12C). Their position and number are as follows. In chromosome pair 1, one chromosome exhibited two DAPI-bands in the interstitial region of the long arm, while the other chromosome exhibited one DAPI-band. In addition, a cluster of pale DAPI-bands resembling very small dots was widely dispersed in the long arm, 1.5 μ-m in length. In chromosome pair 2, a cluster of pale DAPI-bands resembling very small dots was widely distributed about 1.5 μm in distance in the short arm and about 2.0 μm in length in the long arm. In chromosome pair 3, a DAPI-band was located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was widely distributed about 1.5 μm in length in both arms. In chromosome pair 4, two DAPI-bands were located in the interstitial region of the long arm. A weak DAPI-band was located in the terminal region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was scattered in the short arm, 0.5 μm in length.

In chromosome pair 5, a DAPI-bands were located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small-sized dots was scattered in the short arm, 0.5 μm in length. In one chromosome, a weak DAPI-band was located in the terminal region of the long arm. In chromosome pair 6, two DAPI-bands were located in the interstitial region of the long arm. In addition, a cluster of pale DAPI-bands resembling very small dots was scattered in the short arm, 1.0 μm in length.

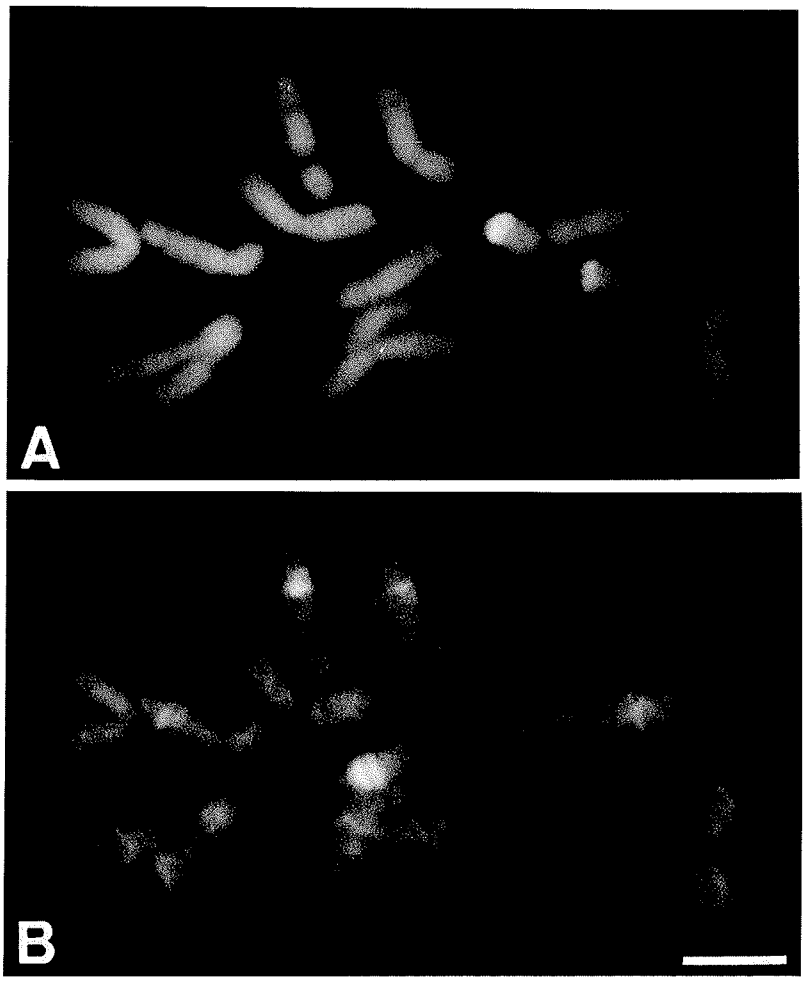

Fig. 7. Root tip metaphase chromosomes from *E. sagittatum* after fluorochrome staining. A : CMA staining, B : DAPI staining. Scale bar represents 5 μm.

8. *E. pinnatum* Fisch. subsp. *β colchicum* Boiss.

Four plants of this taxon were examined from three sources (Table 1). All of the plants were morphologically placed within the taxon ranges without any exception. The chromosome number in all of the five plants was 2n=12, and the chromosomes at the mitotic metaphase were similar to those of *E. diphyllum*.

The CMA-band was placed only at the secondary constriction of chromosome pair 1 (Figs. 8A and 13A-1). DAPI-bands were present in all of the somatic chromosomes (Figs. 8B and 13A). Their position and number are as follows. In chromosome pair 1, DAPI-bands were observed in the interstitial region and in the terminal region of the long arm. One chromosome showed two DAPI-bands in

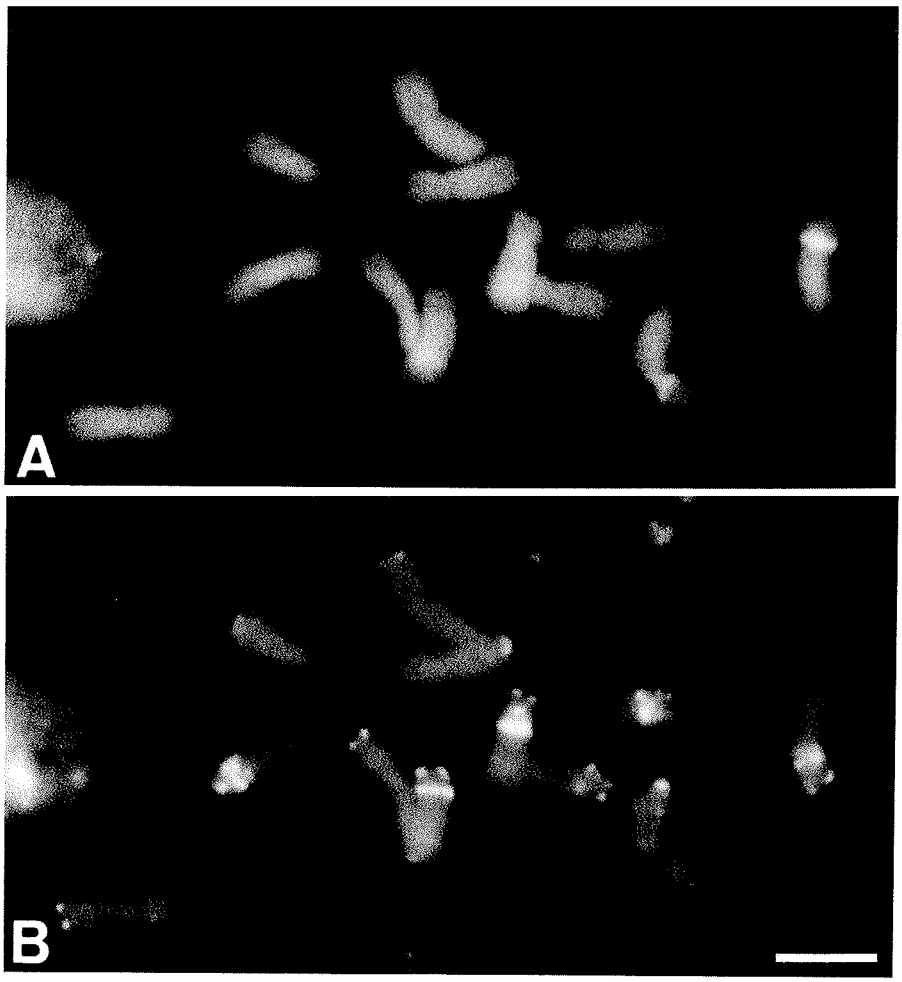

Fig. 8. Root tip metaphase chromosomes from *E. pinnatum* subsp. *ß colchicum* after fluorochrome staining. A : CMA staining, B : DAPI staining. Scale bar represents 5 μm.

the interstitial region, while the other chromosome did not display any DAPI-bands in that region. In chromosome pair 2, two DAPI-bands were located respectively in the terminal region of the both arms. In chromosome pair 3, a DAPI-band was located in the terminal region of the long arm. In chromosome pair 4, three DAPI-bands were located very close together in the interstitial region and one in the terminal region of the long arm. In chromosome pair 5, each homologue showed a DAPI-band in the terminal region of the long arm. One chromosome showed a weak DAPI-band in the interstitial region of the long arm. In chromosome pair 6, DAPI-bands were observed in the interstitial region and in the terminal region of the long arm. In the interstitial region, one chromosome showed two bands, while the other chromosome showed one.

9. *E. perralderianum* Coss.

Two plants of this species were examined from two sources (Table 1). All of the plants were morphologically placed with the typical species ranges. The chromosome number of the species was 2n=12 and orcein-stained chromosomes at the mitotic stage were similar to those of *E. diphyllum*.

The CMA-band was placed only at the secondary constriction of chromosome pair 1 (Figs. 9A and 13B-1). DAPI-bands were present in all of the somatic chromosomes (Figs. 9B and 13B). Their position and number are as follows. In chromosome pair 1, 2, 3, 4 and 5, DAPI-bands were observed only in the terminal region of the long arm. In chromosome pair 6, two DAPI-bands were located respectively in the interstitial region and terminal regions of the long arm.

10. *E. alpinum* Linn.

Five plants of this species were examined from four sources (Table 1). Morphological features of these plants were within the species ranges. The chromosome number in the plants was 2n=12 and the chromosome length and position of the centromere at the mitotic stage were similar to those of *E. diphyllum*.

The CMA-band was placed only at the secondary constriction of chromosome pair 1 (Figs. 10A and 13C-1). DAPI-bands were present in all of the somatic chromosomes (Figs. 10B and 13C). Their position and number are as follows. In chromosome pair 1, two DAPI-bands were located respectively in the interstitial region and in the terminal region of the long arm. The interstitial band was smaller than those of other species. In chromosome pair 2, three DAPI-bands were located respectively in the interstitial region of the long arm and in the terminal region of both arms. In chromosome pair 3, two DAPI-bands were located respectively in the interstitial region and in the terminal region of the long arm. In chromosome pair 4, two DAPI-bands were located in the interstitial region and one in the terminal region of the long arm. In chromosome pair 5, DAPI-bands were observed in the interstitial region and in the terminal region of the long arm. In the interstitial region, one chromosome showed two bands while the other chromosome showed one. In chromosome pair 6, DAPI-bands were observed in the interstitial region and in the terminal region of the long arm. In the interstitial region, one chromosome showed two bands while the other chromosome showed one.

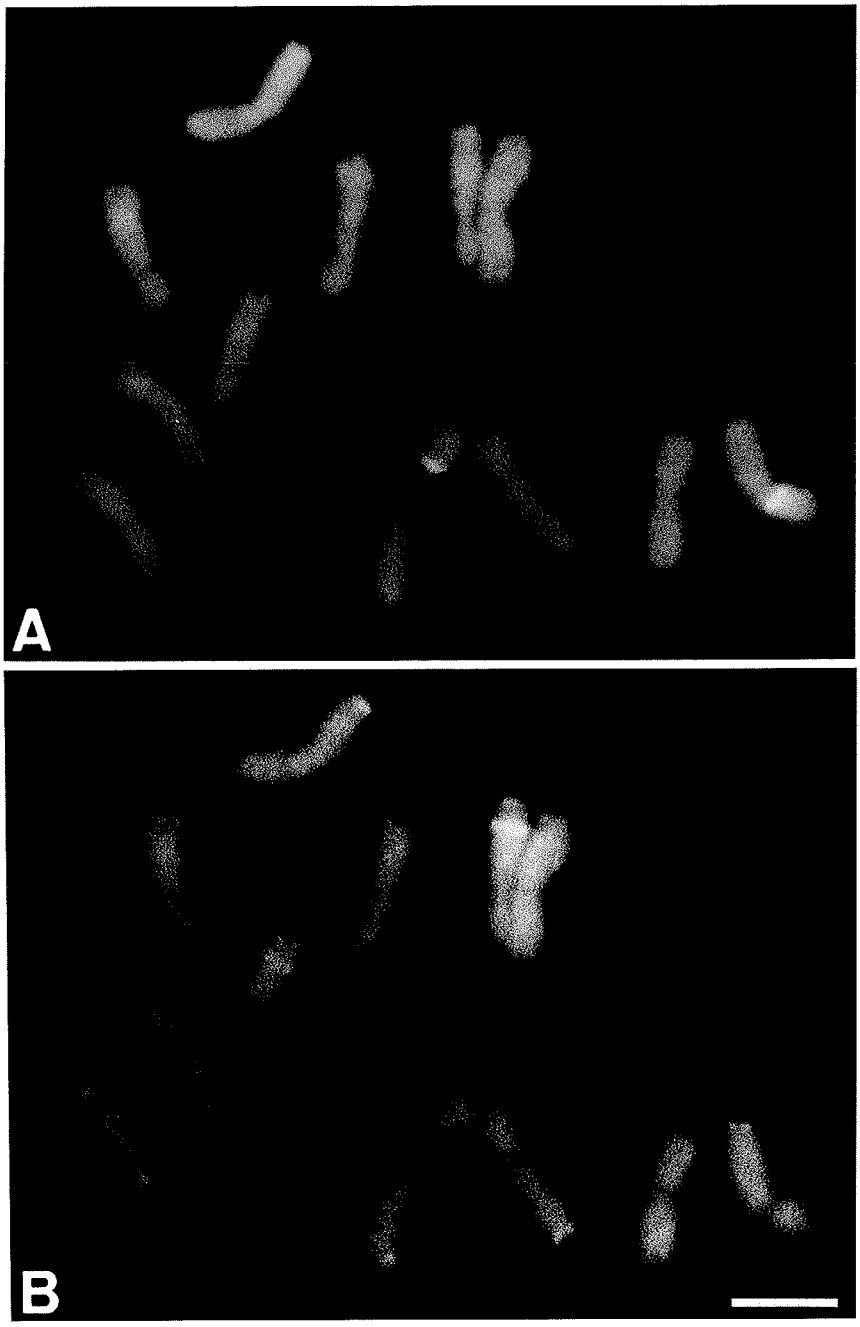

Fig. 9. Root tip metaphase chromosomes from *E. perralderianum* after fluorochrome staining. A： CMA staining, B： DAPI staining. Scale bar represents 5 μm.

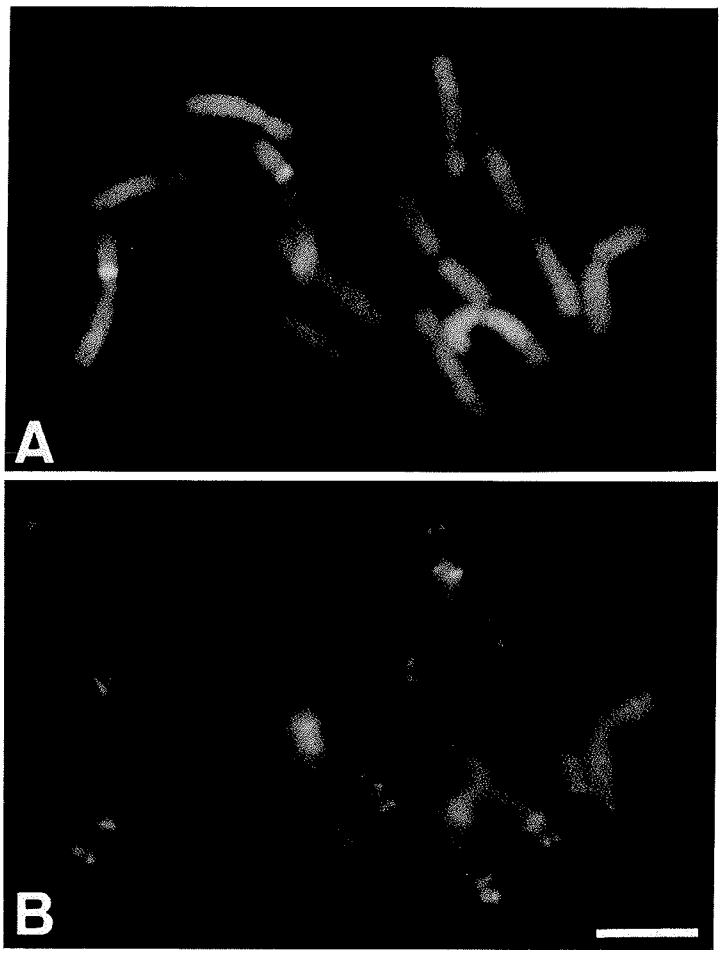

Fig. 10. Root tip metaphase chromosomes from *E. alpinum* after fluorochrome staining. A : CMA staining, B : DAPI staining. Scale bar represents 5 μm.

Discussion

The fluorescent banding sequentially using CMA and DAPI allowed the identification of individual chromosomes in *Epimedium*. Furthermore, the base composition of the banded regions became clear using this banding technique.

Sumner (1990) described all C-bands as heterochromatic, but some heterochromatin is not stained by C-banding methods. Therefore, some fluorescence methods may reveal bands not demonstrated

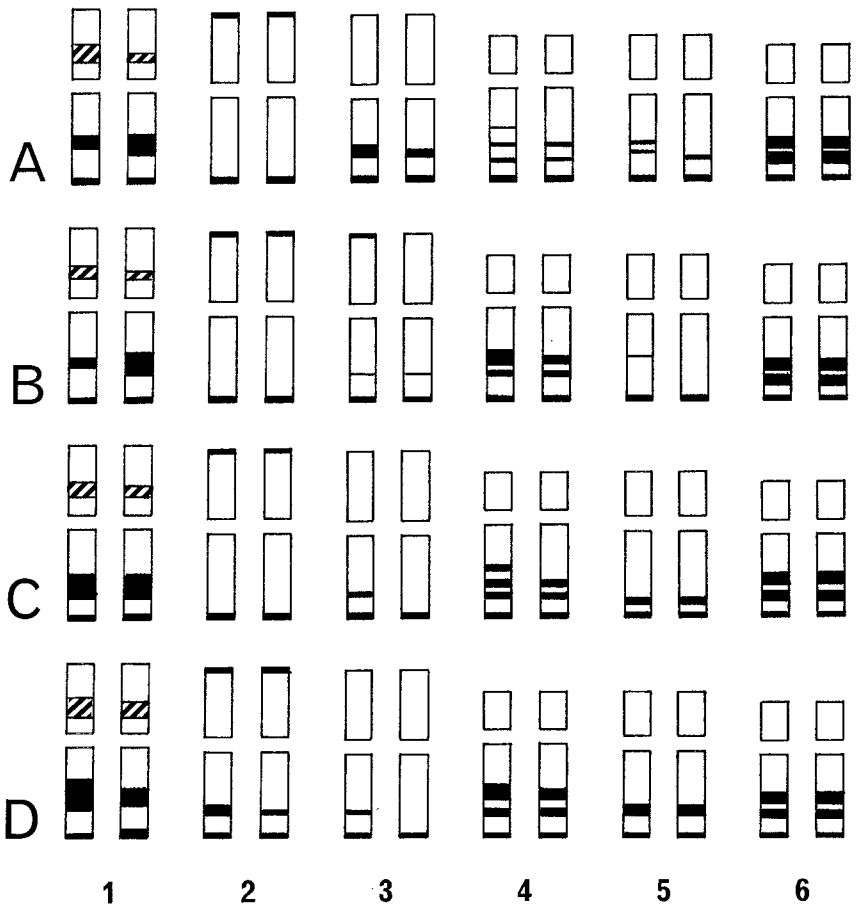

Fig. 11. Idiograms of the fluorescent banded chromosomes in four taxa of *Epimedium* from Japan. A : *E. diphyllum*, B : *E. grandiflorum* var. *thunbergianum*, C : *E. sempervirens* var. *hypoglaucum*, D : *E. trifoliatobinatum*. The hatched regions represent CMA-bands and the solid regions represent DAPI-bands.

by C-banding. In pine, Hizume *et al.* (1989) also found that thin CMA- and DAPI-bands were not detected at all by the C-banding technique. The present results reported here did not correspond with these. In *Epimedium*, fluorescent bands which were not stained by C-banding although C-bands which did not show differential staining with either type of fluorochrome were found. The size and distribution of fluorescing DAPI segments in the present work agreed well with those found previously using C-banding except that the terminal regions of the chromosomes did not stain strongly in *E. acuminatum*, *E. pubescens* and *E. sagittatum* of chinese species. However, such correspondence did not extend to the bands in the centromeric regions. Thus, no centromeric Giemsa C-bands noted previously were detected by the fluorescent banding method used in the present work. This result suggests that the base composition of heterochromatin at the centromeric region in *Epimedium* species is repetitive DNA, neither GC-rich nor AT-rich. The CMA-bands clearly coincided in position with the secondary constriction of the satellited chromosomes (chromosome pair 1) in all of the species investigated. These bands could be related to the brightly fluorescent nucleolus associated dots seen at the interphase. The CMA-banded regions were negative in DAPI-banding. It was concluded that the secondary constricted regions of *Epimedium* chromosomes are C-band positive and contain GC-rich DNA.

From the results described above, it was possible to distinguish three different types of hetero-

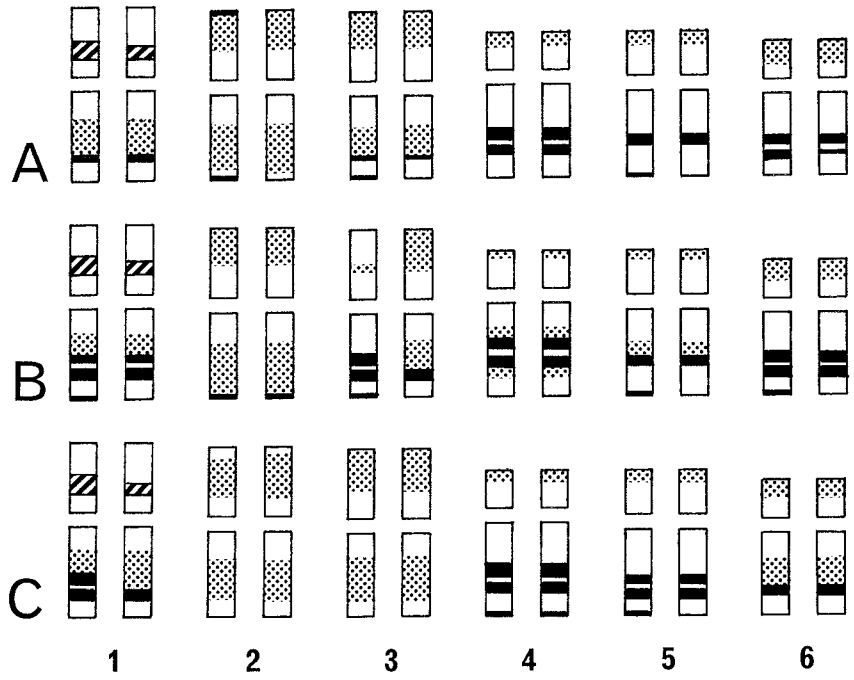

Fig. 12. Idiograms of the fluorescent banded chromosomes in three taxa of *Epimedium* from China. A : *E. acuminatum*, B : *E. pubescens*, C : *E. sagittatum*. The hatched regions represent CMA-bands and the solid regions represent DAPI-bands. The dotted regions represent a cluster of pale DAPI-bands.

chromatin in the karyotypes of *Epimedium* : C-band positive / CMA positive, C-band positive / DAPI positive and C-band positive / CMA negative / DAPI negative.

With regard to position, number, size and total length of CMA- and DAPI-bands, ten taxa of *Epimedium* could be divided into three types. Type 1 had more bands in the interstitial region than Type 3, especially unique bands in the interstitial regions characterized by a cluster of very small, dot-like bands spread throughout every chromosome. Type 1 was observed in *E. acuminatum*, *E. pubescens* and *E. sagittatum* (Fig. 11). Type 2 had more bands in the interstitial region than Type 3, especially large DAPI-bands in the satellited chromosomes (chromosome pair 1). Type 2 was observed in *E. diphyllum*, *E. grandiflorum* var. *thunbergianum*, *E. sempervirens* var. *hypoglaucum* and *E. trifoliatobinatum* (Fig. 12). Type 3 had fewer bands in the interstitial region than the other types, especially no or only thin DAPI-bands in the satellited chromosomes (chromosome pair 1). Type 3 was observed in *E. pinnatum* subsp. *ß colchicum*, *E. perralderianum* and *E. alpinum* (Fig. 13). The species of Type 1 are distributed in China, Type 2 in Japan and Type 3 in the Mediterranean regions. Thus, the karyological groupings by DAPI-bands can be correlated with differences in geographical distribution rather than with differences in morphological characteristics. These were compatible with the grouping by C-bands.

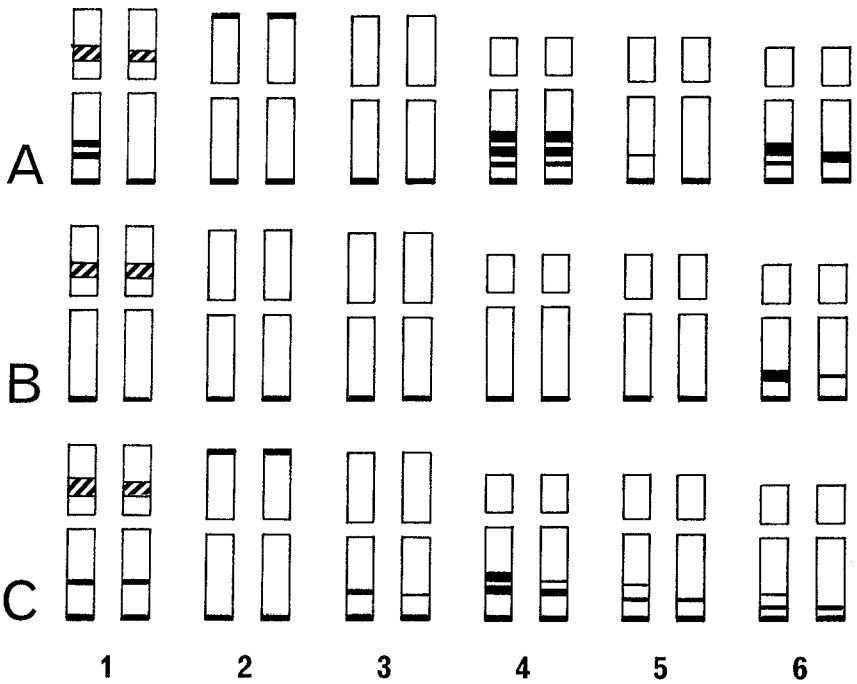

Fig. 13. Idiograms of the fluorescent banded chromosomes in three taxa of *Epimedium* from the Mediterranean regions. A : *E. pinnatum* subsp. *ß colchicum*, B : *E. perralderianum*, C : *E. alpinum*. The hatched regions represent CMA-bands and the solid regions represent DAPI-bands.

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