Phylogeny and phytogeography in the genus *Eupatorium* (Asteraceae)

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The interspecific relationships of the temperate genus *Eupatorium* in Northern Hemisphere were assessd by restriction site analysis of chloroplast DNA and karyotype analysis. Molecular and cytological data provided strong evidences that the origin of *Eupatorium* was in North America and the genus diversified well into three morphological species groups there and extended its range to temperate regions of eastern Asia and Europe. Asian species group is monophyletic. Agamospermous reproduction and hybridization make species recognition very difficult and the distributional range of each species very ambiguous. Cytogeographical study combined with morphological analysis on the agamic complex and related species could reveal the population structure, the discrete circumscription of species, their distributional ranges, and the evolutionary and phytogeographical history in this genus.

Key words: agamic complex, divergence, *Eupatorium*, intercontinental disjunction, phylogeny, phytogeography

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The genus *Eupatorium* L. is a member of Eupatorieae, Asteraceae. Twenty-three species occur in south-eastern North America, 25 species in eastern Asia and one species in Europe (Fig. 1). This distribution matches a disjunction pattern of many temperate species and genera well-known as the Arcto-Tertiary elements in Northern Hemisphere (Halenius, 1750, Gray, 1859, Li, 1952, Graham, 1972, Boufford and Spongberg, 1983). A classical theory for this disjunct distributional pattern assumed a circumpolar ancestral flora which had migrated southwardly during climatical deterioration in Tertiary. According to King and Robinson (1970b), however, the origin of *Eupatorium* was probably in North America, where the greatest number of species and neareast related genera occurred, and the genus then extended its range to the temperate regions of eastern Asia and Europe very late in Tertiary. Thus the study on phylogeny and phytogeography of this genus will provide a better understanding of the evolution of the temperate flora in Northern Hemisphere in general.
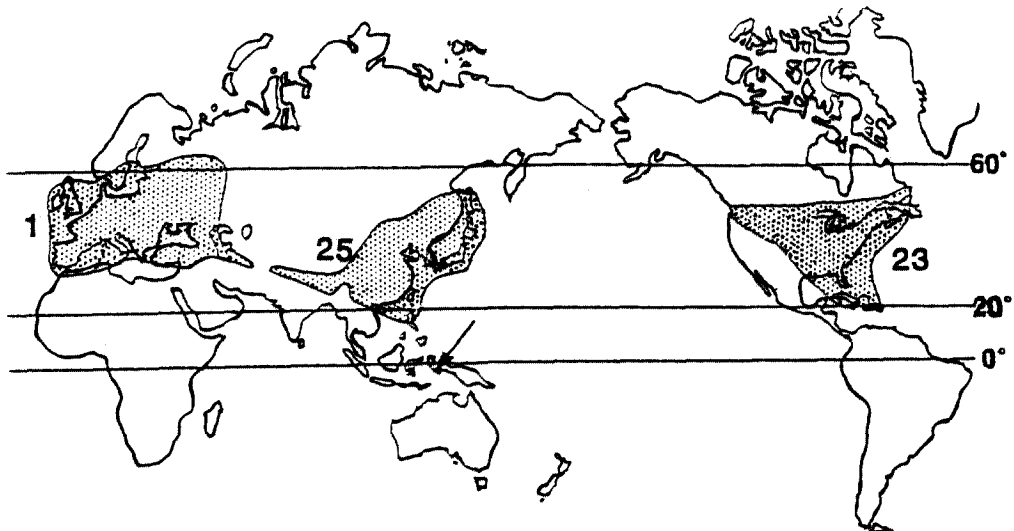

Fig. 1. Total distributional range and species number in the genus *Eupatorium s. str.* An arrow indicates the distribution of *E. toppingianum* Elm. to the Seram Isl. (Watanabe, 1986).

North American eupatoria are subdivided into three morphological groups (King and Robinson, 1989). The *Uncasia* group has non-dissected decussate leaves. The *Eutrochium* group has non-dissected verticillate leaves, enlarged style bases, stomata on the corolla lobes and reddish-purple flowers. It was, once, segregated as a different genus *Eupatoriadelphus* by King and Robinson (1970a). The *Traganthes* group has finely dissected alternate or decussate leaves and the south-biassed geographical distribution. Asian eupatoria consist of one species with verticillate leaves and 24 species with decussate leaves. An European species has decussate leaves. Following questions on the phylogeny and phytogeography in *Eupatorium* were addressed for molecular and cytological analyses. 1) Does evolutionary history of *Eupatorium* support the classical theory of Arcto-Tertiary geoflora? 2) Are three morphological species groups in North America monophyletic? 3) Are Asian and European species monophyletic?

One European species, 7 Asian species, 13 North American species of *Eupatorium* and *Fleishmannia sideritides* (Benth. in Orsted) R. M. King and H. Rob. as an outgroup were analyzed by the RFLPs of chloroplast DNA. The cladogram obtained (Fig. 2) supported the following points (Ito *et al.*, in preparation). 1) Asian species group is monophyletic and has evolved from North American species with decussate leaves. 2) European species, *E. cannabinum* L., has derived from North American species with decussate leaves or from a common ancestor with Asian lineage. 3) The *Traganthes* group with finely dissected leaves is monophyletic. 4) The *Eutrochium* is a sister group to the rest of species. This means that the verticillate leaves of Asian species, *E. glehni* Fr. Schm. ex Trautv., has originated independently from those of North American *Eutrochium* species. In conclusion, the origin of *Eupatorium* was in North America and the genus diversified well into three morphological groups there and migrated to eastern Asia and Europe. This result suggests that the disjunction for *Eupatorium* not has accomplished by the disjornting of the Arct-Tertiary geoflora at the time of climatical deteriolation in Tertiary but more recently by long distance dispersal, although the RFLP analysis can not estimate the precise divergence time. Recent molecular phylogenetic studies also showed that the formations of disjunctions of some temperate species and genera in Northern Hemisphere were attained through multiple migrations during considerably long term, not the result of a single historical event (Qiu *et al.*, 1995, Lee *et al.*, 1996).

The karyotypic comparison for 22 diploid species of *Eupatorium* were also
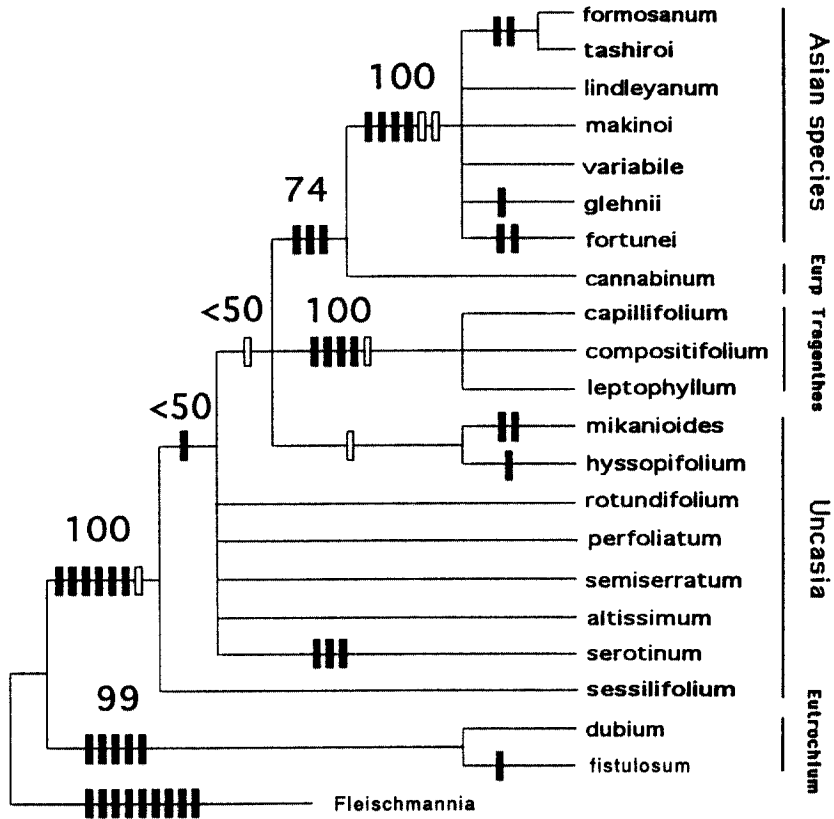
Fig. 2. One of the most parsimonious trees from RFLP analysis in the genus *Eupatorium*. The non-homoplasious apomorphic characters are indicated by black vertical stripes and homoplasious characters are indicated by white vertical stripes. Length=84 steps, CI=0.881, R I=0.896. Bootstrap probabilities (precent) of 1000 bootstrap replications are given for nodes. Among the North American three morphological groups, *Eutrochium* and *Traganthes* groups are monophyletic, respectively. Asian species group is also monophyletic (Ito *et al.*, in preparation).

made using newly developed index, Minimal Chromosomal Distance (Watanabe *et al.*, 1990). Minimal Chromosomal Distance was calculated as follows. At first, relative long arm length (RLL) and relative short arm length (RSL) were calculat-

ed for each chromosome by the following equations; $RLL = 2a_i / \sum_{j=1}^{2n} (a_i + b_i)$, $RSL =$

$2b_j / \sum_{j=1}^{2n} (a_i + b_j)$, where ai was long arm length of chromosome i and bi was short arm length of chromosome i. Chromosomal Distance (CD) was calculated by the following equation; $CD = \sum_{j=1}^{n} \{(ax_i - ay_i)^2 + (bx_i - by_i)^2\}$, where ax_i was the RLL of chromosome i in species x and by_i was the RSL of chromosome i in species y.
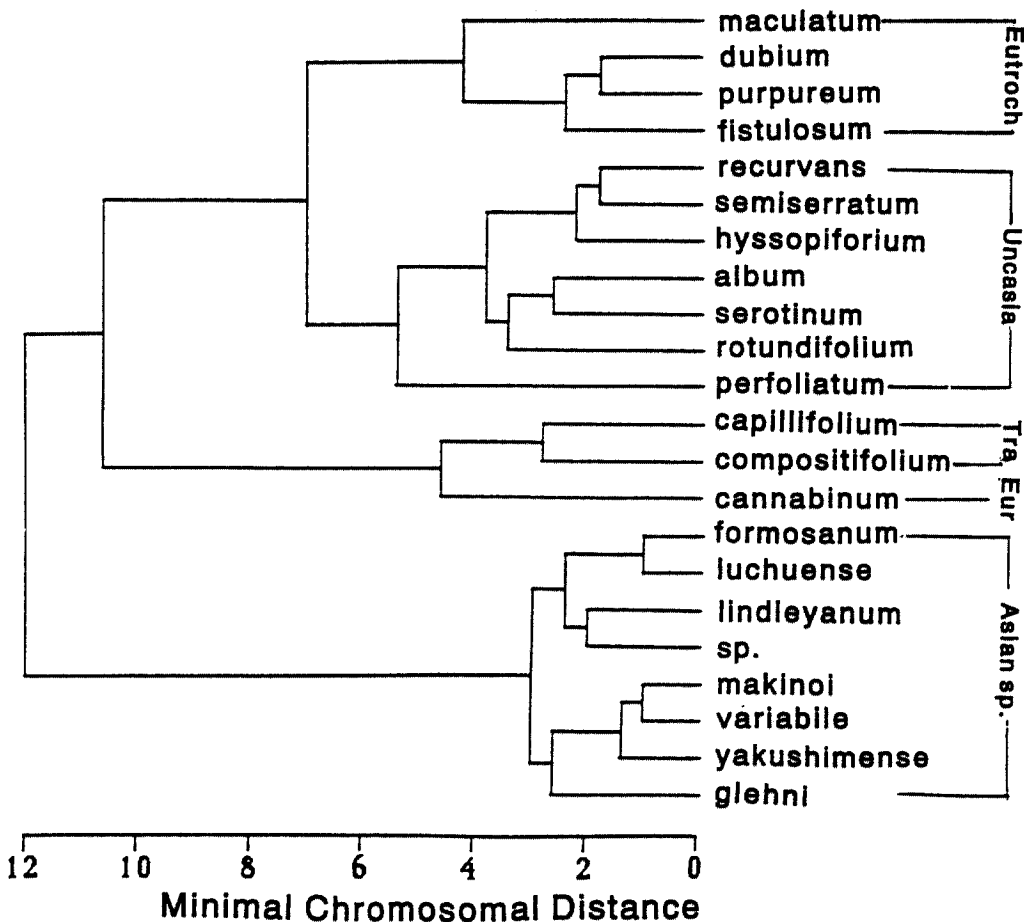

Fig. 3. Phenograms (UPGMA) of taxa of *Eupatorium* based on the index of minimal chromosomal distance. North American three morphological groups and Asian species group are monophyletic, respectively. European species clustered together with the members of North American *Traganthes* group. The sp.: undescribed species from Thailand (Watanabe *et al.*, 1990).

We computed CDs between two species for all 10×10 combinations of haploid chromosome sets, and the minimum value was defined as Minimal Chromosomal Distance (MCD). Figure 3 shows the UPGMA phenogram based on MCD between karyotypes. North American eupatoria well diversified in karyotype corresponding three morphological species groups. Namely, North American *Eutrochium* species with verticillate leaves clusterd together. North American *Uncasia* species with decussate leaves also had similar karyotypes and grouped into a discrete cluster. European species and North American *Traganthes* species clustered together. Eight Asian species grouped into one cluster and their karyotypic diversity among species was lower than that among North American species. Namely, Asian species seem to have radiated more recently than North American species. This result is concordant with the molecular and morphological data on the origin and evolutionary history in the genus *Eupatorium* mentioned above.

Taxonomy and geographycal distribution in Asian *Eupatorium*

The morphological similarity between species, hybridization and morphological and cytological variations associated with agamospermy make species recognition in Asian species group very difficult and the distributional range of each species very ambiguous. For example, Kitamura (1949, 1957) recognized several taxa as species, subspecies and varieties in the *Eupatorium chinense* complex. This complex consists of diploid, triploid, tetraploid and pentaploid based on $x = 10$. In diploid, ten bivalents formed regularly. In polyploid, univalents or very complex multivalents were formed in MI and various size of pollen grains were produced. The mode of reproduction of diploids was sexual and that of polyploids agamospermous (Watanabe *et al.*, 1982). The following differences in pollen morphology make it possible to discriminate sexual plants (possibly diploids) and agamospermous polyploids on herbarium specimens. Polyploid specimens were characterized by the presence of aceto-carmine or cotton blue unstained pollen grains smaller than $16 \mu m$ and of significantly larger or deformed pollen grains (Kawahara *et al.*, 1989a). Although overall morphological comparisons without cytological information failed to discriminate any distinct groups within this complex, four taxa could be recognized by morphology and esterase allozyme polymorphism in sexual, possibly diploid populations (Kawahara *et al.*, 1989a, b, Kawahara, 1990). They are sexual *E. chinense s. str., E. tozanense*

Hayata, *E. makinoi* Yahara et Kawahara and *E. glehni*, with discrete distributional ranges (Kawahara, 1990). The principal component analysis using 23 morphological characters on the *E. chinense* complex showed that sexual *E. chinense s. str.*, was distinct from sexual specimens of the other three taxa but agamospermous polyploids concealed this discreteness. Sexual three taxa were also separated in the first and second principal components. These results indicate that sexual four taxa are recognizable as species in morphology as well as in their allopatric distributions (Kawahara, 1990).
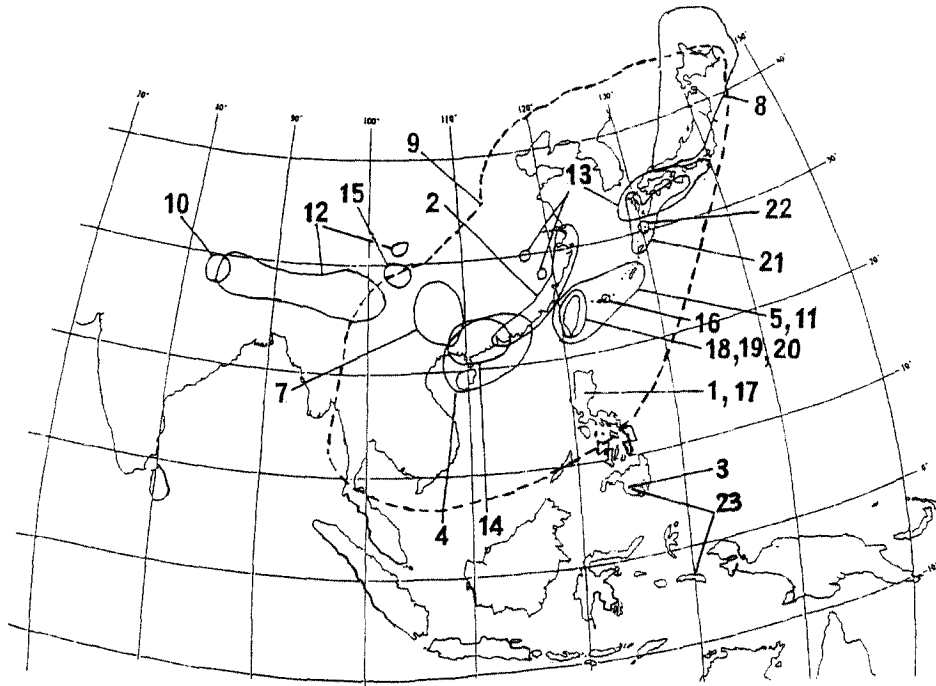
Fig. 4. Geographical distributions of 22 Asian sexual (possibly diploid) species of *Eupatorium* (Kawahara *et al.*, 1989). 1: *E. benguense* Rob., 2: *E. caespitosum* Migo, 3: *E. catense* Elm., 4: *E. chinense*, 5: *E. formosanum* Hayata, 7: *E. fortunei* var. *angustilobum* Ling, 8: *E. glehni*, 9: *E. lindleyanum*, 10: *E. longicaule* DC., 11: *E. luchuense* Nakai, 12: *E. mairei* Levl, 13: *E. makinoi*, 14: *E. melanadenium* Rob., 15: *E. omeiense* Ling et Shih, 16: *E. sp.*, 17: *E. sambucifolium* Elm., 18: *E. shimadai* Hayata, 19: *E. tashiroi* Hayata, 20: *E. tozanense*, 21: *E. variabile* Makino, 22: *E. yakushimense* Makino et Kitam., 23: *E. toppingianum*. The sp.: undescribed species from Ishigaki Isl. (Kawahara, 1990).

The same method was applied to 573 specimens. As a result, 388 specimens were estimated to be sexual diploids and 185 agamospermous polyploids. Morphological comparisons were made on the specimens estimated as sexuals, and 25 morphologically distinct taxa were recognized as species in Asian eupatoria. Figure 4 shows the geographical ranges of 22 Asian diploid species. This geographical distribution suggests that the speciation in Asian eupatoria has been induced predominantly by geographical isolation into the islands along the Eurasian continental arc.

Eupatorium makinoi segregated from *E. chinense* s. str. is a perennial occurring widely in central to northern China, Korea and Japan and is common in open disturbed places along roadsides, forest margins and in half-shaded understories.

Analyses of 1024 individuals collected from 29 populations throughout the Japan Archipelago proved that this species consisted of eight cytotypes comprising diploid, triploid, tetraploid and pentaploid based on $x=10$, a dysploid with $2n=29$ and polyploids with a partial deficient chromosome. Two Korean *E. makinoi* collected at Juk-Ryung were tetraploids.

Figures 5-8 show the horizontal and vertical distribution of polyploids and diploids. Polyploids occur widely and rather continuously throughout the Japan Archipelago (Figs. 5 and 6). On the other hand, diploids occur from the upper warm- to the lower cool-temprate zone at the Pacific Ocean side of southwestern Japan (Figs. 7 and 8). Diploids occur at fragile gravitational slopes with shallow soil on rocks and in half-shaded understories which are poor in species number and devoid of tall competitors. In contrast, polyploids are often associated with tall grasses and forbs such as *Miscanthus sinensis*. Figure 13 shows the size class structure of sexual and agamospermous populations of *E. makinoi*. Clearly diploids tend to be shorter than polyploids. Solid bars indicate the number of flowering plants. The reproduction is size-dependent. In polyploids larger biomass is needed for reproduction than in diploids (Yahara and Oyama, 1993). Figure 14 shows the number of florets per plant and the number of achenia per plant. The fecundity of agamospermous polyploids is significantly higher than that of sexual diploids. Polyploids have tall and stout stems to disperse achenia effectively. In addition polyploids can reproduce agamospermously with high fecundity (Tsunemi, 1992). They are obviously superior in colonizing newly cleared dry and open habitats.

Tabacco leaf curl geminivirus (TLCV) is transmitted by two species of whitefly (*Bemica tabaci* and *B. lonicerae*) (Osaki and Inoue, 1979) and never

Fig. 5-12. The horizontal and vertical distribution of *Eupatorium makinoi*, *E. glehni* *and E. lindleyanum* in Japan. 5 and 6: polyploid *E. makinoi*, 7 and 8: diploid *E.* *makinoi*, 9 and 10: *E. glehni*, 10 and 11: *E. lindleyanum*. Open circles represent the data on Horikawa's map (Horikawa, 1972, 1976) and closed circles based on herbarium specimens (Watanabe and Yahara, 1984, Watanabe, 1986, 1989).
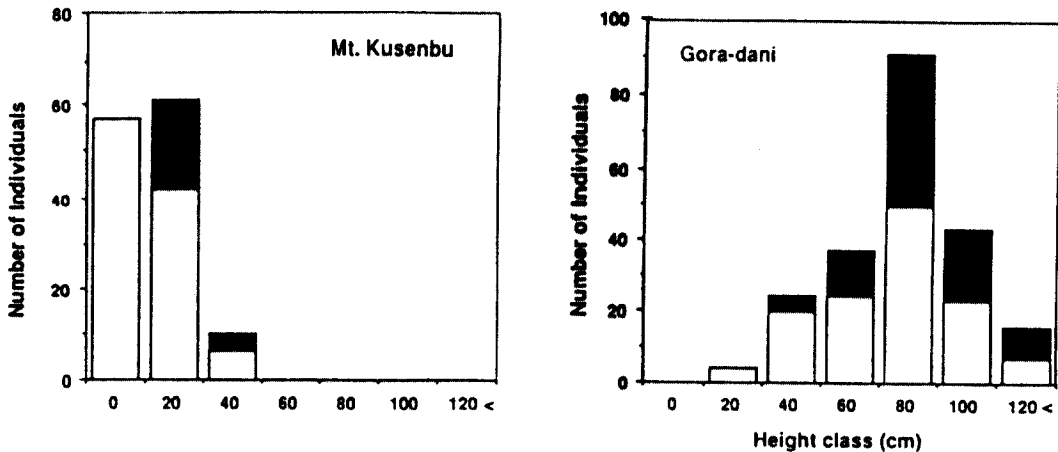
Fig. 13. Size class structure of sexual (Mt. Kosenbu) and agamospermous (Gora-dani) populations of *Eupatorium makinoi*. Solid bars indicate the number of flowering plants (Yahara & Oyama, 1993).
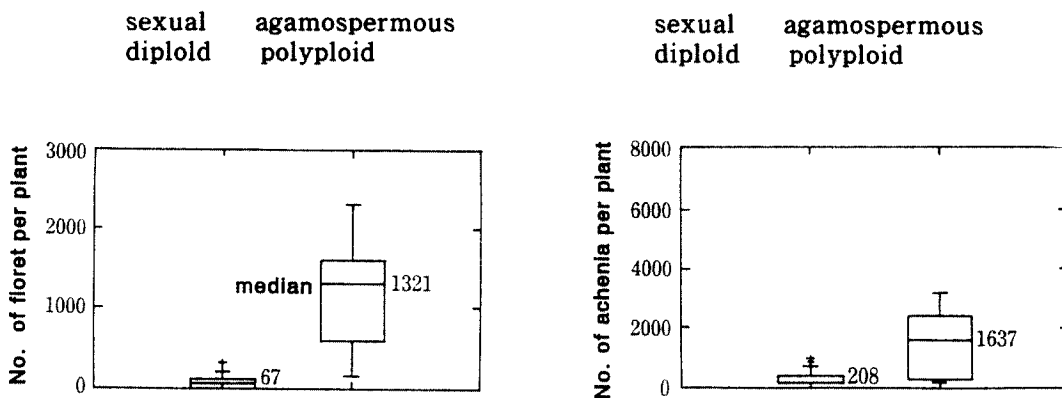
sexual	agamospermous		sexual	agamospermous
diplold	polyploid		diplold	polyploid

Fig. 14. Numbers of florets and achenia per plant in diploid and polyploid *Eupatorium makinoi* (Tsunemi, 1992).

transmitted through host seeds. It was firstly identified as a disease of tobacco and tomato plants (Osaki and Inoue, 1981). In Japan *Eupatorium makinoi* and *Lonicera japonica* (Caprifoliaceae) are only known as wild host plants (Osaki and Inoue, 1979, Osaki *et al.*, 1979). Tobacco leaf curl geminivirus impaired survivorship and growth of *Eupatorium makinoi*. This infection is more frequent in agamospermous populations than in sexual diploid populations (Yahara and Oyama, 1993). Agamospermous populations might be genetically uniform and

Table 1. Effects of tobbaco leaf curl virus infection on the survivourship and growth of *Eupatorium makinoi* grown under high-and low-light conditions. means± SD are shown.

Characteristic	High light			Low light		
	Infected (N=29)	Uninfected (N=29)		Infected (N=29)	Uninfected (N=29)	
Final survivorship(%) a	100	100	– –	46.7	93.3	∗ ∗ ∗
Plant biomass(g) b	9.80±5.74	15.0±6.3	∗ ∗	0.856±0.616	1.27±0.85	NS
Plant biomas/fresh mass Feb. c	3.00±2.12	4.51±2.04	∗ ∗	0.19±0.09	0.33±0.10	∗ ∗
RGR[g.g-1(9mo)-1] c	1.83±0.82	2.23±0.53	∗	-0.930±0.34	-0.077±0.293	∗ ∗ ∗
Root/Shoot c	1.19±0.39	1.24±0.28	NS	2.21±1.90	1.32±0.40	NS
Leef/Shoot c	0.586±0.046	0.493±0.073	∗ ∗ ∗	0.387±0.118	0.514±0.121	∗

a Fisher's exact probability test., b Student's t test., c Mann-Whitney U test.,

∗p<0.005, ∗ ∗p<0.001, ∗ ∗ ∗p<0.001, NS not significant (Funayama *et al.* 1997).

thus they would become infected more frequently and seriously (Ooi *et al.*, 1997). The survivorship of infected plants strongly depends on light condition. Damage caused by virus infection was severe under low light condition as shown in Table 1 (Funayama *et al.*, 1997). Plant biomass in polyploids also extremely decreased under low light condition. This, as well as the requirement of larger biomass for reproduction in polyploids, may be one of the reason why polyploids are scarecely found in half-shaded understories and other unfavorable habitats where diploids occur. Pathogen pressure is one of some hopeful factors which provide advantages for sexual reproduction (Levin, 1975, Hamilton *et al.*, 1990).

Figure 15 shows the frequency of various cytotypes in each population. Most of populations exhibited various combination of polyploid cytotypes irrespective of their temporal population size. The agamospermous propagation, rare sexuality and random colonization could result in an intricate mixture of various polyploid cytotypes within local populations based on their geographical backgrounds at disturbed and temporary habitats. All of the cytotypes were found in south-western regions of Japan. This distributional pattern suggests that the direction of migration from south-western to northern regions of Japan. New polyploids probably spread beyond the range of diploids. Palynological evidence showed the large southward shift of vegetation during the last Ice Age (Tsukada, 1982). The overall distribution of Japanese eupatoria also appears to

Fig. 15-17. Localities of the populations studies and fan diagrams of cytotypic composition of each population in Japanese *E. makinoi* (Fig. 15), *E. glehni* (Fig. 16) and *E. lindleyanum* (Fig. 17). The size of a circle corresponds to the number of plants examined in each locality. Cytotypes 3, 5 and 7 in *E. makionoi* and Cytotypes 3 and 5 in *E. lindleyanum* involved a partially deficient chromosome(Watanabe, 1986).

have withdrawn to the south-western region of Japan, corresponding with the southward shift of vegetation. After the last Ice Age, most of species including polyploid *E. makinoi* began to migrate north-ward and to higher elevation from south-western refuges accompanying the climatical warming. Diploid *E. makinoi*, however, seemed to have migrated less than the other successful colonizers from the refuges, due to its low fecundity of achenia and less competitive habits.

Eupatorium glehni is similar to polyploid *E. makinoi* in plant size, but it has verticillate leaves. Figures 9 and 10 show the distribution of *E. glehni*. It has a more north-biassed distribution reached to Sachaline in Russia and come in contact with *E. makinoi* at the southern and lower marginal regions. It occurs in the cool-temperate forest zone and grows usually in mountain paths, clearings or canopy gaps. Figure 16 shows the cytotypic constitution of *E. glehni* from 13 populations. It consists of five cytotypes comprising diploid, triploid, tetraploid and pentaploid based on $x=10$ and a hypo-tetraploid $2n=38$. The abundance and widespread distribution of diploids were confirmed. In contrast, the occurrences of polyploids were confined to sympatric or adjacent populations with polyploid *E. makinoi*. Most polyploids of *E. glehni* had intermediate morphological traits between diploid *E. glehni* and polyploid *E. makinoi* and suggested to be of hybrid derivatives between them. The difference in morphology and growth habit between diploids and "polyploids" of *E. glehni* are less conspicuous than the difference between diploids and polyploids of *E. makinoi*.

Eupatorium lindleyanum DC. occurs in wet places such as swamps, moors, marshy grounds and fallow rice fields. It has the widest distributional range among Asian eupatoria. Sexual diploids are predominant. *Eupatorium lindleyanum* has the similar geographical distribution to that of polyploid *E. makinoi* in Japan (Figs. 11 and 12) but is usually allopatric to *E. makinoi* occurring in drier places. Figure 17 shows the cytotypic constitution of *E. lindleyanum* from 15 populations. It consists of five cytotypes comprising diploid, triploid and tetraploid based on $x=10$ and polyploids with a partial deficient chromosome. Diploids are abundant and widely distributed, except for Northern Honshu at the Pacific Ocean side and Hokkaido Island. Most polyploids of *E. lindleyanum* have intermediate morphological traits between diploid *E. lindleyanum* and polyploid *E. makinoi* and occur in drier habitats than those inhabited by diploid *E. lindleyanum*. These habitats have also intermediate soil moisture between the habitats of putative parents, created by road construction or reclamation into the wet places. Thus, they seem to be hybrid derivatives. During the range

extension of agamospermous polyploid *E. makinoi*, they had permitted many secondary contacts with *E. glehni* and *E. lindleyanum* which had been geographically or ecologically separated previously, and their contacts had given rise to new polyploid hybrids and hybrid derivatives. Consequently, such disturbed habitats as forest margins, roadsides, cultivated fields may have been occupied by these new polyploid derivatives. Morphological, ecological and distributional intermediacy of agamospermous polyploids and their hybrid derivatives among species makes species recognition of this genus very difficult and distributional range of each species very ambiguous. Thus the cytogeographical study on the agamic complex and related species helps to reveal the population structure, the circumscription of species, their distributional ranges, and the evolutionary and phytogeographical history.

Acknowledgements

Literature Cited

Boufford, D. E. and S. A. Spongberg. 1983. Eastern Asian-eastern North American phytogeographical relationships --A history from the time of Linnaeus to the twentieth century. Ann. Mo. Bot. Gard. 70: 423-439.

Funayama, S., K. Hikosaka and T. Yahara. 1997. Effects of virus infection and growth irradiance on fitness components and photosynthetic properties of *Eupatorium makinoi* (Compositae). Amer. J. Bot. 84: 823-829.

Graham, A. 1972. Outline of the origin and historical recognition of floristic affinities between Asian and eastern North America. *In* Graham, A. (ed.), Floristic and paleofloristics of Asia and eastern North America, pp.1-18. Elsevier, Amsterdam.

Gray, A. 1859. Diagnostic characters of new species of phanerogamous plants in Japan by Charls Wright, Botanist of the U. S. North Pacific Exploring Expedition. (Published by request of Captain James Rodgers, Commanda of the Expedition). With observations upon the relations of the Japanese flora to that of North America, and of other parts of the North Temperate

Zone. Mem. Amer. Acad. Arts. 6: 377-452.

Halenius, J. 1750. Plantae Rariores Camsctatcenses. Thesis University of Uppsala, Uppsala, Sweden.

Hamilton, W. D., Axelrod, R. and Tanase, R. 1990: Sexual reproduction as an adaptation to resist parasites (A review). Proc. Natl. Acad. Sci. (USA). 87: 3566-3573.

Horikawa, Y. 1972. Atlas of the Japanese Flora I. Gakken, Tokyo.

Horikawa, Y. 1976. Atlas of the Japanese Flora II. Gakken, Tokyo.

Ito, M., T. Kawahara, K. Watanabe, D. J. Crawford, and T. Yahara. in preparation. Evolution and phytogeography of the genus *Eupatorium* (Eupatorieae, Asteraceae): Insight from cp DNA RFLPs analysis.

Kawahara, T. 1990. A study of phylogenetic taxonomy of Asian *Eupatorium* (Compositae). Ph. D. dissertation, The University of Tokyo.

Kawahara, T., T. Yahara, and K. Watanabe. 1989a. Distribution of sexual and agamospermous populations in Asian Eupatorium (Compositae). Pl. Sp. Biol. 4: 37-46.

Kawahara, T., T. Yahara, and K. Watanabe, 1989b. Distinction on morphology and esterase isozyme between *Eupatorium chinense* var. *oppositifolium* and *E. glehni* (=*E. chinense* subsp. *sachalinense*). Bot. Mag. (Tokyo) 102: 165-179.

King, R. M. and H. Robinson. 1970a. Studies in the Eupatorieae (Compositae). 25. A new genus *Eupatoriadelphus*. Phytologia 19: 431-432.

King, R. M. and H. Robinson. 1970b. *Eupatorium*, a composite genus of Arcto-Tertiary distribution. Taxon 19: 769-774.

King, R. M. and H. Robinson. 1987. The genera of the *Eupatorieae* (Asteraceae). Monogr. Syst. Bot. Missouri Bot. Gard. 22: 1-581.

Kitamura, S. 1949. Taxonomic notes on Chinese *Eupatorium*. Japan. J. Bot. 24: 76-80.

Kitamura, S. 1957. Compositae Japonicae. Pars sexta. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. 24: 49-50.

Lee, N. S., T. Sang, D. J. Crawford, S. H. Yeau and S. C. Kim. 1996. Molecular divergence between disjunct taxa in eastern Asia and eastern North America. Amer. J. Bot. 83: 1373-1378.

Levin, D. A. 1975. Pest pressure and recombination system in plants. Amer. Nat. 109: 437-451.

Li, H. L. 1952. Floristic relationships between eastern Asia and eastern North America. Trans. Amer. Phil. Soc. 42: 371-429.

Ooi, K., S. Oshita, I. Ishii and T. Yahara. 1997. Molecular phylogeny of geminivirus infecting wild plants in Japan. J. Plant Res. 110: 247-257.

Osaki, T. and T. Inoue. 1979. Whitefly-transmitted virus isolated from variegated *Eupatorium japonicum* Thunb. Ann. Phytopath. Soc. Japan 45: 111-112.

Osaki T. and T. Inoue. 1981. Tobacco leaf curl virus. In Descriptions of Plant Viruses, No. 232. Commonwelth Mycol. Inst. & Assoc. Appl. Biol., Kew.

Osaki, T., H. Kobatake, and T. Inoue. 1979. Yellow vein mosaic of honeysuckle (*Leonicera japonica* Thunb.) a disease caused by tabacco leaf curl virus in Japan. Ann. Phytopath. Soc. Japan 45: 62-69.

Qiu, Y. L., C. R. Parks, and M. W. Chase. 1995. Molecular divergence in the eastern Asia-eastern North America disjunct section *Rytidospermum* of *Magnolia* (Magnoliaceae). Amer. J. Bot. 82: 1589-1598.

Tsukada, M. 1982. Late-Quarternary shift of *Fagus* distribution. Bot. Mag. (Tokyo) 95: 203-219.

Tsunemi, N. 1992. Comparative ecological research on sexual and agamospermous plants in *Eupatorium makinoi* (Asteraceae). MS thesis, The University of Tokyo.

Watanabe, K. 1986. The cytogeography of the genus *Eupatorium* (Compositae). Pl. Sp. Biol. 1: 99-116.

Watanabe, K. 1989. Evolutionary history of the Japanese flora--Cytogeographycal evidences on some composites at disturbed habitats. *In* Hong, D. (ed.) Plant Chromosome Research 1987, Pp. 25-34.

Watanabe, K., T. Fukuhara, and Y. Huziwara. 1982. Studies on the Asian eupatorias I. *Eupatorium chinense* var. *simplicifolium* from the Rokko mountains. Bot. Mag. (Tokyo) 95: 261-280.

Watanabe, K., M. Ito, T. Yahara, V. I. Sullivan, T. Kawahara, and D. J. Crawford. 1990. Numerical analyses of karyotypic diversity in the genus *Eupatorium* (Compositae, Eupatorieae). Pl. Syst. Evol. 170: 215-228.

Watanabe, K., and T. Yahara. 1984. Studies on the Asian eupatoria II. Cytogeography of *Eupatorium chinense* subsp. *sachalinense* var. *oppositifolium*. Bot. Mag. (Tokyo) 97: 87-105.

Yahara, T. and K. Oyama. 1993. Effects of virus infection on demographic traits of an agamospermous population of *Eupatorium chinense* (Asteraceae). Oecologia 96: 310-315.

Yahara, T., T. Kawahara and K. Watanabe. 1995. *Eupatorium*. In K. Iwatsuki *et al.* (eds.) Flora of Japan, Vol. IIIb., Kodansha Scientific, Tokyo, Pp. 110-114.