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CHROMOSOMAL CYTOLOGY AND EVOLUTION IN EUPATORIEAE (ASTERACEAE)¹

Kuniaki Watanabe,² Robert M. King,³
Tetsukazu Yahara,⁴ Motomi Ito,⁵
Jun Yokoyama,⁶ Takeshi Suzuki,⁷
and Daniel J. Crawford⁸

ABSTRACT

Reports of 68 new chromosome counts attributed to 53 species from 25 genera of Eupatorieae of the Asteraceae, based mostly on determinations of mitotic materials, include first counts for 2 genera (*Acanthostyles* and *Lepidesmia*) and 14 species and new reports for 8 species. B chromosomes are reported for 4 genera and 12 species. Karyotype analyses made on 20 species of Eupatorieae and one species of Heliantheae showed that total karyotypic lengths of the taxa with $n = 16$ –19 of helianthoid and eupatorioid taxa are comparable to those of some eupatorioid taxa with $n = 10$. This is contrary to the previous hypothesis that the higher chromosome numbers $n = 16$ –19 were derived from $n = 10$ by polyploidization followed by dysploid loss. Cytological data supplement and are consistent with the following conclusions predicted from molecular phylogenetical and biochemical data: (1) The ultimate base number of Eupatorieae is 17, and the lower numbers are derived by successive dysploid reductions; (2) A reduction in chromosomal and total karyotypic length accompanied by evolutionary advancement has been revealed for some genera and species within this tribe; (3) A high base number of $x = 17$ in Eupatorieae is considered to be derived directly from one of the members of Heliantheae with $n = 17$ to 19.

The Eupatorieae are a very diversified tribe and consist of more than 2300 species and 180 genera in 18 subtribes (King & Robinson, 1987). In spite of its representing a large element of Asteraceae, the tribe Eupatorieae has remained poorly understood because its centers of diversity are in Central America and South America, which were remote from the early centers of study. Recent monographical (King & Robinson, 1987), cladistical (Karis, 1993; Bremer, 1987, 1994; Bremer et al., 1992), and molecular phylogenetic works (Jansen et al., 1991; Watson et al., 1991; Kim et al., 1992) have stimulated us to study the origin and evolution of Eupatorieae. A broad range of base chromosome numbers, from $n = 4$ to 25, has been reported (Fig. 1). The remarkable constancy of chromosome morphology and number within genera has been invaluable in the study of plant systematics. In addition, many of the groups that have distinctive

chromosome numbers are among the most readily defined taxonomically. Therefore, we attempted to find clues regarding phylogenetic relationships throughout the tribe by assessing chromosomal variation, restriction site mutations in chloroplast DNA (Ito et al., in prep.), and the utility of isozyme number for determining ploidy level (Suzuki et al., unpublished).

Our purposes in this paper are to report original counts of chromosome numbers in Eupatorieae, to compare them with numbers reported previously, to analyze karyotypes of selected representative species, and to apply the results to a better understanding of the origin, phylogeny, and chromosomal evolution in the tribe.

MATERIALS AND METHODS

Cytological observations were made primarily on mitotic cells of root tips obtained from seedlings

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² Department of Biology, Faculty of Science, Kobe University, Tsurukabuto 1-2-1, Kobe, 657, Japan.

³ Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A.

⁴ Department of Biology, Faculty of Science, Kyushu University, Fukuoka, 812, Japan.

⁵ Department of Biology, Faculty of Science, Chiba University, Yayoi-cho, Inage-ku, Chiba, 260, Japan.

⁶ Botanical Gardens, University of Tokyo, Hakusan, Bunkyo-ku, Tokyo, 112, Japan.

⁷ Museum of Nature and Human Activities, Hyogo, 6 Yayoigaoka, Sanda, 669-13, Japan.

⁸ Department of Plant Biology, Ohio State University, Columbus, Ohio 54321, U.S.A.

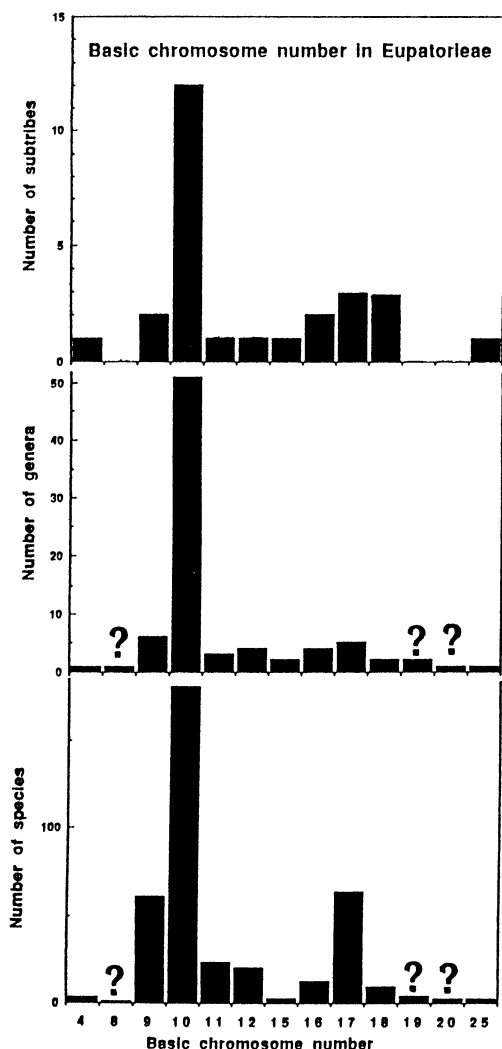


FIGURE 1. Distributions of base chromosome numbers in the species, genera, and subtribes of Eupatorieae.

grown from fruits of known provenance in the greenhouse of Kobe University. For the cultivation of specimens and the preparation of materials for cytological work, the procedures of Watanabe et al. (1975) and Watanabe et al. (1990) were followed. The measurements of karyotypic length were made on root tip mitoses, and each measurement is the mean of measurements of five metaphase plates. Voucher specimens have been deposited in US and MAK.

RESULTS

CHROMOSOME NUMBER

All chromosome number determinations are presented in Table 1. Figures 2–9 are photographs of

somatic chromosomes of eight representative species.

Chromosome number determinations from 68 populations attributed to 53 species from 25 genera of Eupatorieae are reported. First reports are given for *Acanthostyles buniifolius* ($2n = 20$), *Adenostemma cuatrecasii* ($2n = 20$), *Ageratina isolepis* ($2n = 34$), *Decachaeta thieleana* ($2n = 32$), *Fleischmannia sideritides* ($n = 10$, $2n = 20$), *Lepidesmia squarrosa* ($2n = 20$), *Koanophyllon longifolium* ($2n = 30$), *Mikania congesta* ($2n = 34+0$ to $3B$), *Neomirandea arthodes* ($n = 17+0$ to $5B$), *N. guevarii* ($2n = 50+2$ to $12B$), *N. parasitica* ($2n = 34+1$ to $2B$), *N. standleyi* ($2n = 50+1$ to $4B$), *Stevia connata* ($2n = 44$), and *S. suaveolens* ($2n = 33+0$ to $2B$), and new reports are given for *Ageratina rothrockii* ($2n = 51$ and 68), *Chromolaena laevigata* ($2n = 50$), *Mikania scandens* ($2n = 34+0$ to $1B$), *Praxelis clematidea* ($2n = 30$), *Stevia eupatoria* ($2n = 36$ and 48), *S. monardaefolia* ($2n = 33$), *S. pilosa* ($2n = 22$), and *S. tomentosa* ($2n = 33$). Remaining counts confirm the chromosome numbers of all previous reports in some species or are consistent with one of variable numbers reported by several authors in other species. Of the 68 accessions in which chromosome number determinations were made, 53 (78%) are diploids and 15 (22%) are polyploids. The presence of B chromosomes is reported for *Bartlettina sordida*, *Mikania congesta*, *M. scandens*, *Neomirandea angularis*, *N. arthodes*, *N. biflora*, *N. guevarii*, *N. parasitica*, *N. standleyi*, *Stevia salicifolia*, *S. serrata*, and *S. suaveolens*. The B chromosomes are distinctly smaller than autosomes (Fig. 3), except in *Mikania* and *Stevia*, and show the early condensation typical of the centromeric regions of autosomes at prometaphase. Both *Mikania* and *Stevia* have several small autosomes nearly the same size as B chromosomes (Fig. 7).

KARYOTYPE

Figures 10–30 are haploid karyotypic idiograms. Drawings are based on the means of ten measurements for each. Each idiogram is arranged in descending order of chromosome number within Eupatorieae next to helianthoid *Podachaenium eminens* (Lag.) Sch. Bip.

In Table 2, we report the mean total karyotypic lengths with their standard errors, the ranges from the longest to shortest chromosome and their ratio for each karyotype, and the mean arm ratio (total long arm length/total short arm length). The two latter measurements indicate the degree of karyotypic asymmetry. Significance of differences be-

Table 1. Chromosome number determinations in Eupatorieae (Asteraceae). *n* counts are from pollen mother cells (PMCs) and *2n* counts are from root tips.

Species	Chromosome number		Collection data or reference
	<i>n</i>	<i>2n</i>	
<i>Acanthostyles buniifolius</i> (Hook. & Arn.) R. M. King & H. Rob.	—	20	Argentina. Buenos Aires: Parana Delta at Tigre, <i>King 10288</i> (US)
<i>Adenostemma cuatrecasasii</i> R. M. King & H. Rob.	—	20	Venezuela. Mérida: 4.1–6.3 km E of Santo Domingo, <i>King 10538</i> (US)
<i>A. viscosum</i> J. R. & G. Forst.	—	20	U.S.A. Hawaii: Kawaihau Dist., Makaleha Mtns., SSW of Puu Eu, <i>Flynn 5461</i> (US)
<i>Ageratina anisochroma</i> (Klatt) R. M. King & H. Rob.	—	34	Costa Rica. 10 km W of San Ramon, 15 Aug. 1991, <i>Yahara et al. s.n.</i> (MAK)
<i>A. bustamenta</i> (DC.) R. M. King & H. Rob.	17II	—	Costa Rica. 27 km N of San Isidro, <i>Yahara et al. 39</i> (MAK)
<i>A. havanensis</i> R. M. King & H. Rob.	—	34	Mexico. Coahuila: near the University in Saltillo, <i>King 10379</i> (US)
<i>A. herbacea</i> (A. Gray) R. M. King & H. Rob.	—	34	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, <i>Yahara et al. 13</i> (MAK)
<i>A. isolepis</i> (B. Rob.) R. M. King & H. Rob.	—	34	Mexico. Hidalgo: 13 km from Pachuca to El Chico on Hwy. 105, 17 Oct. 1993, <i>Yahara & Ito s.n.</i> (MAK)
<i>A. rothrockii</i> (A. Gray) R. M. King & H. Rob.	—	51	U.S.A. Texas: Jeff Davis Co., Old Fort Davis, <i>Yahara et al. 7</i> (MAK)
	—	68	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, 9 Aug. 1991, <i>Yahara et al. s.n.</i> (MAK)
<i>A. scorodonoides</i> (A. Gray) R. M. King & H. Rob.	—	34	Mexico. Coahuila: 2 km E of Los Lirios, <i>King 10326</i> (US)
<i>A. wrightii</i> (A. Gray) R. M. King & H. Rob.	—	34	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, <i>Yahara et al. 14</i> (MAK)
<i>Ageratum corymbosum</i> Zuccagni	—	40	Mexico. Coahuila: 3 km S of Saltillo, <i>King 10368</i> (US)
<i>A. microcarpum</i> (Benth. in Orsted) Hemsl.	10II	—	Costa Rica. 15 km S of Cartago, <i>King 10236</i> (US)
	10II	—	Costa Rica. 27 km N of San Isidro, <i>King 10241</i> (US)
	10II	—	Costa Rica. Volcan Irazu, 16 Aug. 1991, <i>Yahara et al. s.n.</i> (MAK)
	10II	—	Costa Rica. 0.5 km NE from Mt. Orosi, 17 Aug. 1991, <i>Yahara et al. s.n.</i> (MAK)
	10II	20	Costa Rica. The summit of Mt. Orosi, 17 Aug. 1991, <i>Yahara et al. s.n.</i> (MAK)
<i>Austroeupatorium inulaefolium</i> (HBK) R. M. King & H. Rob.	—	20	Argentina. Buenos Aires: city limit of Buenos Aires at the municipal train station, <i>King 10282</i> (US)
<i>Bartlettina sordida</i> (Less.) R. M. King & H. Rob.	16II	—	Australia. Victoria: cultivated in Royal Botanic Gardens, <i>King s.n.</i> (US)
	—	32 + 1B	Australia. Victoria: cultivated in Royal Botanic Gardens, <i>Watanabe s.n.</i> (MAK)
<i>Brickellia betonicifolia</i> A. Gray	—	18	U.S.A. Texas: Jeff Davis Co., Old Fort Davis, <i>Yahara et al. 8</i> (MAK)
<i>B. chlorolepis</i> (Wooten & Standl.) Shinnery	—	18	Mexico. Coahuila: ca 9 km from Los Lirios, <i>King 10320</i> (US)
<i>B. grandiflora</i> (Hook.) Nutt.	—	18	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, 9 Aug. 1991, <i>Yahara et al.</i> (MAK)
<i>Carminatia tenuiflora</i> DC.	—	20	U.S.A. Texas: Jeff Davis Co., Davis Mts., <i>Yahara et al. 4</i> (MAK)

Table 1. Continued.

Species	Chromosome number		Collection data or reference
	<i>n</i>	<i>2n</i>	
<i>Carphochaete bigelovii</i> A. Gray	—	22	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, 9 Aug. 1991, <i>Yahara et al. s.n.</i> (MAK)
<i>Chromolaena laevigata</i> (Lam.) R. M. King & H. Rob.	—	50	Argentina. Buenos Aires: Parana Delta at Tigre, <i>King 10287</i> (US)
<i>Conoclinium gregii</i> (A. Gray) Small	—	20	U.S.A. Texas: Brewster Co., Chisos Mts., Mt. Emory, <i>Yahara et al. 15</i> (MAK)
<i>Decachaeta thieleana</i> (Klatt. ex T. Durand & Pittier) R. M. King & H. Rob.	—	32	Costa Rica. San José: San Ramon de Tres Rios, Nov. 1993, <i>Castro s.n.</i> (US)
<i>Fleischmannia microstemon</i> (Cass.) R. M. King & H. Rob.	4II	—	Costa Rica. 3 km S of Santa Cruz, <i>King 10243</i> (US)
	4II	—	Costa Rica. 0.5 km NE and on the foot of Mt. Orosi, 17 Aug. 1991, <i>Yahara et al. s.n.</i> (MAK)
<i>F. sideritides</i> (Benth. in Orsted) R. M. King & H. Rob.	10II	20	Costa Rica. Tapanti National Wildlife Refuge, <i>Yahara et al. 128</i> (MAK) & <i>King 10262</i> (US)
<i>Hebeclinium macrophyllum</i> (L.) DC.	—	20	Trinidad & Tobago. St. George: along North Coast Road, 5 km W of Maracas Bay Village, <i>Wasshausen 1811</i> (US)
<i>Isocarpha atriplicifolia</i> (L.) R. Br. ex DC.	—	20	Costa Rica. Santa Rosa N. P., 15 Aug. 1991, <i>Yahara et al. s.n.</i> (MAK)
<i>Koanophyllon longifolium</i> (B. Robinson) R. M. King & H. Rob.	—	30	Mexico. Coahuila/Nuevo Leon: just E of Laguna de Sanchez, <i>King 10341</i> (US)
<i>Lepidesmia squarrosa</i> Klatt.	—	20	Venezuela. Falcon: ca. 80 km S of Coro, <i>King 10270</i> (US)
<i>Liatris spicata</i> (L.) Willd.	—	20	U.S.A. North Carolina: Spring Lake, 7 Oct. 1991, <i>Ito et al. s.n.</i> (MAK)
<i>Microspermum debile</i> Benth.	—	24	Mexico. Oaxaca: 47.7 km from Guelatao, 30 Sep. 1993, <i>Yahara & Ito 231</i> (MAK)
var. <i>arsenei</i> Benth.	—	24	Mexico. Michoacán: near Patzcuaro, <i>Medina 2610</i> (US)
<i>Mikania congesta</i> DC.	—	34 + 0 ~ 3B	Argentina. Buenos Aires: city of Buenos Aires, banks of the Rio de La Plata, <i>King 10285</i> (US)
<i>M. scandens</i> L. Willd	—	34 + 0 ~ 1B	U.S.A. North Carolina: Spring Lake, 6 Oct. 1991, <i>Ito et al. s.n.</i> (TI)
<i>Neomirandea angularis</i> (B. L. Rob.) R. M. King & H. Rob.	—	50	Costa Rica. Cartago: Volcan Turrialba, <i>King 9695</i> (US)
	—	50 + 1B	Costa Rica. San José: La Lucha, 27 July 1987, <i>Lellinger s.n.</i> (US)
	25II	—	Costa Rica. 15 km S of Cartago, <i>Yahara et al. 22</i> (MAK) & <i>King 10238</i> (US)
	25II	—	Costa Rica. 0.5 km NE from Mt. Orosi, <i>Yahara et al. 66</i> (MAK) & <i>King 10244</i> (US)
<i>N. arthodes</i> (B. L. Rob.) R. M. King & H. Rob.	25II	—	Costa Rica. Near the summit of Mt. Orosi, <i>King 10246</i> (US)
	17II + 0 ~ 5B	—	Costa Rica. Cartago to San Isidro, <i>King 10239</i> (US)
	17II + 0 ~ 2B	—	Costa Rica. Cartago: Tapanti National Wildlife Refuge, <i>King 10261</i> (US)
<i>N. biflora</i> R. M. King & H. Rob.	—	50 + 2 ~ 6B	Costa Rica. Volcan Turrialba, <i>Yahara et al. 90</i> (MAK) & <i>King 10251</i> (US)
<i>N. guevarii</i> R. M. King & H. Rob.	—	50 + 12B	Costa Rica. Cartago: ca. 7 km from the dam at Tapanti, <i>King 9674</i> (US)

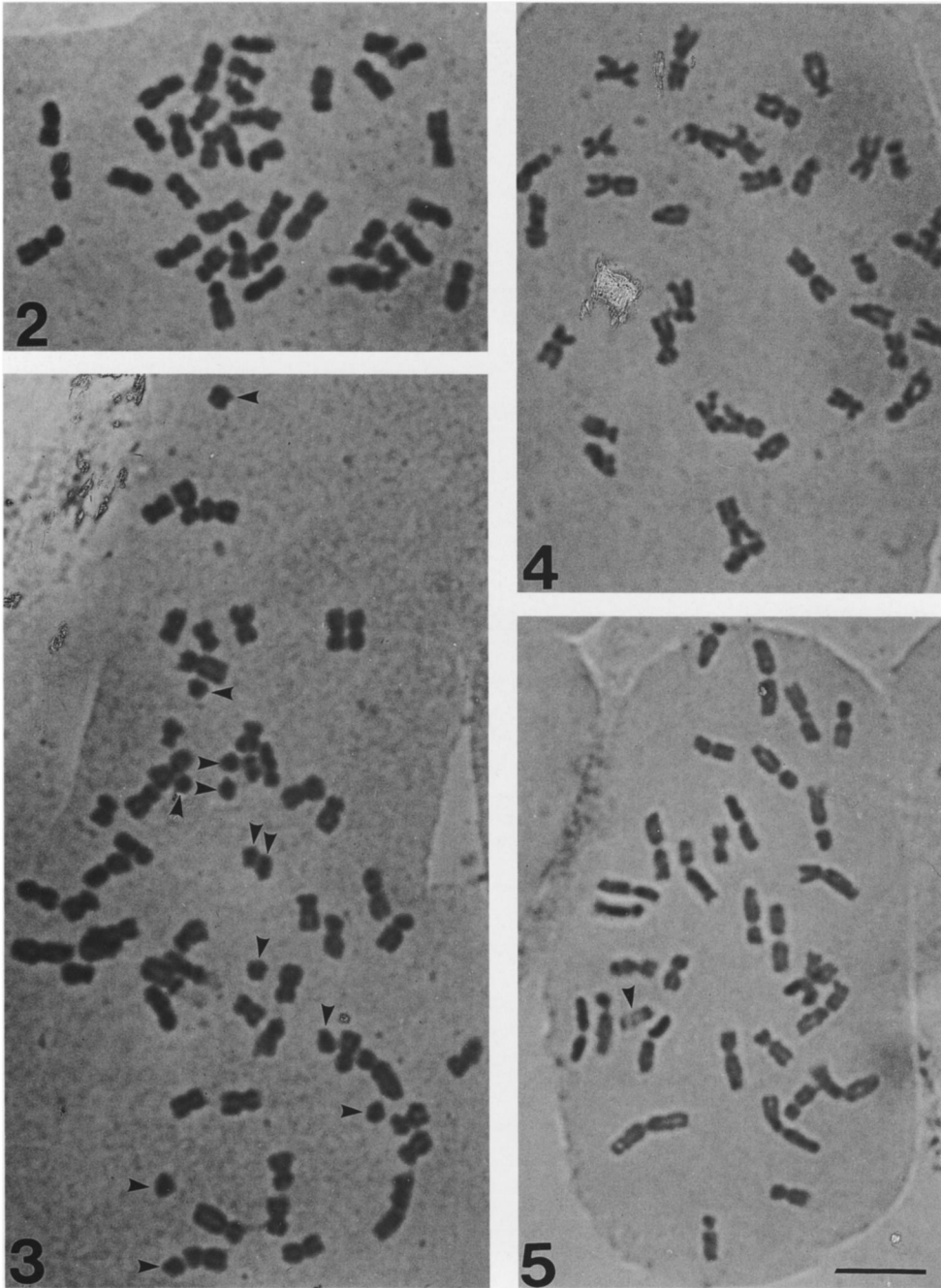
Table 1. Continued.

Species	Chromosome number		Collection data or reference
	<i>n</i>	<i>2n</i>	
	—	50 + 2 ~ 6B	Costa Rica. Cartago: Tapanti National wild-life Refuge, <i>King 10260</i> (US)
<i>N. parasitica</i> (Klatt) R. M. King & H. Rob.	—	34 + 1 ~ 2B	Costa Rica. 8.9 mi. NW of centers of Sanramin, <i>Croat 68077</i> (US)
<i>N. standleyi</i> (B. L. Rob.) R. M. King & H. Rob.	—	50 + 1 ~ 4B	Costa Rica. La Hondura, Bruallio Carrillo N. P., <i>King 10263</i> (US)
<i>Polyanthina nemorosa</i> (Klatt) R. M. King & H. Rob.	10II	—	Costa Rica. On the way from Cartago to Tapanti, W limit of Paraiso, <i>King 10247</i> (US)
<i>Praxelis clematidea</i> (Griseb.) R. M. King & H. Rob.	—	30	Brazil. Rio de Janeiro: City limit of Rio de Janeiro, <i>King 10281</i> (US)
<i>Sclerolepis uniflora</i> (Walter) Britten, Sterns & Poggenb.	—	30	U.S.A. Delaware: Sussex Co. W of Bethamy Beach, <i>King 10235</i> (US)
<i>Stevia connata</i> Lag.	—	44	Mexico. Oaxaca: 4.9 km from El Punto. <i>Yahara & Ito 142</i> (MAK)
<i>S. eupatoria</i> (Spreng.) Willd.	—	36	Mexico. 80 km from Mexico City to Tulancingo, <i>Yahara & Oyama 414</i> (MAK)
	—	48	Mexico. Hidalgo: 11 km from entrance to El Chico, <i>Yahara & Oyama 451</i> (MAK)
<i>S. microchaeta</i> Sch. Bip.	—	24	Mexico. Oaxaca: 54.3 km N from Guelatao, <i>Yahara & Ito 251</i> (MAK)
<i>S. monardaefolia</i> HBK	—	33	Mexico. Puebla: 4 km N from State border, Ixta-Popo N. P., <i>Yahara & Ito 381</i> (MAK)
<i>S. ovata</i> Willd.	—	33	U.S.A. Texas: Brewster Co., Chisos Mts. Mt. Emory, 9 Aug. 1991, <i>Yahara et al. s.n.</i> (MAK)
<i>S. pilosa</i> Lag.	—	22	Mexico. Hidalgo: 10 km from Pachuca to El Chico on Hwy. 105, <i>Yahara & Ito 425</i> (MAK)
<i>S. salicifolia</i> Cav.	—	24 + 0 ~ 1B	Mexico. Coahuila: 2 km E of Los Lirios, <i>King 10327</i> (US)
<i>S. serrata</i> Cav.	—	33 + 0 ~ 2B	Mexico. 80 km from Mexico City to Tulancingo, <i>Yahara & Oyama 412</i> (MAK)
<i>S. suaveolens</i> Lag.	—	33 + 0 ~ 2B	Mexico. 80 km from Mexico City to Tulancingo, 16 Oct. 1993, <i>Yahara & Oyama s.n.</i> (MAK)
<i>S. tomentosa</i> HBK	—	33	Mexico. 80 km from Mexico City to Tulancingo, <i>Yahara & Oyama 411</i> (MAK)
	—	33	Mexico. Hidalgo: 10 km from Pachuca to El Chico on Hwy. 105, <i>Yahara & Oyama 422</i> (MAK)

tween mean total karyotypic lengths were evaluated by a t-test.

Although there is some variation in the total karyotypic lengths and chromosomal lengths between and within species, there is a significant length difference between genera within Eupatorieae. The chromosomal and total karyotypic length of *Mikania congesta* have been shown to be significantly shorter than those of *Neomirandea parasitica* ($t = 11.137$; $p < 0.001$), *Ageratina scorodonioides* ($t = 10.943$; $p < 0.001$), and *A. havanensis*

($t = 9.976$; $p < 0.001$) with the same chromosome number, $2n = 34$ (Table 2 and Figs. 4–7). Similarly, those of *Koanophyllon longifolium* ($t = 5.027$; $p < 0.01$), *Lepidesmia squarrosa* ($t = 3.851$; $p < 0.02$), and *Fleischmannia sideritides* ($t = 5.868$; $p < 0.005$) have also been shown to be significantly shorter (compared with the total karyotypic length in *Hebeclinium macrophyllum*) than those of other species and genera with the same chromosome number, $2n = 20$ (Figs. 8, 9). The chromosomal and total karyotypic lengths of *Carphochaete bige-*

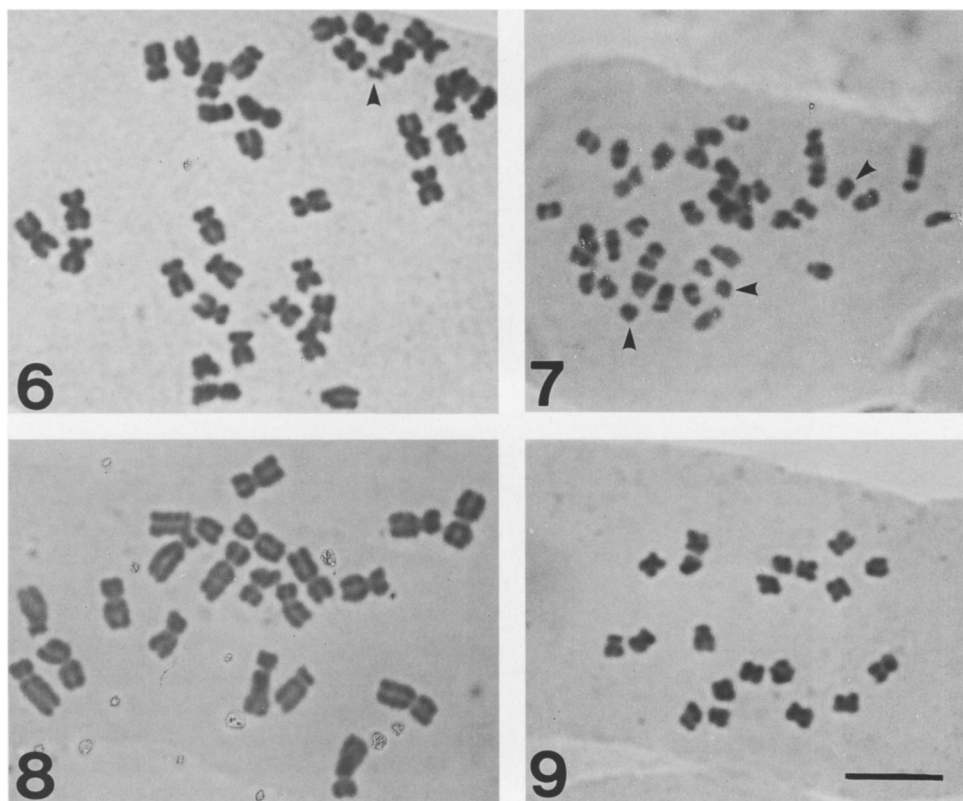


FIGURES 2–5. Photomicrographs of somatic metaphase chromosomes.—2. *Podachaenium eminens* ($2n = 38$).—3. *Neomirandea guevarii* ($2n = 50 + 12B$).—4. *Ageratina scorodonioides* ($2n = 34$).—5. *Bartlettina sordida* ($2n = 32 + 1B$). Scale bar = 5 μm . Arrow heads indicate B chromosomes.

lovii with $2n = 22$ are the longest among the taxa examined in this tribe.

Total karyotypic length of *Podachaenium eminens* ($2n = 38$, Heliantheae) is comparable to that

of *Ageratina scorodonioides* ($t = 2.189$; $0.10 > p > 0.05$) with $2n = 34$, as well as to that of *Bartlettina sordida* with $2n = 32$ ($t = 0.374$; $p > 0.50$). In addition, total karyotypic lengths of those spe-



FIGURES 6–9. Photomicrographs of somatic metaphase chromosomes.—6. *Neomirandea parasitica* ($2n = 34+1B$).—7. *Mikania congesta* ($2n = 34+3B$).—8. *Eupatorium purpureum* ($2n = 20$).—9. *Fleischmannia sideritides* ($2n = 20$). Scale bar = 5 μ m. Arrow heads indicate B chromosomes.

cies with higher chromosome numbers are also comparable (compared with the total karyotypic length in *Ageratina havanensis*) to those of *Austroeupatorium inulaefolium* ($t = 1.212$; $0.40 > p > 0.20$) and *Acanthostyles buniifolius* ($t = 1.703$; $0.20 > p > 0.10$), with $2n = 20$. In contrast, the difference between the total karyotypic length of species with $2n = 50$ and 34 within the genus *Neomirandea* is significant ($t = 4.399$; $p < 0.02$).

DISCUSSION

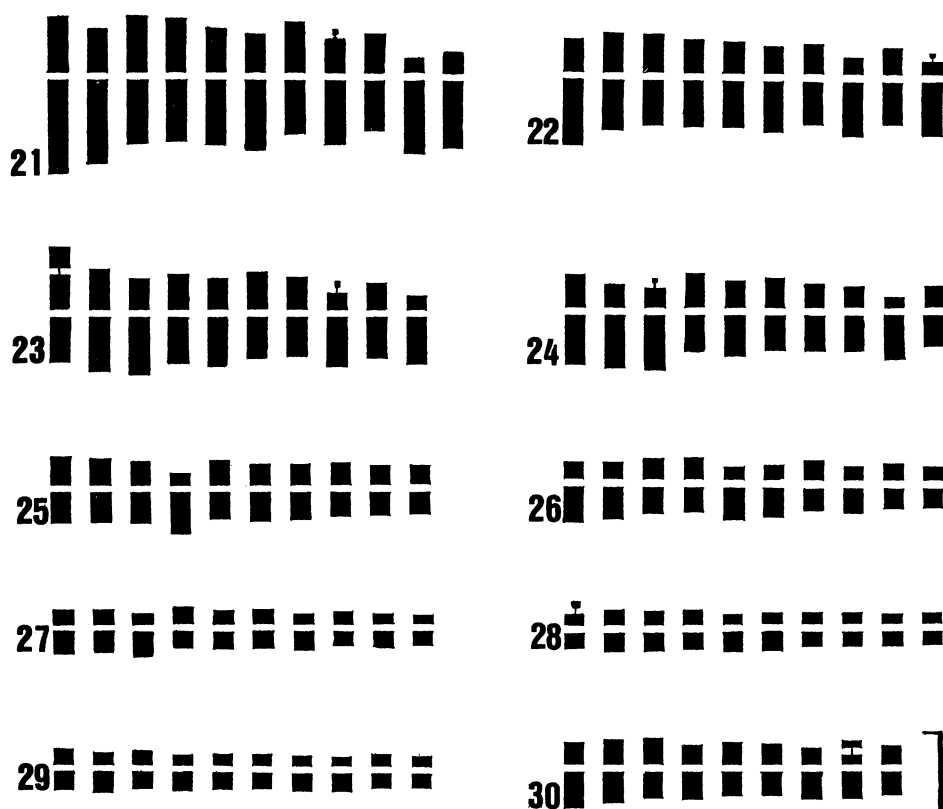
Although previous reports for chromosome numbers have included many miscounts or have been based on misidentified plant materials, chromosome numbers, including the present data, are now known for over 467 species in 96 genera of all 18 subtribes of Eupatorieae (compiled from the indexes to chromosome numbers for 1958–1991: Fedorov, 1974; Goldblatt, 1981, 1984, 1985, 1988; Goldblatt & Johnson, 1990, 1991). These represent over 20% of the species and 53% of the genera in the tribe. Excluding ambiguous and erratic counts

from previous reports, Table 3 shows the revised distribution of base chromosome numbers in Eupatorieae according to the subtribes of King & Robinson (1987); underlined base chromosome numbers were confirmed in the present study. We have not yet determined chromosome numbers for Hofmeisteriinae and Oaxacaniinae ourselves, and we are not certain if the base number $x = 18$ is correct.

The overall distribution of chromosome numbers in Eupatorieae is characterized by having few species and genera with numbers smaller than $n = 10$, compared to all other tribes in Asteraceae except Barnadesieae, Mutisieae, and Cynareae. Also, Eupatorieae is unusual because the lower chromosome numbers are completely lacking in six of the subtribes, and the occurrences of B chromosomes are confined to the groups with higher chromosome numbers. In groups with lower chromosome numbers ($n = 4, 9$, and 10), we have not found any B chromosomes, although we have examined the chromosomes of more than 2000 individuals (Wa-



FIGURES 10–20. Haploid karyotypic idiograms.—10. *Podachaenium eminens* ($n = 19$).—11. *Neomirandea biflora* ($n = 25$).—12. *Neomirandea guevarii* ($n = 25$).—13. *Neomirandea angularis* ($n = 25$).—14. *Ageratina scorodonioides* ($n = 17$).—15. *Neomirandea parasitica* ($n = 17$).—16. *Ageratina havanensis* ($n = 17$).—17. *Mikania congesta* ($n = 17$).—18. *Bartlettina sordida* ($n = 16$).—19. *Stevia salicifolia* ($n = 12$).—20. *Stevia pilosa* ($n = 11$). Scale bar = 4 μ m.



FIGURES 21–30. Haploid karyotypic idiograms.—21. *Carphochaete bigelovii* ($n = 11$).—22. *Austroeupatorium inulaefolium* ($n = 10$).—23. *Acanthostyles buniifolius* ($n = 10$).—24. *Eupatorium purpureum* ($n = 10$).—25. *Adenostemma cuatrecasasii* ($n = 10$).—26. *Hebeclinium macrophyllum* ($n = 10$).—27. *Koanophyllon longifolium* ($n = 10$).—28. *Lepidesmia squarrosa* ($n = 10$).—29. *Fleischmannia sideritides* ($n = 10$).—30. *Brickellia chlorolepis* ($n = 9$). Scale bar = 4 μm .

tanabe, 1986; Watanabe et al. 1990; the present and unpublished data).

In the genus *Neomirandea*, all of the species examined have a variable number of B chromosomes between individuals. It is uncertain at present what role B chromosomes play in chromosomal evolution in the groups with higher chromosome numbers.

To obtain an accurate picture of the nature of higher base numbers in Eupatorieae, we have studied the genus *Neomirandea* more intensely. In previous studies, a wide range of chromosome numbers, from $n = 14$ to 26, has been reported, and it has been uncertain what the true base chromosome number is for this genus. The six species examined have a chromosome number of either $2n = 34$ or 50. Previously reported chromosome numbers are also around $n = 17$ or $n = 25$, except for $n = 14$ in *N. ovadensis* R. M. King & H. Rob. (Strother, 1983) and $n = 20$ in *N. burgeri* R. M. King & H. Rob. (King et al., 1976). Thus, two different lineages based on dibase chromosome numbers have

been revealed within this genus. The subdivision of *Neomirandea* based on chromosome number is supported by many synapomorphic mutations in chloroplast DNA (Yahara et al., in prep.).

Dibase chromosome numbers of 4 and 10, and of 11 and 12, are also confirmed in *Fleischmannia* and *Stevia*. Although there are many reports of $2n = 34$, suggesting the occurrence of $x = 17$ in *Stevia*, those reports appear to be miscounts.

In the genus *Ageratina*, we examined eight species. *Ageratina rothrockii* has two cytotypes $3x$ and $4x$ based on $x = 17$. *Ageratum corymbosum* ($2n = 4x = 40$), *Chromolaena laevigata* ($2n = 5x = 50$), *Koanophyllon longifolium* ($2n = 3x = 30$), and *Praxelis clematidea* ($2n = 3x = 30$) are polyploids based on $x = 10$.

The distribution of base chromosome numbers in the species, genera, and subtribes is shown in Figure 1. Among them, the base numbers of $x = 8$, 19, and 20 reported in a few species and genera appear to be miscounts. Although there is a small

Table 2. Karyotype data for the helianthoid species *Podachaenium eminens* and 20 representative eupatorioid species. *: B chromosomes are excluded from these measurements. **: Because *Koanophyllon longifolium* is regarded as an autotriploid, its total karyotypic length is calculated as two-thirds of total genome.

Species	Chromo- some number (2n)	Total karyotypic length (μm) mean ± S.E.	Range longest–shortest (ratio)	Mean arm ratio long arms/ short arms
<i>Podachaenium eminens</i>	38	96.22 ± 2.25	3.21–1.91 (1.68)	1.47
<i>Neomirandea biflora</i> *	50	107.06 ± 4.02	3.30–1.40 (2.36)	1.51
<i>N. guevarii</i> *	50	103.60 ± 2.93	3.24–1.20 (2.70)	1.48
<i>N. angularis</i> *	50	102.22 ± 3.23	2.73–1.62 (1.69)	1.52
<i>N. parasitica</i> *	34	83.84 ± 1.87	3.30–1.81 (1.82)	1.71
<i>Ageratina scorodonioides</i>	34	88.20 ± 2.38	3.57–1.96 (1.82)	1.90
<i>A. havanensis</i>	34	83.48 ± 2.20	3.37–1.87 (1.80)	1.38
<i>Mikania congesta</i> *	34	52.62 ± 1.67	2.40–1.14 (2.11)	1.31
<i>Bartlettina sordida</i> *	32	97.62 ± 2.48	4.51–2.05 (2.20)	1.41
<i>Stevia salicifolia</i>	24	53.24 ± 1.63	2.82–1.60 (1.76)	1.52
<i>S. pilosa</i>	22	42.50 ± 0.60	2.87–1.43 (2.01)	1.40
<i>Carphochaete bigelovii</i>	22	117.14 ± 4.01	7.12–4.43 (1.57)	1.70
<i>Austroeupatorium inulaefolium</i>	20	77.14 ± 4.13	4.89–3.27 (1.50)	1.79
<i>Acanthostyles buniifolius</i>	20	76.98 ± 2.61	4.83–2.96 (1.63)	1.56
<i>Eupatorium purpureum</i>	20	63.78 ± 3.06	3.98–2.53 (1.57)	1.74
<i>Adenostemma cuatrecasasii</i>	20	47.78 ± 0.76	2.82–1.92 (1.47)	1.34
<i>Hebeclinium macrophyllum</i>	20	42.60 ± 2.15	2.54–1.64 (1.55)	1.63
<i>Lepidesmia squarrosa</i>	20	30.98 ± 1.63	1.68–1.16 (1.45)	1.32
<i>Koanophyllon longifolium</i> **	30	29.46 ± 0.91	1.72–1.14 (1.51)	1.49
<i>Fleischmannia sideritides</i>	20	27.68 ± 0.73	1.59–1.17 (1.36)	1.42
<i>Brickellia chlorolepis</i>	18	42.66 ± 1.33	2.87–2.07 (1.39)	1.31

peak at $x = 17$, 12 of the 18 subtribes, 73% of the reported genera, and 53% of the reported species have chromosome numbers based on $x = 10$. Thus, the predominant chromosome number among species, genera, and subtribes, $x = 10$, was doubtlessly regarded as the ultimate base number in the

tribe by most previous workers. In addition, this number has been proposed as the base number for the entire Asteroideae (Robinson et al., 1981). High chromosome numbers, such as $n = 15$, 16, 17, and 25, have been considered to be the result of polyploid increase followed by dysploid loss (King &

Table 3. Distribution of base chromosome numbers in Eupatorieae according to subtribe (King & Robinson, 1987). Chromosome numbers underlined are confirmed by the present study.

Adenostemmatinae		10			
Eupatoriinae		<u>10</u>			
Disynaphiinae		<u>10</u>			
Gyptidinae		<u>10</u>			
Ageratinae	9	<u>10</u>	<u>11</u>	<u>12</u>	
Ayapaninae		<u>10</u>			
Alomiinae	9	<u>10</u>			
Liatrinae		<u>10</u>			
Fleischmanniinae	<u>4</u>	<u>10</u>			
Critoninae		<u>10</u>			
Praxeiinae		<u>10</u>			
Hebeclininae		<u>10</u>			
Trichoroninae			<u>15</u>	<u>16</u>	
Neomirandeiinae				17	<u>25</u>
Mikaniinae				<u>17</u>	18
Oxylobinae			16	<u>17</u>	
Hofmeisteriinae					18
Oaxacaniinae					18

Robinson, 1987). Taxa with high chromosome numbers that are classified as different subtribes are, however, distinct morphologically from the taxa with $n = 10$. This is in contrast to the present polyploid derivatives based on $x = 10, 11$, and 17 , which are rather similar morphologically to their diploid ancestors. On the other hand, some workers (Grant, 1953; Turner & King, 1964) have postulated that species with the ancestral base number of $x = 5$ and 4 might have given rise through successive allopolyploidy to those taxa with base numbers of $9, 10$, and 17 . It is difficult to imagine, however, that the Costa Rican species of *Fleischmannia* having $n = 4$ are primitive because of their specialized annual habit and weediness.

In the course of electrophoretic studies on *Eupatorium*, we found that diploid species of *Eupatorium* with a chromosome number of $n = 10$ had extensive gene duplications (Yahara et al., 1989). This finding casts further doubt on the hypothesis that $x = 10$ was the ultimate base number for *Eupatorium*, as well as Eupatorieae, because diploid vascular plants have a minimum highly conserved number of isozymes (Gottlieb, 1981).

The chloroplast DNA data provide sufficient resolution to determine the ancestral chromosome number and the directionality of dysploidy within Eupatorieae (Ito et al., in prep.). Specifically, in the cpDNA phylogeny, those taxa with $n = 17$ in Eupatorieae occur at the base of the tribe next to three helianthoids, and taxa with lower numbers, such as $n = 10$, are derived groups. Therefore, $x = 17$ is accepted as the ultimate base number in the tribe, and chromosome number evolution has proceeded in a descending direction only in this tribe, except for $n = 25$ in *Neomirandea*. The highest base number of $x = 25$ in *Neomirandea* could be derived from $x = 17$ by ascending dysploidy as suggested from the higher degree of gene duplications (Suzuki et al., unpublished) and the significantly longer total karyotypic length in species with $n = 25$ as compared to species with $n = 17$.

Differences in chromosome size and shape have been mentioned in the studies of Eupatorieae by Grant (1953) and Gaiser (1953, 1954). Grant reported two size ranges for chromosomes. For species in *Eupatorium* with $n = 10$, the size ranged from 2.5 to $6.5 \mu\text{m}$. For species in *Ageratina* with $n = 17$, the size ranged from 1.5 to $3.1 \mu\text{m}$. For species of *Conoclinium* with $n = 10$, the size ranged from 1.0 to $2.0 \mu\text{m}$. We also confirmed the significant chromosome difference between genera (Table 2). Among the species with $n = 17$, the chromosome length of *Mikania congesta* is significantly shorter than others. Among the species with

$n = 10$, the chromosome length of species shows wide successive ranges from 4.89 to $1.14 \mu\text{m}$, although there is a significant length difference between the species with longer and shorter chromosomes.

To assess relationships between the different chromosome numbers and lengths, we have measured the total karyotypic length of these species. Total karyotypic length of *Podachaenium eminens* ($2n = 38$, Heliantheae) is comparable to that of *Ageratina scorodonioides* with $2n = 34$, as well as that of *Bartlettina sordida* with $2n = 32$. In addition, total karyotypic length of those species with higher chromosome numbers is also comparable to that of *Austroeupatorium inulaefolium* and *Acanthostyles buniifolius* with $2n = 20$. It appears that the difference in chromosome number and length between the taxa is due to descending dysploidy caused by very unequal reciprocal translocation with loss of minute centromeric fragments. This contrasts with the previous hypothesis (King & Robinson, 1987) that the higher chromosome numbers of $n = 16$ to 18 have been derived from $n = 10$ by polyploidization followed by dysploid loss. The comparison between the karyotypes with $2n = 24$ and 22 in *Stevia* tends to substantiate partly this conclusion. The shrubby *Stevia salicifolia* with $2n = 24$ has a unimodal karyotype, and the herbaceous *S. pilosa* with $2n = 22$ has a bimodal karyotype with two longer pairs of chromosomes. These longer chromosomes in the species with $n = 11$ may be the result of translocations to their long arms from each arm of one chromosome of the species with $n = 12$. *Sclerolepis uniflora* ($2n = 30$) has also a bimodal karyotype with one longer pair of chromosomes, suggesting a translocated product.

Throughout the process of evolutionary advancement a reduction in chromosome size has also occurred in a similar manner in several genera, such as *Mikania*, *Polyanthina*, *Hebeclinium*, *Fleischmannia*, *Ageratum*, and *Conoclinium*. With the genus *Brickellia*, Gaiser (1953, 1954) noted the correlation between the trend toward reduction in chromosome size and change of habit from shrubby to herbaceous. Annual species have shorter chromosomes. This is true in the genus *Stevia*, too (Table 2, in the comparison between *S. salicifolia* with $2n = 24$ and *S. pilosa* with $2n = 22$, $t = 5.514$; $p < 0.01$). The understory shrub *Carphochaete bigelovii* has the longest chromosomal and total karyotypic length among the examined taxa. In Eupatorieae, chromosomal evolution has followed a general trend of reduction in chromosome number and length.

Of course, polyploidy has occurred frequently in

several lineages as shown in Table 1. Polyploidy accompanied by agamospermy is common in the genera *Ageratina*, *Stevia*, *Chromolaena*, *Praxelis* (King & Robinson, 1987), and *Eupatorium* (Watanabe, 1986). The frequent occurrences of polyploids including odd-ploids in these genera are consistent with their previous inferences of widespread agamospermy.

The shrubby or arborescent habit is characteristic of taxa with high chromosome numbers in relatively stable habitats both in Heliantheae and Eupatorieae. Primitive groups of Eupatorieae follow similar evolutionary attributes to some members of Heliantheae. The reduction in chromosome number correlated with the herbaceous habit in Eupatorieae might promote the exploitation of open habitats with ephemeral resources.

Cytology might prove most helpful in subtribal and generic circumscription, and in reflecting some of the evolutionary trends.

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