ハナノキとアメリカハナノキの交雑実験

J. Phytogeogr. & Taxon.
41 : 63-69, 1993

Tatemi SHIMIZU* and Tomomi UCHIDA** :

Hybridization between North American *Acer rubrum* L. and Japanese *A. pycnanthum* K. KOCH (Aceraceae)

清水建美*・内田智美** : ハナノキとアメリカハナノキの交雑実験

Abstract

In order to confirm the interfertility between *Acer pycnanthum* and *A. rubrum*, one of the typical East Asian-east North American disjunct species pairs, they were hybridized in April 1992 and 1993 in Kanazawa and Matsumoto, Japan. Well matured samaras were produced from interspecific crossings as well as intraspecific ones. Those synthesized in 1992 germinated at high rates up to April 1993 in Kanazawa, and interspecific heterosis was observed in seedlings. The RAPD assay confirmed the interfertility between those two maple species.

Key words : *Acer pycnanthum, Acer rubrum,* interspecific hybridization, RAPD assay.

The section *Rubra* of the genus *Acer* (Aceraceae) includes two extant species, viz. *A. pycnanthum* K. KOCH (Japanese red maple) and *A. rubrum* L. (red maple) (PAX, 1902 ; KOIDZUMI, 1911 ; MOMOTANI, 1962), while OGATA (1967) put also *A. saccharinum* L. in the same section. The Japanese red maple, though widely cultivated in central Japan, is native only to a small area at the border of the prefectures of Gifu, Aichi and Nagano and discontinuously in the suburbs of Omachi, Nagano Prefecture, about 100 km north of the former. Unlike the Japanese species, the red maple is abundant and often weedy over much of its range occupying nearly all of the United States east of the Mississippi River and the Ozark District ranging over the states of Missouri and Arkansas. These two species are one of the typical pairs of East Asia and eastern North American disjuncts. Although a lot of artificial crosses in various tree genera have been reported as shown in *Acer rubrum*×*A. saccharinum* (SMITH and NICHOLS, 1941 ; JOHNSON, 1939 ; ROHMEDER, 1961), no experimental evidence on interfertility between these counterparts is available except for the genus *Liriodendron* (Magnoliaceae) (SANTAMOUR, 1972 ; PARKS *et al.*, 1983). Even in the famous example of *Platanus*×*acerifolia* (AIT.) WILLD., no one seems to have produced this hybrid experimentally (ERNST, 1963). In the present paper, we add one more example to demonstrate interfertility between such disjunctive counterparts despite long isolation.

Materials and Methods

PLANT MATERIALS : In 1982 several young plants of *Acer rubrum* about 30 cm tall were brought from the Ozark District in Arkansas to Matsumoto, Japan. Three of them produced flowers in 1986, and they were all pistillate. Several male scions of *A. rubrum* were sent to Matsumoto from North Carolina by courtesy of Dr. C.R. PARKS of the University of North Carolina, and grafted on a female tree grown in Matsumoto in 1987. One of the scions flowered in 1992 and 1993. Two of those female trees were transplanted to the Botanical Garden of Kanazawa University, Kanazawa in 1989. These female trees and a male

*Department of Biology and Herbarium (KANA), Faculty of Science, Kanazawa University, Kakuma, Kanazawa 920-11, Japan 〒920-11 金沢市角間町 金沢大学理学部生物学教室
** Toyama Technical Centre of Agriculture, Yatsuo-cho, Nei-gun, Toyama 930, Japan 〒939-23 富山県婦負郡八尾町 富山県農業技術センター

TABLE 1. Materials used for crossing experiments

Species	Code	State	Origin	Sex	Location	Voucher
Acer rubrum	A	tree	Ozark	female	Kanazawa	161183*
	B	tree	Ozark	female	Kanazawa	161184
	C	tree	Ozark	female	Matsumoto	161185
	D	scion	N. C.	male	Matsumoto	161186
Acer pycnanthum	E	tree	unknown	female	Kanazawa	161187
	F	tree	Kiso	male	Kanazawa	161188
	G	tree	unknown	male	Matsumoto	161189

*Specimen number in the KANA herbarium.

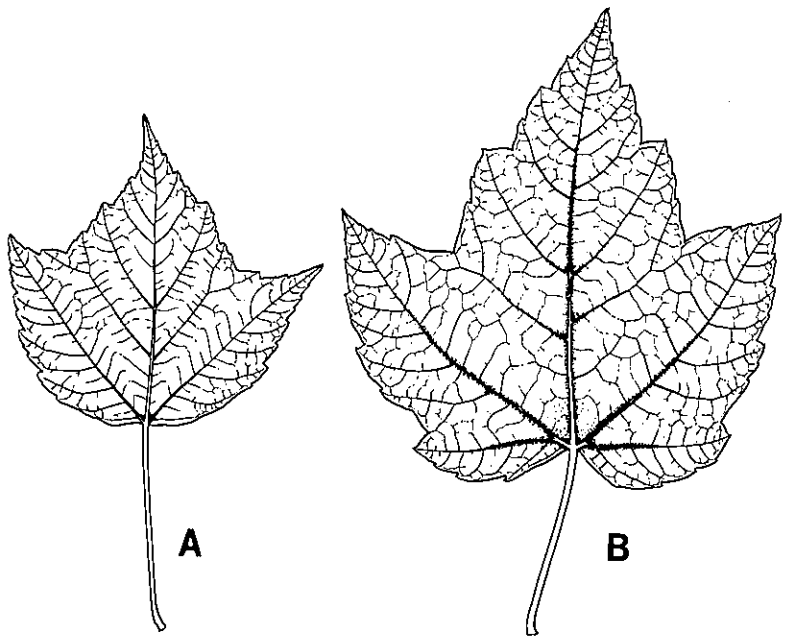

FIG. 1. Leaf shape of *Acer pycnanthum* (A) and *A. rubrum* (B) used in crossing experiments. ×⅔

scion were used for crossing experiments as American parents (Table 1).

In addition to a male tree of unknown origin planted in 1982, several young trees of *A. pycnanthum* were transplanted to Matsumoto from their native habitat in Kiso District, 50 km southeast, in 1984. They have produced staminate flowers since 1987. Two of the latter were again moved to the Botanic Garden of Kanazawa University in 1989. Two male trees, one in Kanazawa and the other in Matsumoto, and an old female tree in the Botanic Garden, were used for experiments as Japanese parents (Table 1). Tree A, B and F in Kanazawa and tree C and G in Matsumoto are cultivated side by side, while tree E is about 200 m far from the other three trees in Kanazawa. Both in Matsumoto and Kanazawa, any other trees of *A. pycnanthum* or *A. rubrum* are not cultivated within distance of 5 km from the experimental sites.

Owing to its wide range of distribution, the American red maple is much more vegetatively polymorphic. The leaves of the Ozark trees used in this experiment are apparently much larger, more hairy on its undersurface and more deeply lobed than those of the Japanese counterpart (Fig. 1). The number of flowers in a cluster is more numerous and the pedicels are longer in the former.

CROSSING EXPERIMENTS: Interspecific crossing between *Acer rubrum* and *A. pycnanthum* as well as their intraspecific crosses were made in 1992 and 1993. The parental combination in the hybridization studies is shown in Table 2.

TABLE 2. Parental combinations in crossing experiments

Crossing	Tree Code	Year
Acer rubrum (♀) × *A. pycnanthum* (♂)	A × F	1992
	B × F	1992
	C × G	1992, 1993
A. pycnanthum (♀) × *A. rubrum* (♂)	E × D	1993
A. pycnanthum × *A. pycnanthum*	E × F	1992
A. rubrum × *A. rubrum*	C × D	1993

In early April of each year, hand pollinations were made by applying pollen to the stigmas of open female flowers. Before pollination, male buds on the scion (Code D) were covered with paper bags to prevent pollen contamination. After pollen application, the clusters of the female flowers were also covered with paper bags for about two weeks, and then with plastic nets until fruits were mature. The same procedure was also performed on several clusters of the non-pollinated pistillate flowers as control. The fruits were harvested at the end of May in each year. The fruiting rate was estimated on the basis of the number of samaras and pedicels collected from the nets.

Some samaras from the 1992 pollinations were sown in flats in the greenhouse of the Botanic Garden, Kanazawa University, immediately after being harvested. The rest were sown in early October after preservation at 5°C for about four months. In both cases, germination rate as well as size of the cotyledons on seedlings were examined.

RAPD ASSAY: The RAPD (Random Amplified Polymorphic DNA) assay commenced by WILLIAMS *et al.* (1990) was performed to confirm hybridization between *Acer rubrum* and *A. pycnanthum*. Two sets of plants, tree C, G and two seedlings of their hybrids, and tree B, F and a seedling of their hybrids, were used for this purpose. The total DNA was extracted from young leaves of the parental trees as well as seedlings of the hybrids by the CTAB method. The CTAB extraction buffer contains 2%(w/v) CTAB (Cetyltrimetylammonium Bromide), 0.1M

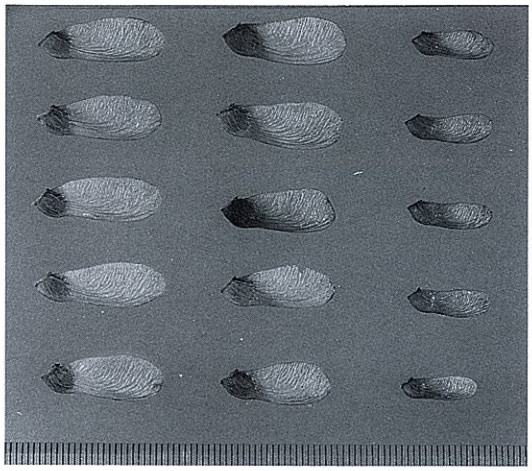

FIG. 2. Samaras obtained from crossing experiments. A., Intraspecific hybrids of *Acer rubrum*, B. Interspecific hybrids between *A. rubrum* and *A. pycnanthum* (♂), C. Inmatured samaras of *A. rubrum* from non-pollinated flowers. Scale: 1mm.

TABLE 3. Percent fruit set in each crossing experiment

Crossings	Tree Code	Flower Count	Samara Count	Fruiting Rate
Interspecific	A × F	1250	1	0.04%
	B × F	339	27	7.9
	C × G	133	180	*67.7
	E × D	70	8	5.7
Intraspecific: *A. pycnanthum*	E × F	395	148	18.7
Intraspecific: *A. rubrum*	C × D	35	55	*78.6

*experiment in Matsumoto in 1993

TABLE 4. Germination rate of the seeds harvested

Crossing	Tree Code	(1) Seed Count	(1) Rate	(2) Seed Count	(2) Rate
Interspecific	B × F	12	41.7%	10	50.0%
	C × G	94	53.2	35	91.4
Intraspecific: *A. pycnanthum*	E × F	93	31.2	50	50.0

(1) Kept in cold room at 5°C; examined on Oct. 29, 1992
(2) Kept in greenhouse; examined on May 28, 1993

Tris-HCl (pH 8.0),1.4M NaCl and 20 mM EDTA. The amplification of random DNA segments was tried with 27 single primers of 10 nucloetides. The amplification reaction was performed in a final volume of 10μl containing 1μl of 10×PCR Buffer (100mM Tris-HCl (pH 8.3), 500 mM KCl, 20 mM $MgCl_2$ and 0.01% gelatin), 1μl of 1mM each deoxynucleotides, 1 μl of 2μM primer, 0.5 ng of genomic DNA and 0.2 unit of Taq DNA polymerase (Perkin Elmer Cetus). Amplification was performed in an incubator (Astec) programmed for 45 cycles of 1 min at 94 °C, 2 min at 40°C and 2.5 min at 72 °C. Amplified products were analyzed by electrophoresis in 3 % Sep Rate-SDF (Amersham) agarose gels and detected by staining with ethidium bromide. Comparison of the electrophoretic patterns of amplified DNA segments was made for confirmation of hybridization.

Results and Discussion

CROSSING EXPERIMENTS: Well matured samaras were obtained from intraspecific as well as interspecific crossing experiments between *Acer rubrum* and *A. pycnanthum*. No mature fruits were obtained from non-pollinated flowers. The mature samaras with well developed em-

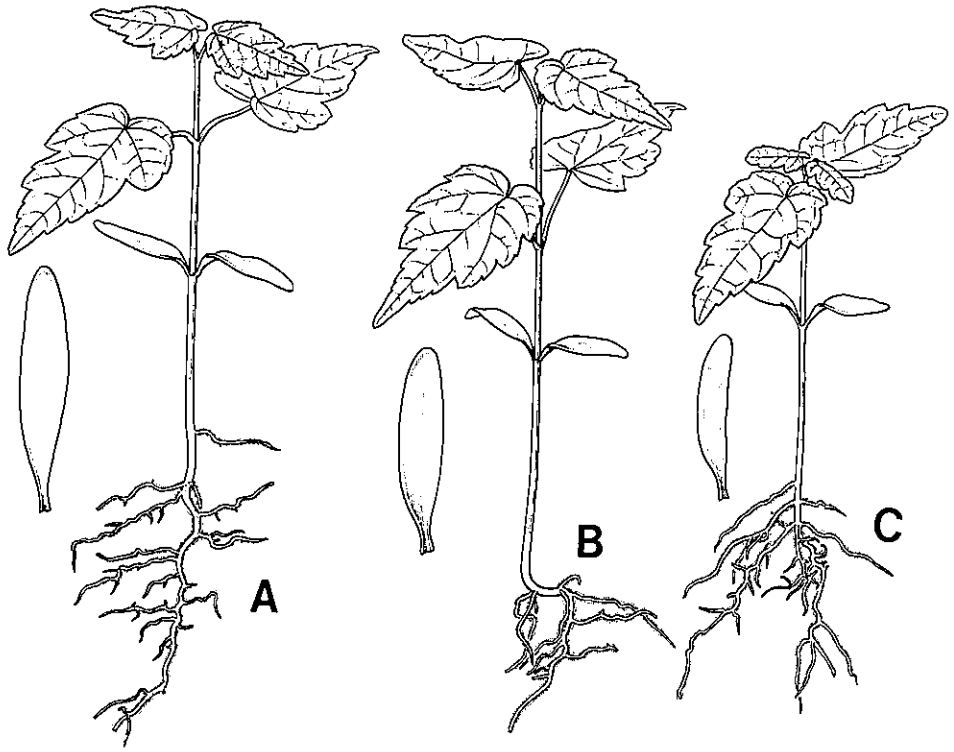

FIG. 3. Seedlings obtained from crossing experiments. A, B. Interspecific hybrid between *A. rubrum* and *A. pycnanthum* (♂), synthesized in Matsumoto (A) and Kanazawa (B). C. Intraspecific hybrid of *A. rubrum*. All germinated in Kanazawa. Seedling: ×1, cotyledon: ×2.

TABLE 5. Length of cotyledons on the seedlings

Crossing	Tree Code	Seedlings Examined	Length of Cotyledon	
			Mean	S. D.
Interspecific	B × F	3	16.3mm	1.7
	C × G	21	16.4	1.9
Intraspecific: *A. pycnanthum*	E × F	11	12.3	0.9

S. D.: standard deviation

bryos are 20.0 to 25.0mm long. The inmature samaras from non-pollinated flowers hardly extend to 15mm long and lacking embryos (Fig. 2). In addition, the fruiting rate was much higher in Matsumoto than in Kanazawa (Table 3).

GERMINATION AND SEEDLINGS: The seeds preserved at 5°C germinated immediately after sown in October 1992, while the rest in flats in the greenhouse did not germinate until April 1993. The germination rate is higher in the case of the latter than the former (Table 4). It should be again noticed that the seeds produced in Matsumoto had a higher germination percentage. Rather dry and cool climatic conditions in Matsumoto seem to be more advantageous for growth and fruiting of the red maples.

All the seedlings are completely glabrous unlike the parental plants. Those of interspecific hybrids between *A. pycnanthum* and *A. rubrum* showed vegetative heterosis. The size of cotyledons on seedlings is apparently different between plants from interspecific and intraspecific hybrids (Fig. 3; Table 5). The result of stochastic test by t-distribution on mean length of cotyledons also confirms significant difference between them at the confidence limit of 95%. The voucher specimen of seedlings is in KANA (161190).

RAPD ASSAY: Twelve among 27 kinds of oligonucleotides produced DNA segments. Only two of them were found to produce DNA segments characteristic of either of the parental plants. Their nucleotide sequences to be named No. 1 and No. 2 primers are 5′-TGGTCGCTGA-3′ and 5′-TGGTCACCGA-3′, respectively.

As mentioned above, the experiments were carried out on two sets of plants. In the first set, both the primers were useful (Table 6) and only one primer was effectively tested in the second set (Table 7). As shown in Tables 6 and 7, 0.63 kb DNA segment was amplified by primer No.1 in all the samples. Interestingly, *A. pycnanthum* (tree F and G) has all the DNA segments produced by

TABLE 6. Amplified DNA segments (in kb) from *Acer rubrum*. *A. pycnanthum*, and their hybrids, using primers No. 1 and No. 2

primes	*A. rubrum* (Tree C)	Hybrid 1	Hybrid 2	*A. pycnanthum* (G)
No. 1	—	0.28	—	0.28
	0.54	—	—	—
	0.63	0.63	0.63	0.63
	0.69	0.69	0.69	—
	0.81	0.81	0.81	—
	0.89	—	—	—
	—	1.01	1.01	1.01
	—	—	1.16	1.16
	—	—	—	1.24
	—	1.37	—	1.37
No. 2	0.48	0.48	—	—
	0.57	—	—	—
	—	0.60	0.60	0.60
	0.94	—	0.94	—
	1.04	1.04	—	—
	1.06	1.06	1.06	1.06
	—	1.44	1.44	1.44
	—	—	1.64	—
	—	—	1.72	1.72

TABLE 7. Amplified DNA segments (in kb) from *Acer rubrum*, *A. pycnanthum* and their hybrid, using primer No. 1

A. rubrum (Tree B)	Hybrid	*A. pycnanthum* (Tree F)
0.28	0.28	—
0.63	0.63	0.63
—	1.01	1.01
—	1.16	1.16
—	—	1.24
—	1.37	1.37

primer No.1 except for the 0.28 kb segment in common, while *A. rubrum* (tree B and C) does not have common DNA segments except for the 0.63 kb segment.

The hybrid, No. 1, has both DNA segments characteristic of either of the parental plants such as 0.28, 0.69, 0.81, 1.01 and 1.37 kb produced by primer No.1 (Table 6). Likewise, such DNA segments as 0.69, 0.81, 1.01 and 1.16 kb, were detected in hybrid No. 2. This fact is molecular evidence for crossability between *Acer rubrum* and *A. pycnanthum*. The other data presented in Table 6 and Table 7 also suggest the utility of the RAPD assay for confirming hybridization, though the source of 1.64 kb DNA segment produced by the primer No. 2 and detected in hybrid No. 2 is questionable (Table 6).

We wish to mention our hearty thanks to Dr. C. R. PARKS for provision of American scions and critical reading of the manuscript, to Dr. K. YAMAGUCHI of Kanazawa University for his correction of the manuscript, to Dr. K. HOSAKA of Kobe University for his valuable suggestions on the RAPD assay, to Dr. E. KINOSHITA, Dr. Y. WATANO, Miss H. TODA and Miss H. ZENTO for their various help throughout the course of this study. Our thanks should be extended to Mr. M. UMEBAYASHI of Kanazawa University for his drawing of plant materials. The present study was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan (No. 03304007), and from the Yamada Science Foundation in 1992.

References

ERNST, W.R. 1963. The genera of Hammamelidaceae and Platanaceae in the Southeastern United States. J. Arnold Arb. **44**: 193-210.

JOHNSON, L.P.V. 1939. A descriptive list of natural and artificial interspecific hybrids in North American forest-tree genera. Canad. J. Res. **17C**: 411-444.

KOIDZUMI, G. 1911. Revisio Aceracearum Japonicarum. Jour. Coll. Sci. Univ. Tokyo **32**(1): 1-75.

MOMOTANI, Y. 1962. Taxonomic study of the genus *Acer*, with special reference to the seed protein. III. System of Aceraceae. Mem. Coll. Sci. Univ. Kyoto, ser. B, **28**: 455-470.

OGATA, K. 1967. A systematic study of the genus *Acer*. Bull. Tokyo Univ. Forests No. 63: 89-206.

PARKS C.R., MILLER, N.G., WENDEL, J.F. and MCDOUGAL, K.M. 1983. Genetic divergence within the genus *Liriodendron* (Magnoliaceae). Ann. Missouri Bot. Gard. **70**: 658-666.

PAX, F. 1902. Aceraceae. *In* Engler's Pfl.-reich IV-163, Ht. 8: 1-89.

ROHMEDER, E. 1961. Praktische Anwendungsmöglichkeiten forstgenstischer Forschungsergebnisse. Schweiz. Z. Forstwesen **112**: 43-71.

SANTAMOUR, F.S., Jr. 1972. Interspecific hybrids in *Liriodendron* and their chemical verification. Forest Sci. **18**: 233-236.

SMITH, E.C. and NICHOLS, C., JR. 1941. Species hybrids in forest trees. J. Arnold Arb. **22**: 443-456.

WILLIAMS, J.G.K., KUBELIK, A.R., LIVAK, K.J., RAFALSKI J.A. and TINGEY, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531-6535.

摘　要

東アジア—北米東部型の分布の典型的な例であるハナノキとアメリカハナノキの交雑試験を1992年

および1993年春金沢と松本で行った。ハナノキ雌親の場合も雄親の場合も，交雑の結果，多かれ少かれ成熟果実を得ることができた。得られた成熟果実を直播および低温処理後播種したところ，前者では翌春，後者では直ちにかなりのともに高率で発芽した。試みに数個の芽生えとそれらの両親個体の葉から全DNAを抽出し，RAPD法を実施したところ，2種のプライマーで両親に特異的なDNA断片が検出された。芽生え個体では，これら両親のDNA断片を共有することが分った。これは，雑種形成の分子的根拠に外ならない。なお，雑種の芽生えは，とくに子葉の大きさにおいてすぐれ，雑種強勢が認められた。
(received June 14, 1993 : accepted Oct. 12, 1993)

○伊那谷自然教育究研会　**中央アルプスと伊那谷の自然**　A 5判，228頁。1993年9月9日発行，信濃毎日新聞社。定価2,600円。

信濃毎日新聞社では，郷土の自然を広く一般に紹介する目的で，地域ごとの自然解説書“自然シリーズ”を発行している。著者は地元に詳しい研究者および自然愛好者である。本書はこのシリーズの5冊目で広く伊那谷の自然を扱ったもので，Ⅰ．自然を形づくるもの，Ⅱ．植物の生活，Ⅲ．動物の生活，Ⅳ．天竜川の生物の5章からなる。植物に直接関係あるのは，Ⅰの第5節生物地理学からみた伊那谷とⅡである。前者では，ハイマツ帯やシラビソ帯では，日本海型分布の植物が大部分を占め，ブナ帯では太平洋型分布が多くなり，クリ帯やカシ帯では大部分が太平洋型分布の植物となるという興味深い事実を指摘している。また，後者では各植生帯ごとに，植生のようすや主な構成種を写真とともに解説してあるばかりでなく，気候や地形などの立地条件と関連させながらその成立要因を説明する努力がなされていることが特筆に価する。　（清水建美）

○　第17回全国育樹祭三重県実行委員会　**郷土の樹木―三重県の樹木誌―**　B 5判，107頁。平成5年10月発行。非売品。

本書は本年「うるおいと　やさしさを伝える　豊かな緑」を大会テーマに行なわれた，第17回全国育樹祭を契機に，先人たちが堂々と築きあげ，今も人々に深いかかわりを持ちながら成長する，貴重な樹木の姿を広く県内外に紹介し，人々の緑への関心をさらに高めることを目的に刊行したという。

書名は「郷土の樹木―三重県の樹木誌―」となっているが，内容は長年の風雪に耐え，地域住民と親しんで来た三重県内の巨樹・巨木林を紹介したもので，選ばれた101件を，笠井道男氏撮影のカラー写真に，武田明正氏が，解説文を執筆している。

選定された101件を，天然記念物指定から見ると，国7件（三多気の桜，東阿倉川のマメナシ，西阿倉川のアイナシ，白子の不断桜，椋本の大椋，果号寺のシブナシガヤ，野村の一里塚のムクノキ〈史跡〉），県25件，市9件，町4件，村2件，計47件である。また樹種から見ると，55種で2件以上の種はスギ・カヤ各8件，ヒガンザクラ・クスノキ各6件，クロマツ・スダジイ各4件，イブキ・コウヨウザン・イチョウ・タブノキ・エノキ各2件である。　（里見信生）

○初島住彦*：**ケイリンサイシン日本に産す**　Sumihiko HATUSIMA*: *Asarum heterotropoides* var. *mandshuricum* Newly Found in Japan

今年5月（1993年）上旬に熊本県阿蘇郡波野村の植物研究家弼（ヅル）　義文氏から変ったサイシンの一種を発見したので鑑定してほしいとの依頼があり，生品3株が筆者の所に送ってきたので調べたところ，従来朝鮮，満州に知られていたケイリンサイシンであることがわかった。ケイリンサイシンは樺太，千島からわが国の東北地方に分布するミチノクサイシンの変種で，母種とは蕚裂片が鋭頭，葉は心状腎形でなく卵心形で，全株無毛なる点で区別されている。蕚筒部が扁円形で裂片が著しく反曲して蕚筒に密着する点ではフタバアオイにも似ているが，根茎は長く横走せず，全株無毛で，葉は大きく幅7 cmに達し，葉柄は長さ15 cmにもなり，花梗も長さ7 cmに達し，花柱は離生，蕚片は下半部がゆ合している点で区別できる。ウスバサイシンとは蕚裂片は卵形でなく，広卵形又は卵状腎形で，上方は開出せず著しく反曲する点と，葉が鋭尖頭でなく鋭頭になる点で区別できる。弼氏によると本変種は阿蘇の原野の5ケ所に多数自生しているという。本変種は朝鮮では済州島，梅加島，蔚島，全南，慶南，江原の諸道，満州では黒龍江，吉林，遼寧の諸省に知られている。ケイリンなる名前は韓国の古都慶州の別名鶏林にもとづき故前川博士が命名したものである。本変種の発見報道後，熊本工業大学の浜田善利氏から便りがあり，同氏は1979年既に本変種を阿蘇の原野で発見され1981年に現地でスライドに撮影していたが詳しく追求しないままであったという。その後深蕊でも発見し，同氏が送った阿蘇のものは東京理科大学の薬草園に栽培されているという。阿蘇には満鮮系の植物が多数知られているが今回の発見で更に一種加えたことになる。最後に筆者の依頼により阿蘇産の本変種の花の解剖図を描いて下さったカンアオイの研究家山幡英示氏に感謝の意を表したい。