台湾・韓国産トリカブト Aconitum bartletii, A. napiforme, A. jaluense の系統学的位置

著者	Kita Yoko, Ito Motomi, Peng Ching-I.
著者別表示	喜多 陽子, 伊藤 元己, Peng Ching-I.
journal or publication title	The journal of phytogeography and taxonomy
volume	45
number	2
page range	75-82
year	1997-12-30
URL	http://doi.org/10.24517/00055550

J. Phytogeogr. & Taxon.
45 : 75-82, 1997

Yoko Kita*, Motomi Ito** and Ching-I. Peng*** : Phylogenetic Position of the Taiwanese and Korean Aconites, *Aconitum bartletii, A. napiforme* and *A. jaluense* (Ranunculaceae)

喜多陽子*・伊藤元己**・Ching-I. Peng***：台湾・韓国産トリカブト *Aconitum bartletii, A. napiforme, A. jaluense* の系統学的位置

Abstract

Aconitum bartletii is a tetraploid aconite species endemic to Taiwan. *A. napiforme* and *A. jaluense* are Korean tetraploid species. In a phylogenetic analysis of East Asian aconites based on chloroplast DNA (cpDNA), these three species were found to be very closely related to the Japanese tetraploid taxa studied. This analysis also revealed that the cpDNA of these tetraploid taxa has only diverged minimally. The cpDNA tree and the distribution of the aconites suggest that two East Asian clades identified by the cpDNA tree, the East Asian tetraploid clade and the Hokkaido-Iide diploid clade, have very different evolutionary histories.

Key words: *Aconitum* subgenus *Aconitum, Aconitum bartletii*, chloroplast DNA, molecular phylogeny, phytogeography.

The genus *Aconitum* L. contains over three hundred species and is divided into three subgenera: *Aconitum, Lycoctonum* Tournefort and *Gymnaconitum* (Stapf.) Rapaics (Kadota 1987). The subgenus *Aconitum*, the largest of these, includes about 260 species (Tamura 1990) and has an Asian center of distribution. Wang (1979) reported that 142 species of the subgenus *Aconitum* are found in China. There are 20 species reported from the Korean Peninsula (Lee 1989). Among them, *A. napiforme* and *A. jaluense* analyzed here are found in Japan as well as in the Korean Peninsula (Kadota 1987). The Japanese aconites are classified into two sections, four series and one sub-series; these include 17 species, 11 subspecies and 4 varieties (Kadota 1987, 1991). In each region, the subgenus *Aconitum* has diversified locally. This subgenus is one of the best groups for studying phytogeography in East Asia.

Four species were described in Taiwan before 1959: *A. bartletii* Yamamoto, *A. fukutomei* Hayata, *A. yamamotoanum* Ohwi and *A. formosanum* Tamura. Three of these species, *A. bartletii, A. fukutomei* and *A. formosanum*, were reported to be tetraploid plants (2 n=32, Kurita 1965). Liu and Hsieh (1976) combined the four Taiwanese species into a single species, *A. bartletii*, with two varieties. The glaborous carpels and erect stem of our *A. bartletii* specimens from Taiwan identify them as '*A. fukutomei*'. Nakai (1953) thought that the Taiwanese aconites were closely related to the Japanese aconites and included some Taiwanese taxa into his section *Japonica*. On the other hand, Tamura (1959) felt that the Taiwanese aconites were very different from the Japanese aconites and that they were more closely related to Himala-

*Graduate School of Science and Technology, Chiba University, Chiba 263-5822, Japan 〒263-5822 千葉市稲毛区弥生町 千葉大学大学院自然科学研究科生命・資源科学専攻
**Faculty of Science, Chiba University, Chiba 263-5822, Japan 〒263-5822 千葉市稲毛区弥生町 千葉大学理学部生物
***Academia Sinica, Institute of Botany, Nankang, Taipei 11529, Taiwan

yan species. The phylogenetic relationships of the Taiwanese aconite, *A. bartletii*, pose an interesting problem in the phytogeography of the East Asian aconites.

In a recent study, we used RFLPs and sequence data from the non-coding regions of chloroplast DNA (Kita *et al.* 1995) to study the phylogeny of aconites. The study included Japanese aconites, *A. ferox* from Nepal, seven species from Yunnan, China, and five species from Siberia. In this study, we focus on the Taiwanese aconite in an attempt to elucidate its phylogenetic relationships, and discuss the implications for the phytogeography of the East Asian aconites.

Materials and Methods

Plant materials

For convenience, in this study, we used the species name and rank for any taxon that was once treated as a species. We examined five indi-

Table 1. Taxa of subgenus *Aconitum* and one outgroup (subgenus *Lycoctonum*) used in this analysis, their ploidy level, origin, specimems

	Taxon	Ploidy	Locality	Specimen
Subgenus *Aconitum*				
Taiwanese and Korean materials newly analyzed				
1	*A. bartletii* Yamamoto	4X	Yakou, Taiton, Taiwan	ITO, Aco4
2	*A. napiforme* Lév. et Van't. (Mt. P'algong)	4X	Mt. P'algong, Kyongsangbukdo, Korea	KITA, 951130
3	*A. napiforme* Lév. et Van't. (Mt. Chiri)	4X	Mt. Chiri, Kyongsangbukdo, Korea	ITO, Aco1
4	*A. jaluense* Kom. (Taegwallyong)	4X	Taegwallyong, Kyongsangbukdo, Korea	ITO, Aco2
5	*A. jaluense* .Kom. (Mt. Sorak)	4X	Mt. Sorak, Kangwondo, Korea	ITO, Aco3
Taxa analyzed Kita et al. (1995)				
	Japanese materials			
	Japanese diploid taxa			
6	*A. yamazakii* Tamura & Namba	2X	Mt. Nipesotu, Hokkaido	KANA180040
7	*A. yuparense* Takeda	2X	Mt. Yupari, Hokkaido	KANA180022
8	*A. apoiense* Nakai	2X	Horoman Riv., Hokkaido	KANA180075
9	*A. sanyoense* Nakai	2X	Asiu, Kyoto	KANA180095
10	*'A. iide-montanum* '*	2X	Mts. Iide, Yamagata	KANA191948
	Japanese tetraploid complex			
11	*'A. nagisoense* '*	4X	Nagiso, Nagano	Kadota *s.n.* (TNS)
12	*A. kiyomiense* Kadota	4X	Kiyomi-mura, Gifu	KANA180110
13	*A. ciliare* DC.	4X	Aso-gun, Kumamoto	KITA, 951120
14	*A. sachalinense* Fr. Schm.	4X	Nemuro, Hokkaido	KANA180063
15	*A. yezoense* Nakai	4X	Mt. Yupari, Hokkaido	KANA180045
16	*A. kurilense* Takeda	4X	Iwaobetsu spa, Hokkaido	KANA180055
17	*A. okuyamae* Nakai	4X	Iwate	TNS9027426
18	*A. subcuneatum* Nakai	4X	Hokkaido Univ. Bot. Gard.	KANA180015
19	*A. napiforme* Lév. et Van't.	4X	Mt. Turugisan, Tokushima	KITA, 951130
20	*A. hakusanense* Nakai	4X	Mt. Hakusan, Ishikawa	KANA180097
21	*A. senanense* Nakai	4X	Mt. Kitadake, Yamanashi	KANA191943
22	*A. paludicola* Nakai	4X	Mt. Kashimayari-ga-take, Nagano	TNS9027427
23	*A. nipponicum* Nakai	4X	Mt. Shirouma-dake, Nagano	KANA180103
24	*A. micranthum* Nakai	4X	Mt. Kisokoma-ga-take, Nagano	KANA180091
25	*A. kitadakense* Nakai	4X	Mt. Kitadake, Yamanashi	KANA191938
	Siberian materials			
26	*A. krasnoboroffii* kadota	4X	Mts. West Tannu-Ola, W. Sayan, Tuwa	TNS9027189
27	*A. baicalense* Turcz. ex Rapaics	4X	Mts. Tumat Taiga, Sayan, Tuwa	TNS9027196
28	*A. decipiens* Worsch. et Anfalov	4X	Mts. West Tannu-Ola, W. Sayan, Tuwa	Wakabayashi et al. 9327102 (TNS)
29	*A. villosum* Reichb.	4X	Mts. Tumat Taiga, W. Sayan, Tuwa	TNS9237144
30	*A. pascoi* Worosch.	4X	Mts. West Sayan, Tuwa	Wakabayashi et al. 9327216(TNS)
	Yunnan materials			
31	*A. hookeri* Stapf.	-	Mt. Baima xueshan, Yunnan	Kadota21366(TNS)
32	*A. nagarum* Stapf.	-	Dali, Yunnan	Kadota21473(TNS)
33	*A. taroense* (H.-Mazz.) Fletch. & Lauen.	-	Tianchi, Zhongdian, Yunnan	Kadota21196(TNS)
34	*A. rockii* Fletch. et Lauener	-	Weixi, Yunnan	Kadota21381(TNS)
35	*A. longtouense* T.L. Ming	-	Mt. Tianbaoshan, Zhongdian, Yunnan	Kadota21178(TNS)
36	*A. pendulum* Busch	-	Wufengshan, Zhongdian, Yunnan	Kadota21224(TNS)
37	*'A. sino-proliferum* '*	-	Bitahai, Zhongdian, Yunnan	Kadota21215(TNS)
	Nepal materials			
38	*A. ferox* Wall. ex Seringe	4X	Mt. Shiwapuri, Nepal	Minaki et al. 9100909 (TI)
Subgenus *Lycoctonum*				
31	*A. gigas* Lév. et Van't.	2X	Mt. Yupari, Hokkaido	KANA180011

*See Kita *et al.* 1995.

vidual plants from one Taiwanese and two Korean species. A single plant represented the Taiwanese species, *Aconitum bartletii*. There were two specimens for each of the Korean species, *A. jaluense* and *A. napiforme*, collected from different localities (Table 1). We extracted total DNA from living and dried leaf tissues, using the CTAB method (Doyle and Dickerson 1987, Doyle and Doyle 1987). In a previous study (Kita *et al.* 1995), there was no RFLP or sequence variation within the 15 Japanese tetraploid taxa examined. In this paper, these 15 taxa are referred to as the Japanese tetraploid complex.

RFLP analysis

DNA samples from the five plants were digested with the following 17 restriction endonucleases in accordance with the suppliers directions: *Apa*I, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Mlu*I, *Pst*I, *Pvu*II, *Sac*I, *Sac*II, *Sal*II, *Sca*I, *Xba*I, *Xho*I and *Hae*III. Twelve cpDNA subclones from tobacco were used as hybridization probes. There were four groups of probes. Two groups were from the LSC region ((B 25, B 7 and B 20) and (B 19, B 29, B 22 and B 1)), and one each from the IR (B 28, B 15, B 10 and B 8) and SSC (B 2) regions (Kita *et al.* 1995). The probes were labeled using the ECL Gene Labeling/Detection System (Amersham) following the manufacturer's instructions. In this paper, the probes are referred to by the name of the first probe in each group.

Sequencing of two non-coding regions of cpDNA

We sequenced the *trnL* intron and the intergenic spacer (IGS) between the *trnL* (UAA) 3'exon and *trnF* (GAA), using four primers (primers *c, d, e* and *f*, Taberlet *et al.* 1991; Kita *et al.* 1995). First, PCR amplification of the total DNA was performed using *Taq* polymerase (TaKaRa), with 25-cycles of 30 sec at 94℃, 30 sec at 50-55℃ and 60 sec at 72℃. The dideoxy sequencing reaction was performed with a Thermo Sequenase Fluorescent-labeled Primer Cycle Sequencing Kit (Amersham) in an automated sequencer (ALF*express*™, Pharmacia).

Phylogenetic analysis

The RFLP and sequence data were combined for the phylogenetic analysis. We treated all gained or lost restriction sites and gaps as binary characters (Kita *et al.* 1995). The phylogenetic tree was generated using the maximum parsimony method with PAUP v. 3. 1. 1 (Swofford 1993) using a branch and bound search.

Chromosome number

Root tips were collected from plants cultivated in the nursery at Chiba University. Somatic chromosomes in the root tips were observed using the method of Kurita (1986).

Results

Chromosome number

All five plants studied had the same somatic chromosome number, 2 n=32 and were considered to be tetraploids (Table 1, Fig. 1). The karyotype of *Aconitum bartletii* was bimodal, with 8 large chromosomes and 24 small ones. This result agrees with a previous report for *A. bartletii* (Kurita 1965).

RFLP data

A total of three hundred and sixty-eight fragments were detected in all the taxa included in this and the previous study (Kita *et al.* 1995). In the previous study, there were 57 polymorphisms. One newly detected polymorphism was a synapomorphy that grouped *A. bartletii* with the *A. jaluense* specimen from Taegwallyong, Korea (Table 2). This was the only variation detected in the five plants examined in this study. Otherwise, there were no differences when compared with the Japanese tetraploid complex.

Sequence data

The intergenic spacer (IGS) region between

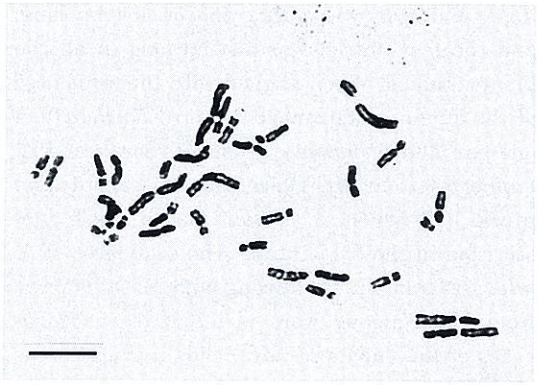

Fig. 1. Somatic chromosomes of *A. bartletii* (2 n=32). × 1940. (bar, 10.3 μm)

Table 2. Restriction fragment length polymorphisms of chloroplast DNA

Enzyme	Region*	Flagment	Taxa
Apa I	B2, 28	12.4---10.8+1.6	Japanese tetraploid complex, *A. bartletii*, *A. napiforme* (Mt. P'algong), *A. napiforme* (Mt. Chiri) *A. jaluense* (Taegwallyongs), *A. jaluense* (Mt. Sorak)
Dra I	B25	3.4+4.6---9.0	Japanese tetraploid complex, *A. bartletii*, *A. napiforme* (Mt. P'algong), *A. napiforme* (Mt. Chiri) *A. jaluense* (Taegwallyongs), *A. jaluense* (Mt. Sorak)
Eco RI	B25	2.5---2.0+0.5	Japanese tetraploid complex, *A. bartletii*, *A. napiforme* (Mt. P'algong), *A. napiforme* (Mt. Chiri) *A. jaluense* (Taegwallyongs), *A. jaluense* (Mt. Sorak)
Eco RI	B28,19	4.8---2.1+2.7	*A. bartletii* *A. jaluense* (Taegwallyongs)

* See text.

Table 3. Base substitutions and a gap in the *trnF* intron and the intergenic spacer between *trnL* and *trnF*, with site numbers after multiple alignment of all sequences analyzed in here and Kita *et al.* (1995) by base position within the each region to be vertically read

	trnF intron	IGS between *trnL* and *trnF*	
	107	37	169
15 Japanese tetraploid taxa	C	T	T
A. bartletii	A	-	*
A. napiforme (Mt. P'algong)	*	*	*
A. napiforme (Mt. Chiri)	*	*	C
A. jaluense (Taegwallyong)	*	*	*
A. jaluense (Mt. Sorak)	*	G	*

* represents the same base as that of 15 Japanese tetraploid taxa.
– represents a gap.

trnL and *trnF* was either 453 or 454 bp long, and the *trnF* intron was 484 bp long in all the plants studied. When aligned with the sequences of the Japanese tetraploid taxa, *A. bartletii* had one gap and *A. jaluense* from Mt. Sorak and *A. napiforme* from Mt. Chiri had one substitution in the IGS region. *A. bartletii* had a single substitution in the *trnF* intron. The sequences of *A. jaluense* from Taegwallyong and *A. napiforme* from Mt. P'algong were identical to the sequences of the Japanese tetraploids in those areas (Table 3).

Phylogenetic tree

Figure 2 is the single most parsimonious (MP) tree for the Asian aconites constructed from the combined RFLP and sequence data. *Aconitum gigas*, from the subgenus *Lycoctonum*, was used as the outgroup. The phylogenetic tree is an unresolved polytomy with six clades radiating from the base (Fig. 2). All six clades are well supported statistically. The relationships between these clades are not resolved with our data. In the MP tree, the Taiwanese aconite, *A. bartletii*, and the four Korean plants clustered with the Japanese tetraploid complex. This clade consists solely of tetraploid plants from Japan, Korea and Taiwan, and is referred to as the East Asian tetraploid clade. The other clades on the MP

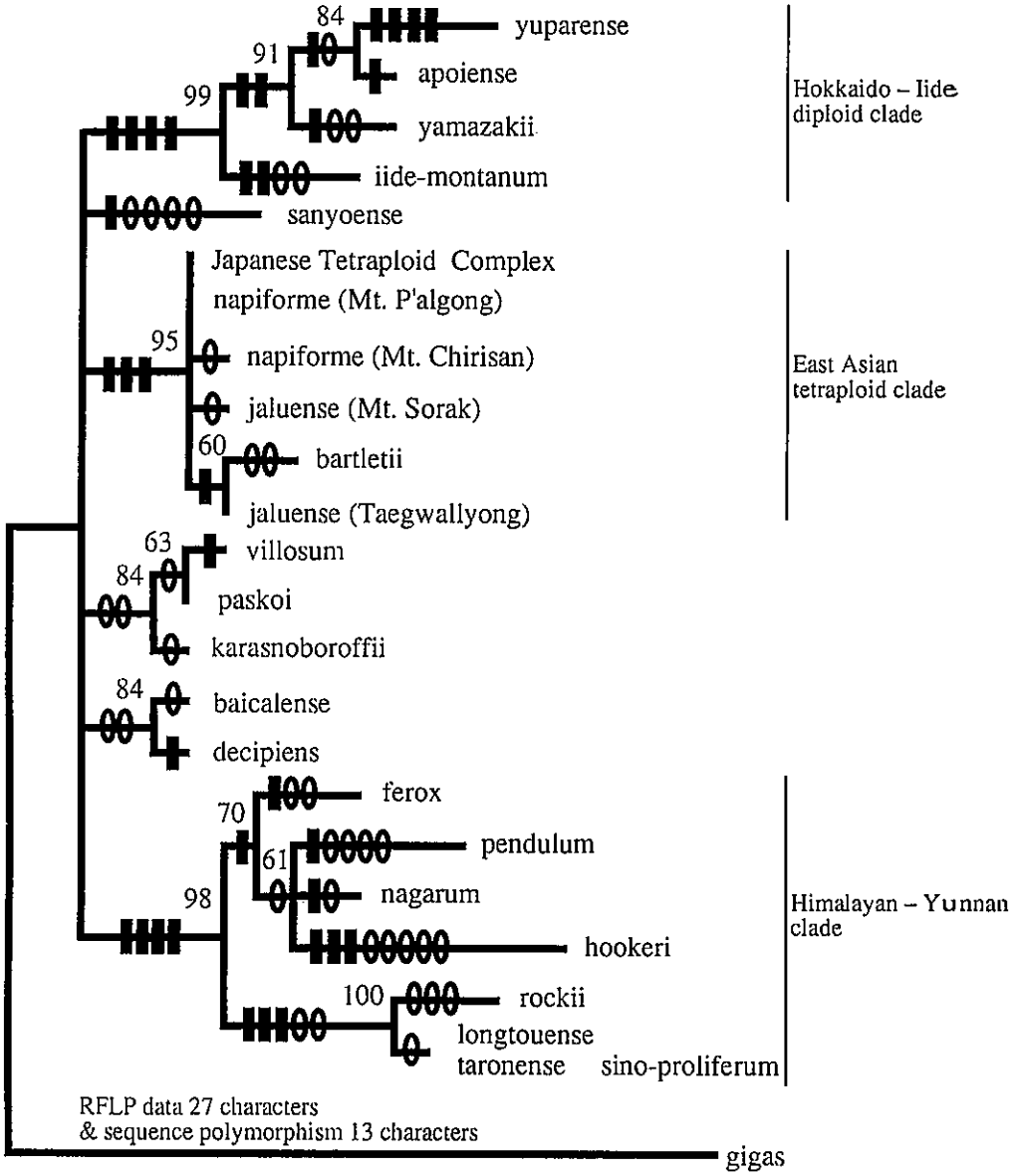

Fig. 2. The single most parsimonious tree produced by a branch and bound search of RFLP and sequence data for chloroplast DNA in *Aconitum*. CI = 0.991. RI = 0.986. Tree length = 114. The branch lengths are proportional to the number of character changes. Closed boxes and open circles represent RFLP data and sequence polymorphism, respectively. Numbers along the branches are 100 replication bootstrap values. The tree was rooted with *A. gigas* (Subgenus *Lycoctonum*).

tree are identical to those reported in Kita *et al.* (1995).

Discussion

Both tetraploid and diploid plants of the subgenus *Aconitum* grow in East Asia. In the MP tree (Fig. 2), the tetraploid taxa and diploid taxa

from East Asia each formed separate clades. One of these clades consisted of the tetraploid taxa from Japan, Korea and Taiwan, referred to as the East Asian tetraploid clade. There was no RFLP or sequence variation between the Japanese tetraploid taxa analyzed (Kita *et al.* 1995), and the Korean and Taiwanese tetraploid taxa were only slightly different. This suggests that the East Asian tetraploid taxa are closely related to each other. If the East Asian tetraploid taxa have multiple origins, however, then a chloroplast capture event involving recent hybridization/introgression must have occurred within this group. Chloroplast capture would make it appear that the East Asian tetraploids share a most recent common ancestor, when they do not. A nuclear ribosomal ITS tree (Kita *et al.* in preparation) also shows that the East Asian tetraploid taxa studied are monophyletic, and minimally diverged. This supports the hypothesis that the East Asian tetraploid taxa are closely related to each other.

Most of the sequence variation observed in the five plants from Taiwan and Korea were autapomorphies, although *A. bartletii* and *A. jaluense* from Mt. Sorak share one RFLP synapomorphy (Table 2). Morphological studies failed to reveal a relationship between these species. There is not enough RFLP and sequence data to discuss the phylogenetic relationships of the East Asian tetraploid taxa.

Tamura (1959) felt that the aconites from Taiwan were quite different from the Japanese taxa. He proposed that they were more closely related

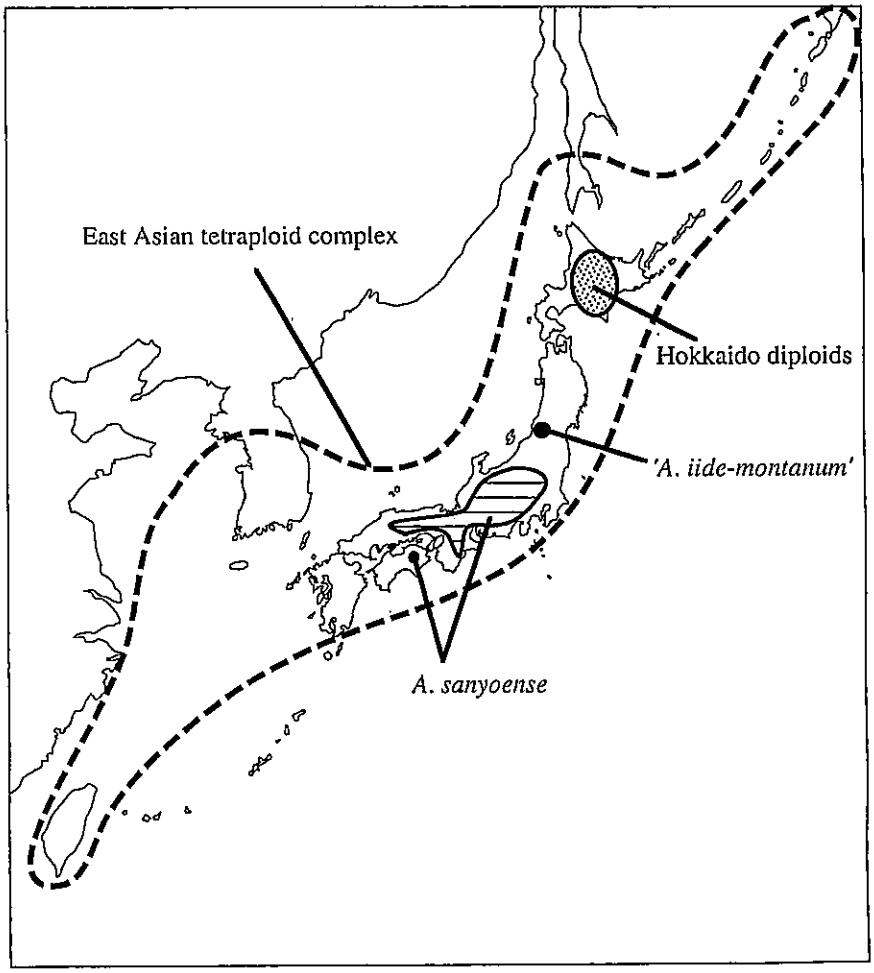

Fig. 3. Distribution of the East Asian aconites.

to Himalayan taxa. Nevertheless, he did not mention any morphological similarity between the Taiwanese and Himalayan taxa (Tamura 1959). In the MP tree (Fig. 2), *A. ferox* from Nepal and eight species from Yunnan, China form a monophyletic clade, the Himalayan-Yunnan clade. The Taiwanese species, *A. bartletii*, was not related to the Himalayan-Yunnan clade, but it was closely related to the Japanese and Korean tetraploid taxa. A nuclear ribosomal ITS tree (Kita *et al.* in preparation) also supports this result.

Only one Himalayan species was included in our studies. There are many *Aconitum* species from the Himalayan region, so we cannot eliminate the possibility that some of these taxa may be closely related to the Taiwanese *A. bartletii*. More taxa from China and the Himalayas must be studied to clarify the affinities of *A. bartletii*.

The ranges of the East Asian tetraploid taxa studied are illustrated in Fig. 3, although the Asian taxa need more study to accurately determine the true distribution of the East Asian tetraploid complex. These taxa are found from Taiwan to Hokkaido, throughout the Japanese archipelago and in the Korean Peninsula (Fig. 3). In contrast to the East Asian tetraploid clade, the taxa in the Hokkaido-Iide diploid clade (Fig. 2) are rare, and their distribution is restricted to alpine and sub-alpine areas of Hokkaido and to Mt. Iide in northern Honshu (Fig. 3). The cpDNA of these species is highly divergent, and they are considered relict species. In contrast, there is little divergence in the cpDNA of the East Asian tetraploid taxa, which range widely throughout East Asia. This suggests that the taxa of the East Asian tetraploid and Hokkaido-Iide diploid clades underwent independent migrations in East Asia, and that the East Asian tetraploid clade diverged more recently than the Hokkaido-Iide diploid clade.

It is necessary to study more species from Asia to clarify the history of the East Asian aconites.

We would like to thank J-H. Pak for helping us to obtain the important foreign specimens. The twelve tobacco cpDNA subclones used as hybridization probes were kindly provided by Prof. T. Sugiura of Nagoya University. This study was partly supported by Grant-in-Aid for Overseas Research No. 06041016 and 07041126 to MI from the Ministry of Education, Science and Culture, Japan and the grant to YK from the Fujiwara Natural History Foundation.

References

Doyle, J. J. and Dickerson, E. E. 1987. Preservation of plant samples for restriction endonuclease analysis. Taxon **36**: 715–722.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19**: 11–15.

Kadota, Y. 1987. A Revision of *Aconitum* Subgenus *Aconitum* (Ranunculaceae) of East Asia. Sanwa Shoyaku, Utsunomiya.

Kadota, Y. 1991. *Aconitum azumiense* (Ranunculaceae), a new species from Nagano Prefecture, Central Japan. J. Jpn. Bot. **66**: 39–45.

Kita, Y., Ueda, K. and Kadota, Y. 1995. Molecular phylogeny and evolution of the Asian *Aconitum* subgenus *Aconitum* (Ranunculaceae). J. Plant Res. **108**: 429–442.

Kurita, M. 1965. Chromosome studies in Ranunculaceae. XXII. Mem. Ehime Univ. **5**: 11–17.

Kurita, S. 1986. Variation and evolution in the karyotype of *Lycoris*, Amaryllidaceae I. General karyomorphological characteristics of the genus. Cytologia **51**: 803–815.

Lee, T.-B. 1989. *Aconitum* L. *In* T.-B. Lee, Illustrated Flora of Korea, Gobunsha, Seoul, pp. 359–365, figs. 1435–1457. (In Korean)

Liu, T.-S. and Hsieh, C.-F. 1976 *Aconitum* L. *In* Li, H.-L., Liu, T.-S., Huang, T.-C., Koyama, T. and Devol, C. E. (eds.) : Flora of Taiwan, vol. 2, pp. 476–479. Epoch Publishing, Taipei.

Nakai, T. 1953. A new classification of *Lycoctonum* and *Aconitum* in Korea, Japan, and their surrounding areas. Bull. Natn. Sci. Mus., Tokyo **32**; 1–53.

Swofford, D. L. 1993. PAUP (phylogenetic analysis using parsimony), version 3. 1. 1. The Illinois Natural History Survey, Champaign.

Taberlet, P., Gielly L., Pautou, G. and Bouvet, J. 1991. Universal primers for amplification of three non-coding region of chloroplast DNA. Pl. Mol. Biol. **17**: 1105–1109.

Tamura, M. 1959. *Aconitum* of Formosa (*Aconitum* of Japan and surrounding regions 1). Sci.

Rep. Osaka Univ. 8: 67–73

Tamura, M. 1990. A new classification of the family Ranunculaceae 1. Acta. Phytotax. Geobot. **41**: 93–101

Wang, W.–T. 1979. *Aconitum* L. *In* Kuan, K.–C., Hsaio, P.–K., Pan., K.–Y., Wang, W.–T., Wang, S.–H. (eds.): Flora Reipublicae Popularis Sinicae, vol. 27, pp. 113–362. Science Press, Beijing. (In Chinese with Latin description)

摘　要

トリカブト属（*Aconitum*）にはトリカブト亜属，レイジンソウ亜属（*Lycoctonum*），*Gymnaconitum* 亜属の3亜属が一般に認められている。このうち，トリカブト亜属は最も種数が多く，それらは主にアジアに分布している。本亜属からは，中国では142種，朝鮮半島では20種が報告されている（Wang 1979; Lee 1989）。このうち，今回解析した *A. napiforme* と *A. jaluense* は日本にも分布している。Kadota (1987, 1991) は，日本産のトリカブト亜属植物を2節4列1亜列17種11亜種4変種に分類している。本亜属の植物はアジアの各地域ごとに固有種が存在し，生物地理学的研究に適していると思われる。

台湾には，1959年までに *Aconitum bartletii* Yamamoto, *A. fukutomei* Hayata, *A. yamamotoanum* Ohwi, *A. formosanum* Tamura の4種が記載されている。Kurita (1965) は，*A. fukutomei*, *A. bartletii*, *A. formosanum* に関して2n=32の4倍体植物と報告している。その後，上記4種は Liu and Hsieh (1976) によって，*A. bartletii* の1種にまとめられている。Nakai (1953) は，台湾のトリカブトは日本のものと近縁であると考え，*A. fukutomei* と *A. bartletii* を日本産のものと一緒に Sect. *Japonica* に分類している。一方，Tamura (1959) は台湾のトリカブトは日本のものとは全く別物であり，ヒマラヤ地方のものとより近縁であると考えている。このように，台湾のトリカブトと東アジアの他地域の種との系統関係は興味を持たれてきた。Kita *et al.* (1995) では，葉緑体 DNA の RFLP と葉緑体 DNA 上の2つの non-cording 領域（*trnL* のイントロンと *trnL* (UAA) 3' exon～*trnL* (GAA) の遺伝子間領域）の塩基配列を用いて，日本産の種を中心にシベリア産、中国雲南省産，ネパール産のトリカブト亜属植物の系統解析を行った。本研究は，台湾の *A. bartletii* と韓国の *A. jaluense, A. napiforme* から Kita *et al.* (1995) と同じ方法で系統情報を得て，すでに解析した他のアジア産の種と合わせて最節約法を用いて系統解析を行った。

台湾の *A. bartletii* は Yakou 産の1個体，韓国の *A. jaluense* は Taegwallyong 産と Mt. Sorak（雲岳山）産の2個体，韓国の *A. napiforme* は Mt. Chiri（智異山）産と Mt. P'algong 産の2個体を系統解析に用いた。また，これら5個体については，根端を用いて染色体数を数えた。Kita *et al.* (1995) では，解析した日本産の4倍体15種間には，葉緑体 DNA の RFLP 解析，non-cording 領域塩基配列に多型は全く見られなかったので，以下では日本産4倍体種群と示す。

今回の解析では以下の結果が得られた。

1) 染色体数は，解析した5個体全てが2n=32で，4倍体植物と考えた。台湾産の *A. bartletii* の染色体は長い染色体が8本，短い染色体が24本で Kurita (1965) の報告と一致した。

2) 葉緑体 DNA の RFLP 解析の結果，台湾産の *A. bartletii*，韓国 Taegwallyong 産の *A. jaluense* は共有派生となる多型を新たに唯一示したが，他の韓国産の3個体は日本産4倍体種群と全く同じバンドパターンを示した。

3) 葉緑体 DNA の2つの non-cording 領域の塩基配列解析の結果，日本産4倍体種群と比べ，*A. bartletii* は1塩基の gap と1塩基の置換を持っていた。Taegwallyong 産の *A. jaluense* と Mt. P'algong 産の *A. napiforme* は日本産4倍体種群と全く同じ配列を持っていた。

4) 最節約系統樹では，台湾産の *A. bartletii*，韓国の *A. jaluense, A. napiforme* は日本産4倍体種群と単系統になり，ネパール産の *A. ferox* とは全く関係を示さなかった。

以上の結果から，台湾産の4倍体種の *A. bartletii*，韓国産4倍体種の *A. jaluense, A. napiforme* は日本産4倍体種群と非常に近縁であることが分かった。この非常に近縁な4倍体種群は日本列島，朝鮮半島，台湾に広く分布し，個体数も多い。これとは対照的に，最節約系統樹の Hokkaido-Iide diploid clade の2倍体種は，日本列島の高山帯に隔離して遺存的に分布し，葉緑体 DNA の解析では互いによく分化している。これらのことから，東アジアの4倍体種群と Hokkaido-Iide diploid clade の2倍体種の東アジアでの移住史はお互いに異なっており，また，東アジアの4倍体種群は Hokkaido-Iide diploid clade の2倍体種と比較すると，より近年になって分化したと考えた。

(recieved October 20, 1997; accepted December 1, 1997)