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Numerical analyses of karyotypic diversity in the genus *Eupatorium* (*Compositae, Eupatorieae*)

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Abstract: Somatic metaphase karyotypes were analyzed for 22 diploid species of *Eupatorium*. The karyotypic comparisons were made using two indices: minimal chromosomal distance (MCD), measuring overall dissimilarities, and chromosomal identity (CI), measuring number of morphologically identical chromosomes between species. The resulting phenograms from these indices are largely compatible. The 22 species cluster into four groups in the phenogram using MCD, and the grouping corresponds well with morphology or geographic distribution into the three N. American groups *Eutrochium, Uncasia, Traganthes*, and the E. Asian group. These results suggest that karyotypes in perennial *Eupatorium* have been considerably conservative and changed not through large chromosomal mutations but through small chromosomal mutations gradually fixed.

Karyotypic analyses have been useful in clarifying phylogenetic and evolutionary relationships between some related species and species groups (STEBBINS 1971, JACKSON 1971, WATANABE & SMITH-WHITE 1987). Where differences in karyotypes between taxa are not distinct, however, it has been difficult to evaluate their differences using conventional methods, and interpretations of the differences have often been criticized for lack of statistical analysis (DUNCAN & SMITH 1978). Several indices measuring karyotypic differences are available if homologies of chromosomes and chromosome arms are accurately ascertained (BIANCHI & MERANI 1984, BIANCHI & al. 1988). It is, however, hardly possible to obtain definite evidence on chromosome homologies in plants because G-banding techniques are unmature. Recently, MEEROW (1987) attempted at quantifying karyotypic differences among three genera of *Amaryllidaceae* using multivariate analysis. However, his method is based on classification of chromosomes into artificial categories. More objective method for assessing karyotypic differences is needed. To answer this deficiency, we developed numerical methods for describing karyotypic difference or similarities and applied them to the genus *Eupatorium*.

Eupatorium comprises c. 45 species with 22 in N. America (mainly in the southeastern United States), c. 22 in E. Asia, and one occurring from western India to Europe. This distribution matches a well-known disjunction pattern of many temperate species called Arcto-Tertiary elements (LI 1952, GRAHAM 1972, BOUFFORD

& SPONGBERG 1983). According to KING & ROBINSON (1970b), the origin of *Eupatorium* was probably in N. America, where the greatest number of species and nearest related genera occur, and the genus then extended its range to the temperate regions of E. Asia and Europe very late in the Tertiary. Understanding the phylogeny and evolution of the genus *Eupatorium* will provide a better understanding of the evolution of the temperate flora in the Northern Hemisphere in general.

N. American eupatoria are placed in three groups without nomenclatural rank based on morphology (KING & ROBINSON 1987): (1) the *Eutrochium* group comprises four species having nondissected verticillate leaves, enlarged style bases, stomata on the corolla lobes and reddish purple flowers; the species are *E. dubium* WILLD., *E. fistulosum* J. BARRATT, *E. maculatum* L., and *E. purpureum* L.: (2) the *Uncasia* group comprises 14 species having nondissected decussate leaves; the species are *E. album* L., *E altissimum* L., *E. cuneifolium* WILLD., *E. hyssopifolium* L., *E. lancifolium* (TORREY & A. GRAY) SMALL, *E. leucolepis* TORREY & A. GRAY, *E. mikanioides* CHAPMAN, *E. perfoliatum* L., *E. pilosum* WALTER, *E. resinosum* TORRY & A. GRAY, *E. rotundifolium* L., *E. semiserratum* DC., *E. serotinum* MICHX. and *E. sessilifolium* L.; and (3) the *Traganthes* group comprises three species with finely dissected alternate or decussate leaves; the species are *E. capillifolium* (LAM.) SMALL, *E. compositifolium* WALTER, and *E. leptophyllum* DC.

In the contemporary classification of *Eupatorium* s. str. (KING & Robinson 1970b, 1987), groupings of the E. Asian species and *E. cannabinum* L. were not made. The Asian species are less well understood than those from N. America. Taxonomic studies of these species are now in progress. There are at least 22 species in E. Asia (KAWAHARA & al. 1989). Among them, one species, *E. glehni* FR. SCHM. & TRAUTV. has nondissected verticillate leaves. The remaining species have dissected or nondissected decussate leaves. *Eupatorium cannabinum* has dissected decussate leaves and is isolated geographically from the E. Asian and N. American species because it occurs in Europe, N. Africa, Asia Minor, and western India.

Karyotypes of Asian *E. chinense* var. *oppositifolium* (KOIDZ.) MURATA & KOYAMA, *E. glehni, E. lindleyanum* DC., *E. variabile* MAKINO, and *E. yakushimense* MASAMUNE & KITAMURA have been reported (HUZIWARA 1956, 1968; KAWANO 1961; WATANABE & al. 1982). These workers, however, did not give detailed data for karyotypic comparisons between the taxa. Karyotypes of the other species are reported here for the first time. Also, the karyotypes of 13 N. American species, 8 Asian species and *E. cannabinum* are described and analyzed based on two indices, minimal chromosomal distance and chromosomal identity. The following questions are addressed: (1) Do the two indices give compatible results in phenetic analyses?, (2) Do the three morphological groups in N. America cluster according to karyotypic differences or similarities?, (3) Do Asian species cluster together?, (4) To which group does *E. cannabinum* belong?

Materials and methods

Sources of materials examined are listed in Table 1. Plants of *E. cannabinum, E. dubium, E. fistulosum, E. maculatum, E. perfoliatum, E. purpureum, E. serotinum,* and *E. semiserratum* were grown from achenes, whereas plants of all other taxa were transplanted in the greenhouse of Kobe University. Root tips were treated with 0.004 M 8-hydroxyquinoline at 18 to 20 °C for 2 h. They were then macerated and stained in a mixture of 2% aceto-orcein (9 parts) and 1 N HCl (1 part) overnight at 5 °C. Subsequently, small glass bottles containing

Table 1. Sources of *Eupatorium* spp. investigated and number of individuals examined (No.)

Taxa	No.	Sources
N. American		
E. album L.	1	East of Aucilla River Florida U.S.A.
E. capillifolium (LAM.) SMALL	1	Chicot State Park, Evangeline Parish, Louisiana, U.S.A.
E. compositifolium WALTER	2	0.7 mi west of Suwannee River, Florida, U.S.A.
E. dubium WILLD.	5	Arnold Arboretum, Cambridge, Massachusetts, U.S.A.
E. fistulosum J. BARRATT	3	Morain State Park, Bulter, Pennsylvania, U.S.A.
E. hyssopifolium L.	1	0.7 mi west of Suwannee River, Florida, U.S.A.
E. maculatum L.	1	U.S.A. (from York Co. by seed exchange)
E. perfoliatum L.	4	Powdermill Nature Reserve, Westmoreland Co., Pennsylvania, U.S.A.
E. purpureum L.	5	Arnold Arboretum, Cambridge, Massachusetts, U.S.A.
E. recurvans SMALL	3	10 mi north of Switzerland, along FL 13, Duval Co., Florida, U.S.A.
E. rotundifolium L.	2	10 mi north of Switzerland, along FL 13, Duval Co., Florida, U.S.A.
E. semiserratum DC.	1	9 mi east of Chipola River, Jackson Co., Florida, U.S.A.
E. serotinum MICHX.	3	U.S.A. (from R. M. KING)
Asian		
E. chinense L. var. *oppositifolium* (KOIDZ.) MURATA & H. KOYAMA	5	Mt Tamakiyama, Nara Pref.; Sandankyo-gorge, Hiroshima Pref.; Nagoro, Tokushima Pref.; Mt Raizan and Mt Homanzan, Fukuoka Pref., Japan
E. formosanum HAYATA	2	Ishigakijima Isl. Okinawa Pref., Japan; Mt Arisan, Taiwan
E. glehni FR. SCHM. & TRAUTV.	4	Mt Teine, Hokkaido Pref.; Mt Kurikoma, Iwate Pref.; Abe Pass, Shizuoka Pref., Japan
E. lindleyanum DC.	5	Maiko and Mt Tonomine, Hyogo Pref.; Shigenobu-cho, Ehime Pref., Japan
E. luchuense NAKAI	3	Kumejima Isl. (from M. YOKOTA), Ishigakijima Isl. and Okinawa Isl., Okinawa Pref., Japan
E. spec. (Thailand)	3	Doi Chang, Thailand (from H. KOYAMA)
E. variabile MAKINO	3	Yakushima Isl., Kagoshima Pref., Japan
E. yakushimense MASAMUNE & KITAMURA	2	Yakushima Isl., Kagoshima Pref., Japan
European		
E. cannabinum L.	5	Greifensee, Storen, Switzerland (from C. FARRON); Berkshire, Tatcham, edge of Kennet-Avon, England (from C. FARRON)

macerated root tips in the above solution were placed in a water bath for 30 sec at 80 °C, before meristematic cells were squashed on a glass slide. Five cells containing well spread chromosomes were selected and used for measurements. Photographs of chromosome were magnified × 5000, and the lengths of the arms were measured using a scale with 0.5 mm units. In each cell, 10 pairs of homologous chromosomes were identified based on similarity in size and centromere position. In each species, 5 pairs of chromosomes were identified as homologous from each of 5 cells using the same criteria in each cell. Measurement data from 10 chromosomes (2 homologues × 5 cells) were used for statistical calculation.

The following indices were calculated for each chromosome:

$$\text{relative long arm length (RLL)} = 2a_i / \sum_{j=1}^{2n} (a_j + b_j),$$

$$\text{relative short arm length (RSL)} = 2b_i / \sum_{j=1}^{2n} (a_j + b_j),$$

$$\text{relative chromosome length (RL)} = \text{RLL} + \text{RSL},$$

$$\text{arm difference ratio (AD)} = (a_i - b_i)/(a_i + b_i),$$

where a_i = long arm length of chromosome i,

b_i = short arm length of chromosome i, and

$\sum_{j=1}^{2n} (a_j + b_j)$ = total diploid chromosome length.

Arm difference ratio (AD) defined in this paper can be expressed by traditional measures d, difference between long arm and short arm lengths standardized as total chromosome length = 10 and r, arm ratio (Denver Study Group 1960, LEVAN & al. 1964) as follows:

$AD = d/10 = (r-1)/(r+1)$.

This measure varies from 0 to 1 and is more easily treated statistically than traditional measures.

Based on these values, minimal chromosomal distance (MCD) and chromosomal identity (CI) were computed. MCD is defined as a minimum of chromosome distance (CD) as follows:

$$CD = \sum_{i=1}^{n} \{(a_{xi} - a_{yi})^2 + (b_{xi} - b_{yi})^2\},$$

where a_{xi} = the RLL of chromosome i in species x, and

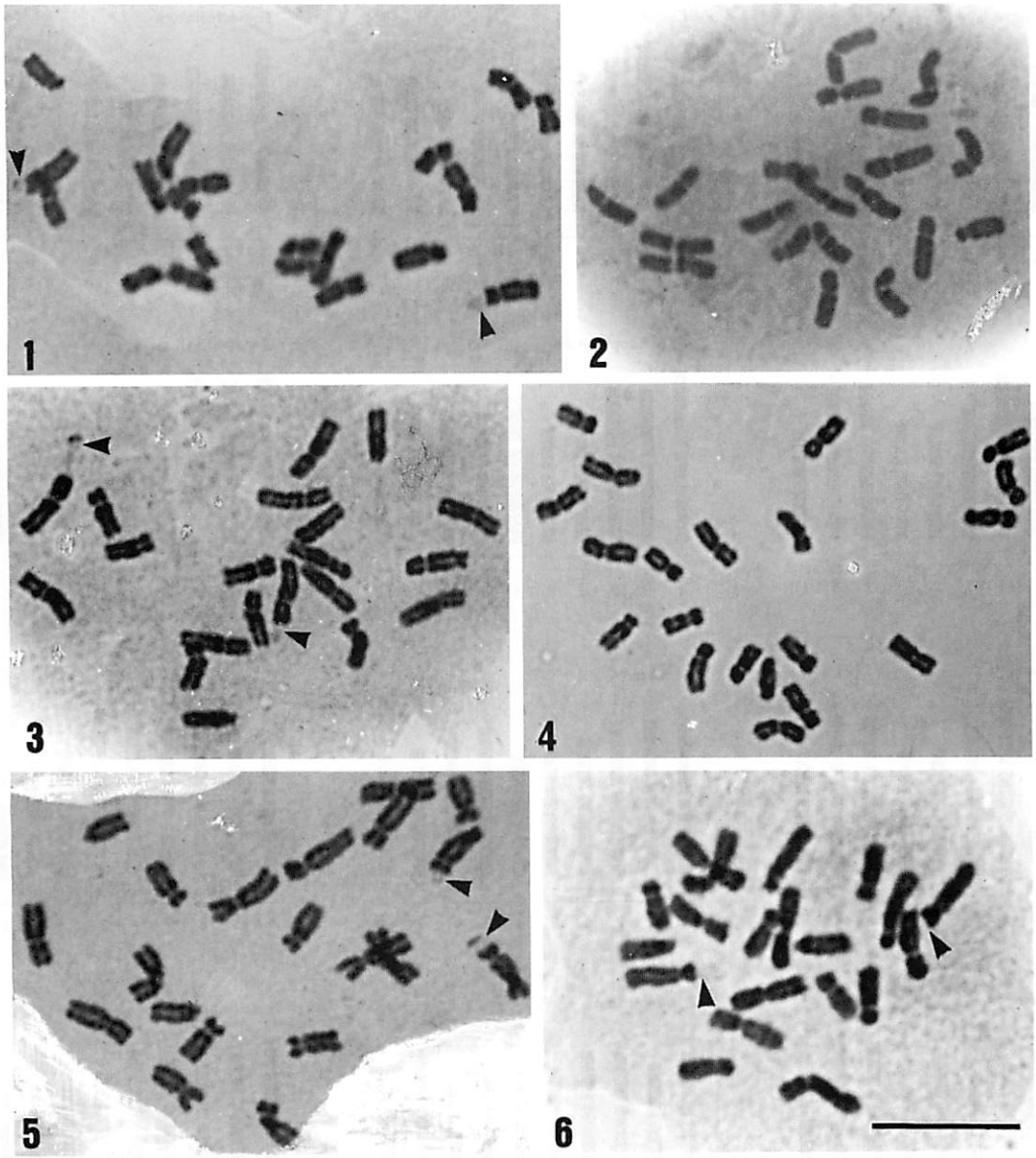
b_{yi} = the RSL of chromosome i in species y.

CDs were calculated for all the possible combinations of haploid chromosomes between species, and the minimum value was used as MCD. The algorithm for computation of MCD will be given by ITO & al. (unpubl.).

CI is defined as the number of morphologically identical chromosomes between species (NIC) divided by the haploid chromosome number (n = 10 in *Eupatorium*). NICs were counted based on t-test of differences in AD and RL between species; chromosome pairs whose ADs and RLs are not significantly different ($p < 0.05$) were judged as morphologically identical. Phenograms based on CI and MCD were generated by UPGMA method using NT-SYS pc (ROHLF 1986). Chromosomes are designated following nomenclature recommended by LEVAN & al. (1964).

Results

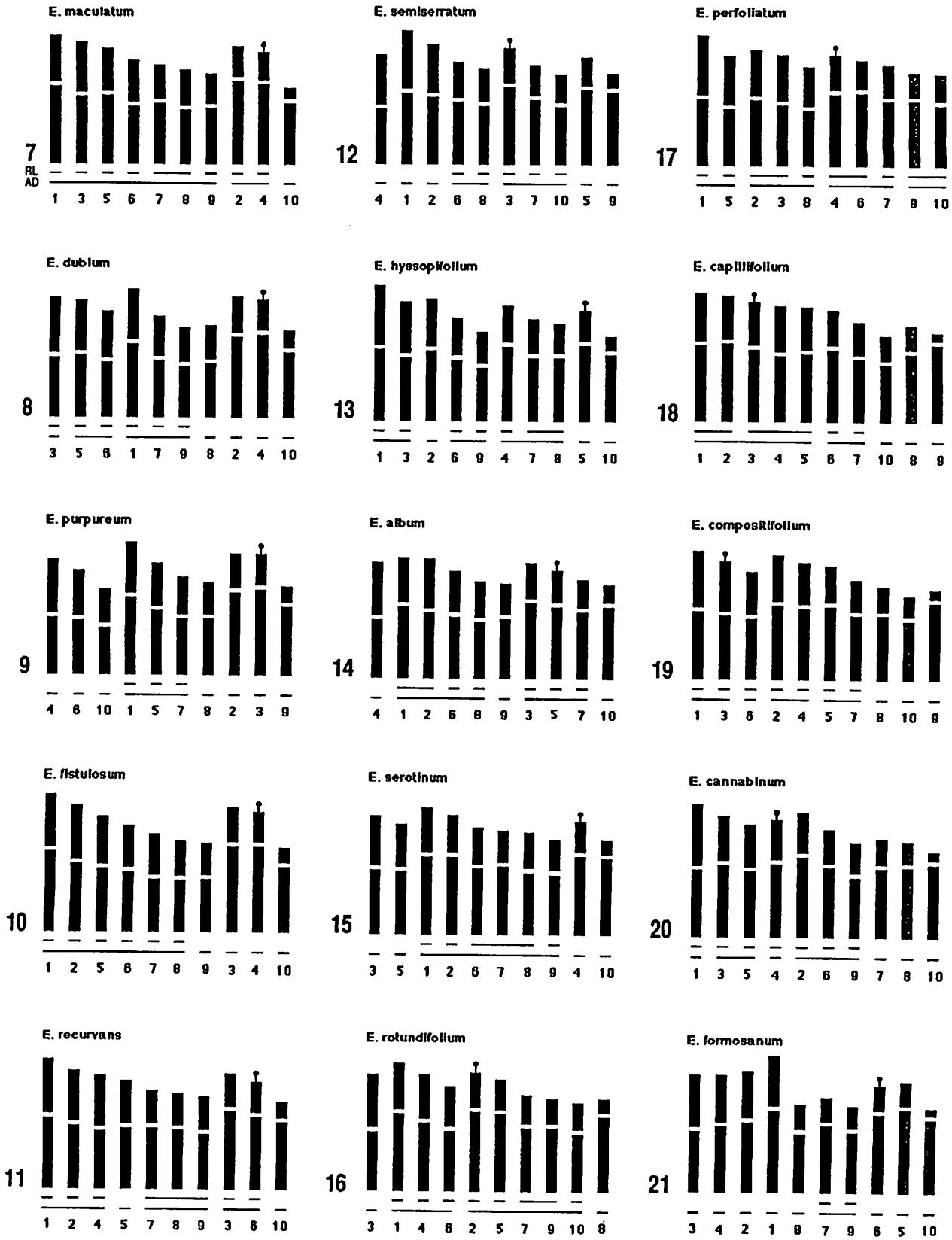
Figures 1 – 6 are photomicrographs of somatic chromosomes of six representative species. Somatic chromosomes of *Eupatorium* are medium-sized, and means of chromosomal lengths range from 2.07 to 4.97 μm. All 22 species examined have the chromosome number 2n = 20 and are thus diploid. Figures 7 – 28 are idiograms and karyotypes. Drawings are based on means of RLL and RSL, and species are arranged in the same order as in Fig. 29 A. Each idiogram is arranged in ascending

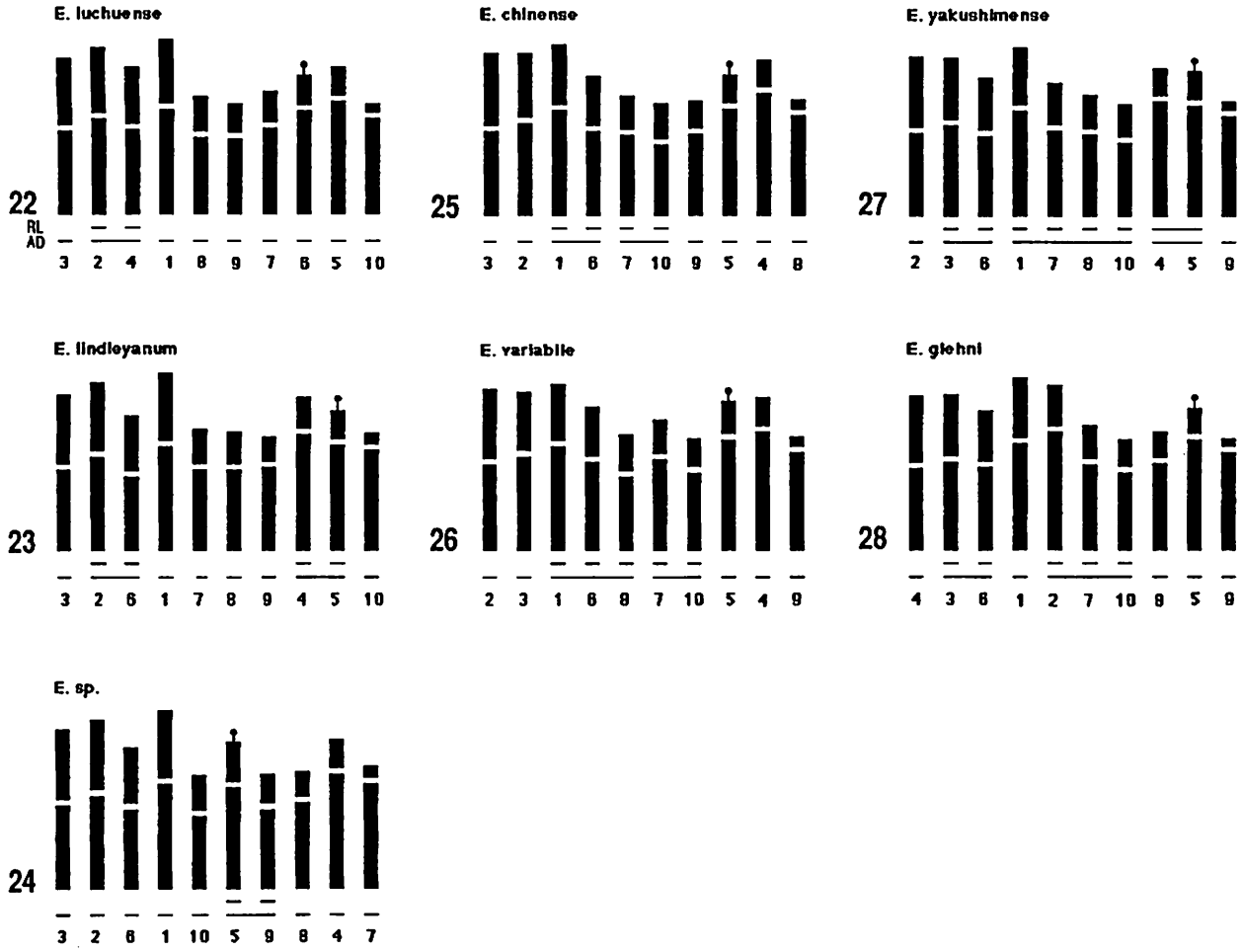


Figs. 1 – 6. Photomicrographs of somatic metaphase chromosomes of *Eupatorium*. – Fig. 1. *E. purpureum*. – Fig. 2. *E. hyssopifolium*. – Fig. 3. *E. capillifolium*. – Fig. 4. *E. recurvans*. – Fig. 5. *E. glehni*. – Fig. 6. *E. chinense* var. *oppositifolium*. ▶ Satellites. – Bar: 10 μm

order of mean AD; and if mean ADs are not significantly different ($p < 0.05$) in a cluster, they are arranged in descending order of mean RL. Clusters within which mean AD or mean RL is not significantly different ($p < 0.05$) are underlined. Chromosomes are numbered in descending order of mean RL regardless of homologies. Table 2 shows the data matrix of MCD and CI, and Fig. 29 includes phenograms drawn using UPGMA based on data in Table 2.

The four N. American species of the *Eutrochium* group with vertillicate leaves,


E. maculatum
7
RL
AD
1 3 5 6 7 8 9 2 4 10
E. semiserratum
12
4 1 2 6 8 3 7 10 5 9
E. perfoliatum
17
1 5 2 3 8 4 6 7 9 10
E. dubium
8
3 5 6 1 7 9 8 2 4 10
E. hyssopifolium
13
1 3 2 6 9 4 7 8 5 10
E. capillifolium
18
1 2 3 4 5 6 7 10 8 9
E. purpureum
9
4 6 10 1 5 7 8 2 3 9
E. album
14
4 1 2 6 8 9 3 5 7 10
E. compositifolium
19
1 3 6 2 4 5 7 8 10 9
E. fistulosum
10
1 2 5 6 7 8 9 3 4 10
E. serotinum
15
3 5 1 2 6 7 8 9 4 10
E. cannabinum
20
1 3 5 4 2 6 9 7 8 10
E. recurvans
11
1 2 4 5 7 8 9 3 6 10
E. rotundifolium
16
3 1 4 6 2 5 7 9 10 8
E. formosanum
21
3 4 2 1 8 7 9 6 5 10



Figs. 7 – 28. Idiograms of somatic metaphase chromosomes. – Fig. 7. *E. maculatum.* – Fig. 8. *E. dubium.* – Fig. 9. *E. purpureum.* – Fig. 10. *E. fistulosum.* – Fig. 11. *E. recurvans.* – Fig. 12. *E. semiserratum.* – Fig. 13. *E. hyssopyfolium.* – Fig. 14. *E. album.* – Fig. 15. *E. serotinum.* – Fig. 16. *E. rotundifolium.* – Fig. 17. *E. perfoliatum.* – Fig. 18. *E. capillifolium.* – Fig. 19. *E. compositifolium.* – Fig. 20. *E. cannabinum.* – Fig. 21. *E. formosanum.* – Fig. 22. *E. luchuense.* – Fig. 23. *E. lindleyanum.* – Fig. 24. *E.* spec. – Fig. 25. *E. chinense* var. *oppositifolium.* – Fig. 26. *E. variabile.* – Fig. 27. *E. yakushimense.* – Fig. 28. *E. glehni.* Species are arranged in the same order as in Fig. 29 A. Underlines indicate clusters within which mean AD or mean RL is not significantly different ($p < 0.05$)

Eupatorium dubium, E. purpureum, E. fistulosum, and *E. maculatum,* have similar karyotypes and are grouped into a discrete cluster at MCD of 0.42 and CI of 0.63. They share five to eight morphologically identical chromosomes (CI = 0.5 – 0.8). These species are characterized by two submetacentric and one subtelocentric chromosomes arranged in right-hand side in Figs. 7 – 10; one of two submetacentric chromosomes is satellited. Among these four species, *E. dubium* and *E. purpureum* are closely paired in both phenograms (Fig. 29) and share eight morphologically identical chromosomes (CI = 0.8).

The seven N. American species of the *Uncasia* group with nondissected decussate leaves (*E. recurvans, E. semiserratum, E. hyssopifolium, E. album, E. perfoliatum,*

Table 2. Data matrix of minimal chromosomal distance (numbers above the diagonal) and

	mac	dub	pur	fis	rec	sem	hys	alb	ser	rot
E. maculatum		5.00	4.53	3.06	5.07	7.19	5.75	6.39	5.06	5.36
E. dubium	0.8		1.74	2.12	3.15	6.73	5.17	5.00	6.65	6.98
E. purpureum	0.5	0.8		2.54	4.90	7.63	7.54	8.04	6.38	7.27
E. fistulosum	0.6	0.7	0.6		5.68	8.43	7.05	8.30	8.26	8.82
E. recurvans	0.2	0.2	0.3	0.1		1.60	2.02	2.70	3.90	3.52
E. semiserratum	0.1	0	0.2	0	0.2		2.38	3.38	4.47	3.05
E. hyssopifolium	0.4	0.3	0	0.1	0.5	0.4		3.38	4.26	5.16
E. album	0.1	0.2	0.3	0.2	0.5	0.4	0.3		2.48	3.61
E. serotinum	0.2	0.3	0.1	0.2	0.4	0.4	0.5	0.3		2.88
E. rotundifolium	0.3	0.1	0.2	0.1	0.1	0.6	0.3	0.4	0.4	
E. perfoliatum	0.1	0.3	0.3	0.2	0.2	0.5	0.3	0.2	0.3	0.3
E. capillifolium	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.3	0.1
E. compositifolium	0	0	0.1	0.2	0.3	0.2	0.2	0.1	0.4	0.4
E. cannabinum	0	0	0.1	0	0.1	0.2	0.1	0.2	0.3	0.1
E. formosanum	0.2	0.1	0	0	0.1	0.1	0.2	0.3	0.1	0.3
E. luchuense	0.2	0.2	0.2	0.2	0.1	0	0.2	0.1	0.2	0.2
E. lindleyanum	0.2	0.3	0.1	0.3	0.1	0	0.1	0.1	0	0.4
E. sp.	0.2	0	0.1	0.3	0.3	0.1	0.1	0.1	0.3	0.4
E. chinense	0.2	0	0	0.1	0	0.3	0.2	0.1	0.1	0.4
E. variabile	0.1	0	0	0.1	0	0.1	0.1	0.3	0.2	0.3
E. yakushimense	0.1	0.1	0	0.2	0.3	0.4	0.4	0.2	0.4	0.6
E. glehni	0.2	0.1	0.1	0	0.2	0.2	0.3	0.3	0.2	0.5

E. serotinum, and *E. rotundifolium*) clustered together in both phenograms (Fig. 29), but at a very low level (0.33) in the CI phenogram: this is lower than the *Eutrochium* and E. Asian groups. In the MCD phenogram, the *Uncasia* cluster formed at 5.3, which is higher than any of the other three cluster groups (Fig. 29 A). Both the CI and MCD phenograms show higher chromosomal diversity among the *Uncasia* species than among the *Eutrochium* and E. Asian species. Species assigned to the *Uncasia* group share one to six morphologically identical chromosomes (Table 2 and Fig. 29 B). They commonly possess a submetacentric satellited chromosome and, except for *E. perfoliatum*, a subtelocentric chromosome. The morphology of other chromosomes varied among species. The phenograms differ in the subgroup clustering; thus it is difficult to subdivide it karyotypically.

Eupatorium cannabinum occurring from western India to Europe, and two N. American species of the *Traganthes* group (*E. capillifolium* and *E. compositifolium*) are grouped into one cluster in the MCD phenogram. In the CI phenogram, *E. cannabinum* and *E. capillifolium* cluster together, but *E. compositifolium* is clustered not with *E. capillifolium* but with E. Asian species. Two species of the *Traganthes* group and the geographically isolated *E. cannabinum* share only two or three morphologically identical chromosomes (CI = 0.2 − 0.3). They are characterized by an acrocentric chromosome and a satellited chromosome with the lowest arm ratio among all species examined.

Eight E. Asian species, *E. lindleyanum*, *E. luchuense* NAKAI, *E. formosanum* HAYATA, *E. chinense* var. *oppositifolium*, *E. variabile*, *E. yakushimense*, *E. glehni*,

chromosomal identities (numbers below the diagonal) of the *Eupatorium* spp. investigated

per	cap	com	can	for	luc	lin	sp	chi	var	yak	gle
8.89	10.96	14.48	10.90	7.68	7.45	8.79	10.38	8.39	7.20	6.85	8.91
6.26	13.64	16.24	15.38	13.83	14.06	13.04	14.34	14.69	14.91	12.42	14.77
10.00	14.54	18.50	16.68	13.45	14.12	13.35	14.93	13.84	13.58	11.19	14.01
9.94	15.12	18.93	17.29	14.08	13.08	12.43	14.27	13.19	13.02	11.61	14.57
3.24	7.75	11.07	9.65	8.66	9.58	10.16	9.66	12.43	11.97	10.31	12.27
4.42	6.02	8.21	6.78	8.08	8.62	8.72	7.86	11.42	11.02	10.04	11.30
4.60	6.70	9.34	6.25	11.20	11.28	11.90	11.25	13.54	13.28	11.92	13.22
3.47	5.94	8.67	6.91	9.75	10.35	10.46	9.38	12.20	11.81	11.21	12.04
7.97	4.81	8.73	6.22	8.77	10.05	10.58	10.78	11.37	9.78	8.79	8.98
8.10	4.47	6.95	4.60	4.66	5.61	5.65	4.71	6.92	6.03	5.52	5.98
	11.21	12.22	10.50	14.22	15.53	15.84	15.17	19.09	18.41	17.17	20.35
0.2		2.79	4.24	9.57	10.50	13.13	8.98	14.75	13.22	13.33	13.10
0.2	0.3		4.95	14.50	16.73	18.11	13.15	20.05	18.56	18.95	18.71
0.4	0.3	0.2		9.10	10.93	13.12	9.77	13.28	11.31	10.54	11.35
0.2	0.1	0.3	0.2		0.88	2.94	2.41	3.35	2.94	3.05	3.42
0.3	0.1	0.2	0.3	0.5		1.79	2.00	1.98	2.01	2.25	2.71
0.2	0.2	0.1	0.1	0.3	0.4		1.96	1.93	3.50	2.41	2.50
0.2	0.2	0.3	0.1	0.5	0.4	0.6		2.62	3.94	4.19	4.62
0.2	0.2	0.3	0.2	0.3	0.4	0.4	0.4		0.90	1.33	2.43
0.3	0.2	0.3	0.3	0.5	0.4	0.2	0.4	0.8		1.32	2.71
0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.5	0.3	0.5		2.38
0.2	0.1	0.2	0.4	0.5	0.4	0.4	0.3	0.5	0.4	0.7	

and an undescribed species from Thailand (*E.* spec. in Figs. 24 and 29), have similar karyotypes and are grouped into one cluster in both phenograms. *Eupatorium chinense* var. *oppositifolium*, *E. variabile*, *E. yakushimense*, and *E. glehni* clustered together in both phenograms, but it is difficult to identify morphological features characterizing these species as a whole. These eight species are characterized by an acrocentric chromosome and two large subtelocentrics. One of the latters is satellited (Figs. 21 – 28). Their karyotypes are more asymmetrical than those of American species and *E. cannabinum*. *Eupatorium chinense* var. *oppositifolium* and *E. variabile* share eight pairs of morphologically identical chromosomes and thus their karyotypic differences are very slight.

Discussion

Advantages of two indices, MCD and CI. The rate and mode of karyotypic changes during speciation remains an unsolved problem of considerable evolutionary interest. A necessary and hopeful approach to this subject is to compare results from karyotypic analysis with those from external morphology and molecular systematics in particular plant groups. Methodologies for computing phenetic or cladistic trees from external morphological and molecular data set have been intensively studied. On the other hand, only a few works have been made on methodology for quantifying karyotypic difference or similarity between taxa. Bianchi & Merani (1984) developed an index, karyological distance (KD) among related species. Bianchi &

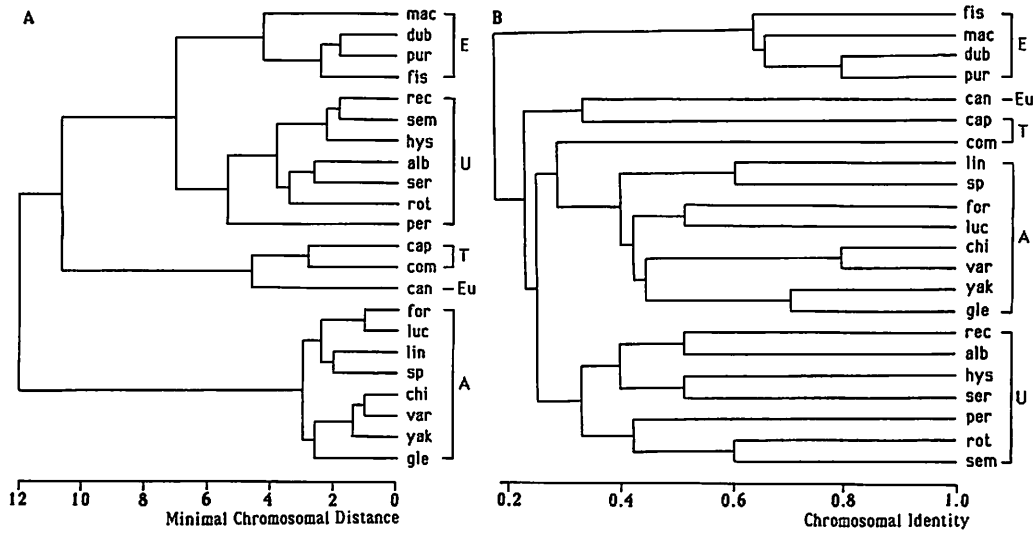


Fig. 29. Phenograms (UPGMA) of taxa of *Eupatorium* based on minimal chromosomal distance (*A*) and chromosomal identity (*B*). mac *E. maculatum*, dub *E. dubium*, pur *E. purpureum*, fis *E. fistulosum*, rec *E. recurvans*, sem *E. semiserratum*, hys *E. hyssopyfolium*, alb *E. album*, ser *E. serotinum*, rot *E. rotundifolium*, per *E. perfoliatum*, cap *E. capillifolium*, com *E. compositifolium*, can *E. cannabinum*, for *E. formosanum*, luc *E. luchuense*, lin *E. lindleyanum*, sp *E.* spec. from Thailand, chi *E. chinense* var. *oppositifolium*, var *E. variabile*, yak *E. yakushimense*, gle *E. glehni*, E *Eutrochium* group in N. America, U *Uncasia* group in N. America, T *Traganthes* group in N. America, Eu European species, A Asian species

al. (1988) further developed an index, karyological conservatism (KC) among related species. These indices are, however, based on identifications of homologous chromosomes, homologous arms, and structural rearrangements by G-banding pattern, and are not generally applicable to karyological data in plants. Although various banding techniques are now available, G-banding technique, the most useful one in chromosomal identification in animals, is hardly applicable to plant chromosomes at present. In addition, Giemsa C and base-specific fluorochrome staining has limited value for identification of homologous chromosomes and segments because most plant groups including *Compositae* studied here lack constitutive heterochromatin in interstitial parts of chromosomes (EHRENDORFER 1986). Due to these limitations, karyotypic analysis has provided conclusive evidence for phylogenetic relationships only in limited plant groups such as annual members of *Brachyscome* (WATANABE & SMITH-WHITE 1987), *Haplopappus* (JACKSON 1962, 1965) and *Crepis* (BABCOCK 1947, TOBGY 1943) where constituting chromosomes are well differentiated in size and centromere position and correspondences between somatic and meiotic chromosomes can be determined. In contrast to these annuals, karyotypes of perennials and woody plants are very stable and less diversified (SCHWEIZER & EHRENDORFER 1976; EHRENDORFER 1983, 1986; BRANDHAM 1983). In these groups, karyotypic differences between species are largely quantitative and have been difficult to be assessed by conventional qualitative methods. Recently, MEEROW (1987) analyzed karyotypes of three perennial genera of *Amaryllidaceae* by principal component and cluster analysis. However, his calculation is based on sorting of chromosomes to 16 arbitrary categories such as long/metacentric, mod-

erately long/subtelocentric and others. More objective method in assessing karyotypic difference or similarity is necessary to compare it with those in external morphology and molecular sequence.

Two indices developed in the present paper do not assume homology of any pair of chromosomes and also do not use artificial categories of karyotypes. MCD evaluates overall karyotypic differences between species, whereas CI scores number of chromosomes that are identical in the statistical sense (these may not be always homologous). Both indices can be widely applied to taxa with the same chromosome number. CI can be applied also to taxa with different chromosome numbers, but the validity of phenetic comparisons between taxa with different chromosome numbers has been criticized because taxa with the same chromosome number tend to cluster together irrespective of actual phylogenetic relationships (ATCHLEY 1972). MCD and CI have merits and demerits, respectively. MCD can overestimate the differences between particular species pairs when a few large chromosomal mutations are responsible for those differences. In such cases, CI may provide a more reliable method for comparing chromosomal differences between species. However, CI is not reliable for quantifying karyotypic similarities where its value is low.

Compared with the two quantitative methods mentioned above, the conventional qualitative method of comparing only distinct chromosomes between species (such as satellited or acrocentric chromosomes in *Eupatorium*) is not as sensitive because it overemphasizes the differences between karyotypes where a few large chromosomal mutations have occurred, and conversely, it ignores many small karyotypic differences. An example of the former situation is found in *E. perfoliatum*, which lacks the subtelocentric chromosome that characterizes other *Uncasia* group species. The latter situation becomes a particular problem when attempting to distinguish among many metacentric and submetacentric chromosomes in *Eupatorium*. The MCD method is advantageous in evaluating all of the constituted chromosomal differences between karyotypes in numerical terms.

Karyological evolution and suggested phylogenetic relationships in *Eupatorium*. Overall karyotypic similarity per se does not always reflect the systematic affinity particularly in organisms where chromosome numbers have changed through large chromosomal mutations (ATCHLEY 1972). In *Eupatorium*, however, all species examined have $2n=20$ and karyotypic differences among species are rather small; the CI phenogram is highly compatible with the MCD phenogram, suggesting that most of species still share morphologically identical homologous chromosome pairs. This result agrees well with a general conservatism of karyotypes in perennials and woody plants (EHRENDORFER 1983, 1986). Also, the general concordance between morphological or geographical group and clustering based on MCD suggests that karyotypes in the genus *Eupatorium* have changed not through large chromosomal mutations but through small chromosomal mutations gradually fixed (additions or deletions of chromatin, and pericentric inversions; cf. BRANDHAM 1983). This view is supported by evidence that 10 bivalents have been formed in meiosis of artificial hybrids ($2n=20$) between American *E. perfoliatum* and Asian *E. chinense* var. *oppositifolium* (WATANABE, unpubl.). Thus, it is plausible in *Eupatorium* that overall karyotypic similarity reflects considerably the systematic affinity. This finding allows the following interpretations of the phenograms (Fig. 29).

Firstly, the karyotypic diversity among E. Asian species is lower than that among N. American species. This suggests that E. Asian species radiated more recently

than N. American ones. Alternatively, it may indicate higher chromosomal conservatism in E. Asian species. However, it is implausible that factors affecting the rate of chromosomal evolution, such as effective population size and kinds and strengths of selection pressures, are different between E. Asian and N. American species. It is remarkable that N. American *E. rotundifolium* shows high chromosomal identity with some E. Asian species (0.6 with *E. yakushimense*, 0.5 with *E. glehni*). We suggest that E. Asian species were derived from a single or closely related N. American ancestor(s).

Secondly, chromosomal identities among four species of the *Eutrochium* group are very high (0.5 − 0.8). These taxa are distinctive among the N. American species in both vegetative and floral morphology and were segregated as the genus *Eupatoriadelphus* (KING & ROBINSON 1970a). KING & ROBINSON (1987), however, reincluded these in *Eupatorium* and stated that the *Eutrochium* group is approached closely in leaf arrangement and head shape to some variant of *E. chinense* (= *E. glehni*, which has been treated as a variety of *E. chinense*). In both CI and MCD phenograms, *E. glehni* and the four *Eutrochium* group species do not cluster together. Asian species including verticillate-leaved *E. glehni* share considerably more karyotypic similarities with N. American *E. rotundifolium* than with the *Eutrochium* spp. (Fig. 29). These results suggest that verticillate leaves have evolved separately in the two lineages.

Thirdly, the geographically isolated *E. cannabinum* clusters with one of two species of the *Traganthes* species. Since *E. cannabinum* is a single species distributed west of India, KING & ROBINSON (1987) believed it to be derived from Asian species. Asian *E. heterophyllum* and *E. formosanum* resemble *E. cannabinum* morphologically. KITAMURA (1961) regarded the Asian *E. formosanum* as a subspecies of *E. cannabinum* and proposed the combination as *E. cannabinum* L. subsp. *asiaticum* KITAMURA. In both CI and MCD phenograms, however, *E. cannabinum* does not cluster with *E. formosanum* or other Asian species examined, and these are doubtfully close. Chromosomal identities between *E. cannabinum* and two species of the *Traganthes* groups are low (CI = 0.2 and 0.3) and are nearly equivalent to those between *E. cannabinum* and the Asian (CI = 0.1 − 0.4) or the *Uncasia* spp. (CI = 0.1 − 0.4). Therefore, it is uncertain whether *E. cannabinum* and the *Traganthes* group belong to the same monophyletic lineage.

These hypotheses should be tested by evidence from different methodologies, especially molecular systematics. These tests are now in progress. Evidence from restriction fragment length polymorphism of chloroplast DNA supports the monophyletic origin of Asian species and the origin of *E. cannabinum* not from Asian species (KAWAHARA, unpubl.).

Chromosome morphology provides different sources of evidence from gross morphological, anatomical, biochemical, and molecular biological methods, and the two indices developed in this study open the way for applying it more widely in plant systematics. However, cluster analysis using these indices has the limitation inherent in the phenetic method; clusters may be paraphyletic or polyphyletic. Cladistic analysis based on identification of homologous chromosomes and their changes is highly desired in order to overcome this limitation. Improving the method in a way that would allow recognition of the chromosomal homology is necessary.

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