INVESTIGATIONS ON JAPANESE *ADENOPHORA*

I. Observations on a Population of *A. petrophila* *

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The Genus *Adenophora* (Campanulaceae), including some seventy species, is broadly distributed in Asia and in Europe and extending from the warm temperate to the frigid zone. In Japan, there have been reported about a dozen species. Because of the great polymorphism, however, the delimitation of certain species and their affinity still remain questionable, the taxonomic treatment of them being often contradictory as exemplified by *A. nipponica*, *A. petrophila*, etc. The ecological situation of the genus, on the other hand, is also of great diversity. Some species having vast areas extended from Japan to the Continent will appear on the grassy stretches or in the open thickets, while some other ones showing limited areas will be found characteristically on the rocky or rubbly places. *A. triphylla* and *A. remotiflora* are the representatives of the former. And the latter includes such examples as *A. howozana*, *A. maximowicziana*, *A. petrophila*, *A. takedai*, *A. teramotoi*, *A. uryuensis*, etc. These members of the latter might have differentiated through isolation on the respective particular habitats. The problem of derivation and affinity of them is of my special interest.

In this and the following papers, therefore, I wish to present any biological materials on Japanese *Adenophora* aiming to approach to the speciation of such local species or races and at the same time to contribute to the taxonomic revision of the genus. The present paper consists of an introduction of the genecological observations on a certain population of *Adenophora*, and a record of the chromosome counts of some Japanese species so far examined.

Material population

The material population has come from the montane ridge near the Jumoji Pass occupying the border-line between Nagano and Saitama Prefecture in

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middle Honshu of Japan (Fig. 1).

Geologically this area is mainly constructed by the siliceous rocks, chert and slate. Through this siliceous ridge, some calcareous fields are occasionally inserted, a great limestone bluff named 'Azusa-shiraiwa' projecting over the ridge and a series of gravelly fields over some hundred meters occurring about 2km off the bluff. In the latter, however, the case is petrologically rather complicated, for the fields consist of somewhere calcareous gravels only but somewhere of siliceous and somewhere of mixture of both. Anyway a brief botanical survey in this area will reveal at least two kinds of *Adenophora*; one is apparently *A. divaricata* and the other referable to *A. petrophila* (Shimizu 1963, Shimizu & Aoki 1964). The material population in this text is that of the latter, standing between 1700m and 1900m in altitude and in the lowest part of the coniferous zone represented by *Tsuga diversifolia*.

A. petrophila was described by Hara (1935) based on the plants from Takaiwa and Mts. Myogi in Gumma Prefecture. Since then, it has remained one of the problematical species in taxonomy. Hara (1937, 1952) recognized its close relationship to *A. nikoensis*, with which he combined it as *A. nikoensis* var. *petrophila*. Ohwi (1953, 1965) supported his treatment. Kitamura et al. (1957), on the other hand, considered it an independent species. My own opinion (1958, 1963) was that this plant would be rather related to *A. pereskiaefolia* to be named *A. pereskiaefolia* f. *petrophila*.

Apart from such taxonomic treatments of *A. petrophila*, the exact geographical record of it I could examine is only from four localities besides the present area at least in middle Honshu, viz. Mt. Akasawa, Mts. Futago, Mts. Myogi and Takaiwa (Fig. 1). The habitat is calcareous in the first two and siliceous in the rest.

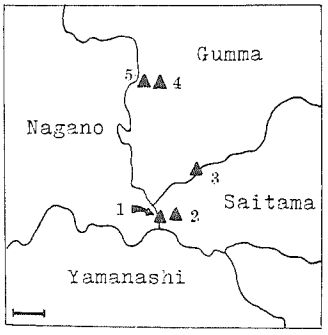


Fig. 1 Distribution of *A. peterophila*
1: Azusa shiraiwa (limestone)
2: Mt. Akasawa (limestone)
3: Mts. Futago (limestone)
4: Mts. Myogi (conglomerate)
5: Takaiwa (conglomerate?)
Scale: 10km

Environment of the population

As previously reported (Shimizu & Aoki 1964), the vegetation on the calcareous field and the siliceous one in this area has a striking contrast. It is worth noticed that the vegetation around the limestone bluff, Azusa-shiraiwa, is characterized first by the calcicolous plants such as *Asplenium ruta-muraria*, *Camptosorus sibiricus*, *Gymnocarpium jessoense*, *Betula chichibuensis*, *Spiraea nip-*

ponica and *Rhamnus costata*, and secondly by the alpine or subalpine uncommon species such as *Asplenium viride*, *Athyrium spinulosum*, *Woodsia glabella*, *Draba oiana*, *Rosa acicularis* var. *nipponensis*, *Hedysarum ussuriensis*, *Tilingia tachiroei* and *Allium splendens*. Also, *A. petrophila* is nearly concentrated on this bluff, growing abundantly side by side with these plants. Beyond the bluff, indeed, we can find it only in a few stations. Contrariwise, *A. divaricata* is almost always restricted to the siliceous habitat, never intruding on the limestone bluff mentioned above. Thus, it was only at two spots, basing on my survey throughout the area, where these two kinds of *Adenophora* were found close by each other.

To clarify such the habitat difference, some soil characters, i.e. water content, ignition loss, pH value and exchangeable calcium content, were examined. The soil samples, brought from the root-sphere of the respective plants, were 16 in number for *A. petrophila* and 7 for *A. divaricata*. Measurement was followed to the ordinary procedures as below:

Water content: Air-dried soils sifted with a 5mm-meshed sieve was dried at 105°C to become constant in weight. Diminished weight was expressed in percentage against the air-dried sample.

Ignition loss: Dried samples obtained through the above procedure were heated at 900°C to become constant in weight. Diminished weight was expressed in percentage against the dried sample.

pH value: Air-dried soils sifted with a 5mm-meshed sieve were digested with distilled water of twice volumes of the sample and concussed. After the solution was left intact overnight, the pH value was measured by the glass electrode pH meter.

Exchangeable calcium content: Five grams of air-dried soils shifted with a 0.5mm-meshed sieve were digested at 70°C with 50cc of normal ammonium acetate, and concussed. After left intact overnight at 70°C in the incubator, the sample was leached out with normal ammonium acetate until 50cc of the leachate was obtained. The precipitates of calcium oxalate formed in the leachate by ammonium oxalate were left overnight and filtered, and then the amount of calcium was determined by volumetric analysis through the titration with 0.01 normal potassium permanganate solution. The value of exchangeable calcium content was expressed in miligram equivalent against 100 grams of dried sample.

The result of the soil analysis is shown in Table 1 and 2. As evident in Table 1, the soil samples from the limestone bluff (No. 7 to No. 16) show the nearly approximate values in any characters examined, indicating the high ratio of water and organic substances and the high value of pH and exchangeable calcium content. Even the lowest values of them, obtained from the marginal spot of the population around the bluff (Sample No. 8), are 6.68 and 67me in the latter two, respectively. With regard to the case of the rubbly ridge off

Table 1 Habitat of *Adenophora petrophila* near the Jumoji Pass

Sample	Water cont. (%)	Ign. loss (%)	pH	Exchang. Ca (me / 100g)
1			7.01	41.5
2			6.99	33.5
3*	3.64	12.47	5.80	16.5
4	4.31	14.65	5.36	9.5
5	2.88	9.26	5.75	7.5
6*	9.78	41.70	6.75	46.5
7			7.08	74.5
8	18.86	61.19	6.68	67.0
9			7.34	102.5
10			7.38	122.0
11			7.97	112.5
12			7.75	110.0
13	13.31	60.03	7.62	96.0
14	19.98	61.67	7.76	
15	17.13	60.06	7.65	119.5
16			8.02	105.5

Sample No. 1～ 6 : from the rubbly ridge
No. 7～16 : from the limestone bluff
* Station mixed with *A. divaricata*

the bluff (Sample No. 1 to No. 6), however, it was proved that the soil characters were fairly different from the above. As far as the samples, No. 3, No. 4 and No. 5, indicate, *A. petrophila* is rather silicicolous. This fact means that the plant would be tolerable also to the siliceous habitat, though its distribution shows a strong preference for the calcareous field.

Meanwhile, the values of the pH and the exchangeable calcium content in the soil sample of *A. divaricata* extend from 5.09 to 7.47 and 8.5 to 50.4 me, respectively (Table 2). The samples, No. 2 and No. 4, are calcareous, and the rest siliceous. Therefore, this plant would be silicicolous, but it has some

Table 2 Habitat of *Adenophora divaricata* near the Jumoji Pass

Sample	Water cont. (%)	Ign. loss (%)	pH	Exchang. Ca (me / 100g)
1			5.20	13.5
2			7.47	50.4
3			5.09	8.5
4			7.44	49.5
5	8.37	23.77	6.71	29.5
6	9.83	24.28	5.62	11.0
7			5.40	10.5

degree of tolerance to the calcareous habitat, for it can never intrude into the area showing high content of the exchangeable calcium such as the limestone bluff here.

Speaking of the edaphic tolerance of the two species, *A. petrophila* and *A. divaricata*, the seedlings of them the seeds of which originated from the present area have been grown in this year in Matsumoto on three kinds of medium: calcareous soils, siliceous soils and 1 : 1 mixture of them. The soils used for cultivation were also brought from the present area, the limestone bluff and the chert bluff. The process of cultivation to the present indicates:

Any kinds of medium do not look deleterious for the seedlings of *A. petrophila*.

As for *A. divaricata*, the calcareous medium looks deleterious, for chlorosis occurred in all the seedlings on it. But, they look sound on two other kinds of media.

Through these experiments, it can be concluded that the physiological tolerance of *A. petrophila* against the edaphic conditions would be superior to that of *A. divaricata*, though the former prefers calcareous habitats to siliceous ones and the latter vice versa. Probably this is the reason why the latter does not occur on the heavily calcareous habitat of the limestone bluff here.

Chromosome numbers

The chromosome numbers of Japanese *Adenophora* were first examined by Matsuura and Suto (1935), who dealt with *A. hakusanensis* (=*A. triphylla* var. *hakusanensis*), *A. thunbergiana* f. *hirsuta* (=*A. triphylla* var. *japonica*) and *A. remotiflora*. Through the observations on the meiotic division, they concluded that the diploid chromosome number of these three species were 51, 37 and 37, respectively. Sugiura (1942) also revealed the chromosome numbers of the genus, and stated that, in spite of Matsuura and Suto's observations, the present genus had the chromosome numbers of 17 or its multiples. According to him, *A. bulleyana*, *A diplodonta*, *A. forrestii* and *A. stricta* are diploid species, viz. n=17, while *A. lilifolia*, *A. ornata*, *A. palustris* and *A. potaninii* are hexaploid, n=51. Two of them, *A. palustris* and *A. stricta* are indigenous to Japan.

Using the squash method of the root-tip cells, I examined the somatic chromosome numbers on 61 samples of *A. petrophila* which were sampled throughout this area; 54 from the limestone bluff and 7 from the rubbly ridge including the siliceous habitats mentioned above. The result was that, indifferently to the habitat difference and also to the morphological difference discussed later, all the materials examined were proved to be same in chromosome number, 2n=68 (Fig. 2; A, B). This number, probably a new

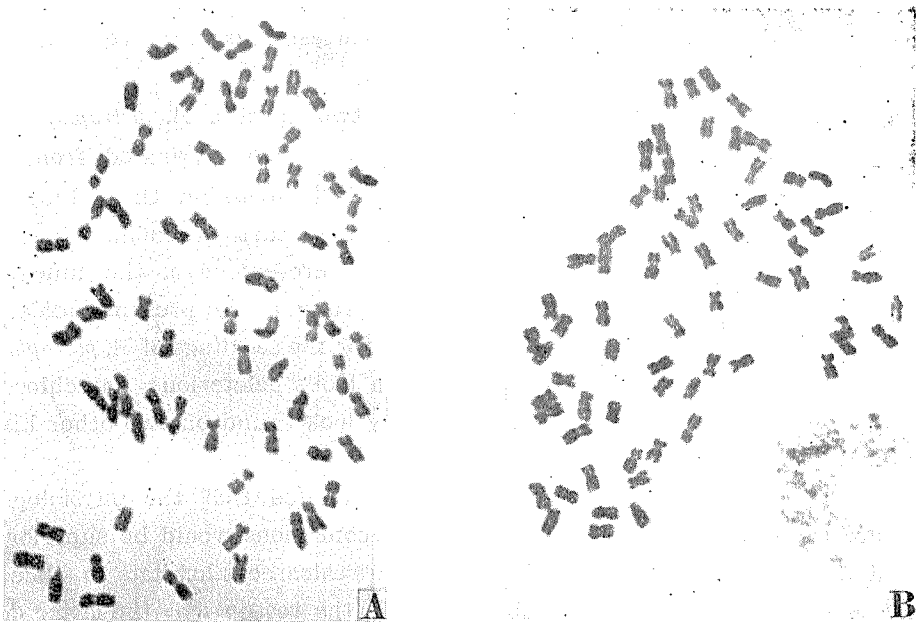


Fig. 2 Somatic chromosomes of *A. petrophila* sampled near the Jumoji Pass (×1500)
A : From the rubbly ridge (Soil sample No. 1)
B : From the limestone bluff

record to the genus, denotes that these material plants are tetraploid. To b interested, in spite of my suggestion of 2n=68 (Shimizu 1965), *A. petrophila* from Mts. Myogi shows a queer number of 2n=70 so far as the several materials examined are concerned (Fig. 3; E).

On the other hand, the chromosomes of *A. divaricata* were counted as to 9 samples from the present area, and found to be all 34 (Fig. 3; D). This number proved same in Mts. Myogi, too.

In addition, I have still more counts on some other Japanese *Adenophora*. They are as follows:

A. nikoensis is diploid, 2n=34 (Fig. 3; A).

A. triphylla var. *japonica* is diploid (Fig. 3; C), likewise in var. *triphylla* from Taiwan (Shimizu 1965).

A. teramotoi is hexaploid, 2n=102 (Fig. 3; F, F').

A. remotiflora shows another exceptional number beyond the series of 17, viz. 2n=36 (Fig. 3; B).

All these available materials on the chromosomes of Japanese *Adenophora* are listed in Table 3.

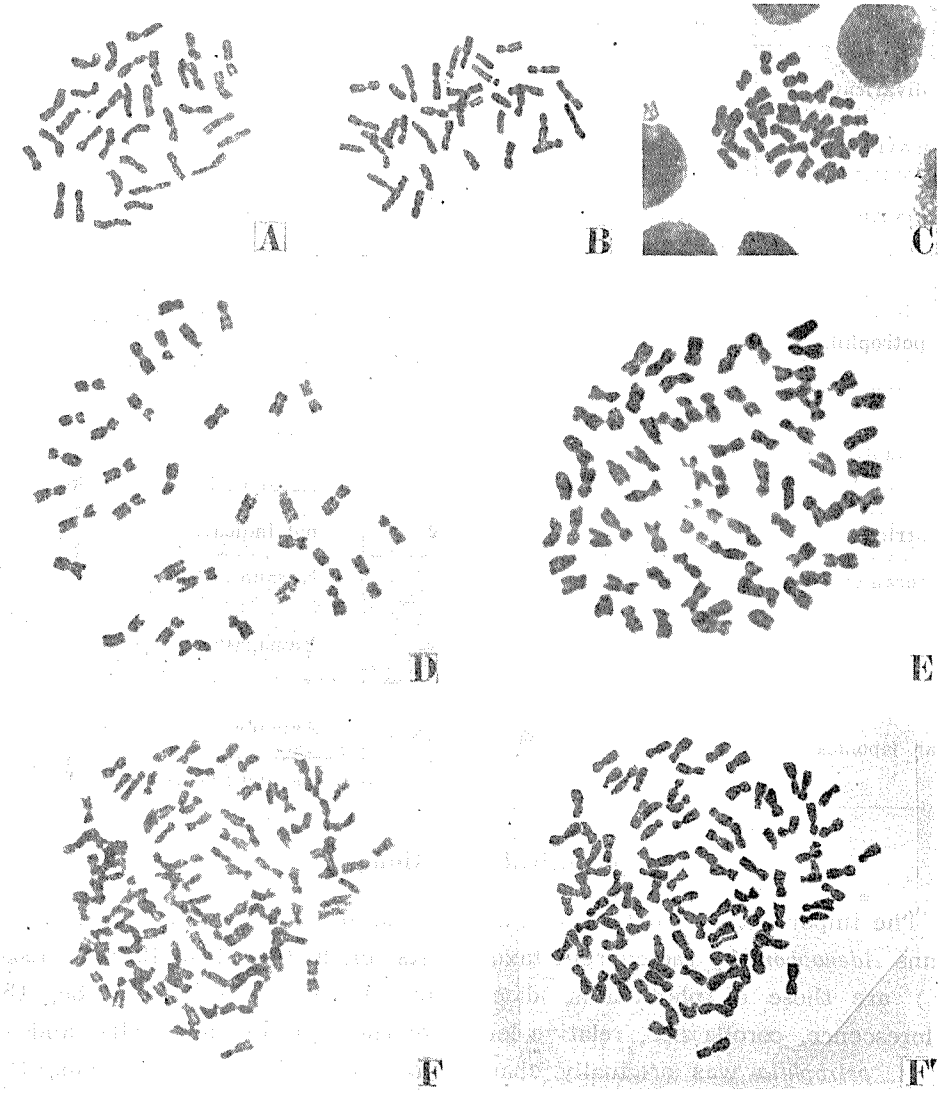


Fig. 3 Somatic chromosomes of some Japanese *Adenophora* (×1500)

A : *A. nikoensis* from the Shiga Heights (2n=34)

B : *A. remotiflora* from Mt. Hakamakoshi (2n=36)

C : *A. triphylla* var. *japonica* from Obasute (2n=34)

D : *A. divaricata* from the Jumoji Pass (2n=34)

E : *A. petrophila* from Mts. Myogi (2n=70)

F, F' : *A. teramotoi* from Todai (2n=102)

Table 3 Chromosome numbers of Japanese *Adenophora*

Plant	Chromosomes		Author	Locality
	n	2n		
A. divaricata		34		Nagano : Jumoji Pass Gumma : Mts. Myogi
A. maximowicziana		34	Shimizu '63	Ehime : Oonogahara
A. nikoensis		34		Nagano : Sugadaira & Shiga Heights
A. palustris	51		Sugiura '42	not indicated
A. petrophila		70		Gumma : Mts. Myogi
		68		Nagano : Jumoji Pass
A. remotiflora	$18_{II}+1_{I}$	(37)	Matsuura & Suto '35	Yamagata : Mt. Zao
		36		Nagano : Mt. Hakamakoshi
A. stricta	17		Sugiura '42	not indicated
A. teramotoi		102		Nagano : Todai
A. triphylla var. hakusanensis	17_{III} 17	(51)	Matsuura & Suto '35	Yamagata : Mt. Gassan
var. japonica	$17_{II}+1_{III}$	(37)	Matsuura & Suto '35	Yamagata : Mt. Zao
		34		Nagano : Obasute

Morphological variation

The important morphological characters used in the classification of the Genns *Adenophora* by the current taxonomists (cf. Fedorov 1957, Baranov 1963, etc.) are those of phyllotaxis, disc form, shape of the calyx lobe, the inflorescence, corolla size, relative length of the style to the corolla, and so on. *A. petrophila* was originally characterized by the glabrous stems, the narrow leaves arranged whorled, the divaricate inflorescence, the entire lanceolate calyx lobes 4~5mm long and 1.5~2mm wide, the corolla about 2cm long, the long exserted styles and the annulate discs 0.8~1.5mm long and about 1.8mm in diameter (Hara 1935). Hara (1937) pointed out that the plants of *Adenophora* besides *A. divaricata* in Mts. Myogi were all provided with the characters mentioned above, so that they could be readily included in *A. petrophila*. Also in my experience, I could not find any plants beyond such a demarcation there around.

However, the condition is completely different within the population of

A. petrophila in the present area. Before further going, the population is desirably divided into two groups ; one standing on the rubbly ridge represented by the soil sample No. 1 to No. 6 and the other around the limestone bluff characterized by the soil sample No. 7 to No. 16 (Table 1). Though few in number, the plants of the former are all provided with most characters of the typical *A. petrophila* ; the whorled leaves, the entire calyx lobes, the annulate discs and the exserted styles. But, it should be noticed that the stems were sometimes hispid with minute hairs, and that the inflorescences were not so spreading (Plate I ; A, B).

In the latter group in turn, the morphological characters are so considerably fluctuated plant by plant that, exaggeratedly speaking, each plant looks like of different kind. Indeed, the stems are sometimes hispid but sometimes completely glabrous, the phyllotaxis sometimes whorled but sometimes completely alternate, the leaves sometimes broad but sometimes narrow, the calyx lobes sometimes entire but sometimes apparently incised, ····· (Plate I ; C, D). And, the various degree of difference and the various combination of such differences seem to occur within this population. For example, the result of complete sampling in an area of 15 square meters on a side of the limestone bluff and precise examination on two kinds of the characters, phyllotaxis and shape of calyx lobes, is as in Table 4, where the figure denotes the number of the plant (not stem) with the respective combination of the characters. The shape of the calyx lobes is, though different plant by plant, uniform within the same plant, both kinds of the calyx lobe, entire and incised, being not found therein. Moreover, it is remarkable that the calyx lobe is much lengthened extending to 10mm or more long whenever incised (Fig. 4). Meanwhile, the phyllotaxis seems to be rather loose, for the different arrangement sometimes occurs on the different stems of the same plant. In such case, the predominant phyllotaxis is considered for Table 3. As evident in the table, the plant with verticillate leaves and entire calyx lobes appears most abundantly in this population (Plate I ; C). Judging from these two characters, it is only these plants that resemble the typical *A. petrophila*. In turn, the plants represented by 11 samples in the table are rather like *A. nipponica* and by one like *A. nikoensis*, while the plants corresponding to the rest 12 samples are unlike any other *Adenophora* hitherto reported in Japan. A plant such as in the photograph (Plate I ; D) will be undoubtedly determined to be *A. nipponica* when only a specimen is shown.

Fig. 4 Showing slender and incised calyx lobes (×2/3)

Table 4 An example of the morphological variation within an *Adenophora petrophila* population

Leaves \ Calyx lobes	entire	incised	Total
verticillate	55	12	68
alternate	11	1	12
Total	66	13	80

Area : 15 sq. m.

Similarly as for the other characters, the situation is approximately same in the population. The leaves are ovate to narrow lanceolate in shape. The corolla size varies from 1.2cm to 3.5cm, though nearly same within the same plant. The styles are mostly exserted, but occasionally inserted. The disc is here fairly uniform in shape, that is, mostly annulate, but in a few plants it is rather cylindrical showing twice length of the diameter.

Moreover, it should be noted that the inflorescences were in any plant here unlike the typical *A. petrophila*. As pointed out by Hara (1935), the inflorescence of *A. petrophila* spreads to be divaricate. Different from it, the plants in the present population have a few flowers at the top of the stem, otherwise some long pedicellate solitary flowers at the axiles of the upper leaves of normal size (Plate I). Therefore it results that the inflorescence of them is same with that of *A. nikoensis* or *A. nipponica*.

Table 5 Morphological comparison of *A. petrophila* between two populations

Characters \ Population	near Jumoji Pass	Mts. Myogi
phyllotaxis	whorled to alternate	whorled
leaf shape (length : width)	ovate to narrow lanceolate 2~8	lanceolate 4~6.5
hairiness of stem	hispid often glabrous	glabrous
inflorescence type	not divaricate	divaricate
shape of calyx lobe (length)	ovate and entire to linear and incised 3~15mm long	ovate and entire 4~8mm long
collora size	15~35mm long	12~20mm long
style condition	exserted often inserted	exserted
disc shape (length : diameter)	annulate rarely cylindrical 0.3~2.0	annulate 0.3~1.0
chromosome number	2n=68	2n=70

The morphological variation within the present population and the comparison with that of Mts. Myogi (the typical *A. petrophila*) are summarized in Table 5. In short, the plants referred to *A. petrophila* are much variable within the present population and far beyond the variation range of the original *A. petrophila* concerning to many characters. The origin of this singular population, I suppose, might be different from that of the typical *A. petrophila*.

Summary

A genecological observation was carried out on a population referable to *Adenophora petrophila* near the Jumoji Pass in middle Honshu of Japan. Around there this perennial is nearly concentrated on the calcareous habitat. Another species of *Adenophora* abundantly found in the same area, *A. divaricata*, is contrariwise inclined to be silicicolous, never intruding on the limestone bluff.

Cytologically the somatic chromosome number was proved to be 68 (tetraploid) as to all the materials of *A. petrophila*, indifferently to the habitat differences and the morphological distinctions (Fig. 2). The two related species, *A. divaricata* and *A. nikoensis*, were found to be diploid, and the typical *A. petrophila* from Mts. Myogi 2n=70 (Fig. 3).

Morphologically the population of *A. petrophila* in the present area shows a great range of variation concerning to many key characters (Table 4 & 5), many a plant standing beyond the demarcation of the species. Therefore, entity of this species and the origin of the present population are much problematical.

Literature Cited

Baranov, A. I. (1963). Quart. Jour. Taiwan Mus. 14 : 143~179.

Fedorov, An. A. (1957). Fl. URSS 24 : 343~372.

Hara, H. (1935). Jour. Jap. Bot. 11 : 825~826, f. 25.

——— (1937). Ibid. 13 : 464~468.

——— (1952). Enum. Spermat. Jap. 2 : 89.

Kitamura, S. et al. (1957). Col. Ill. Herb. Pl. Jap. (Sympetalae) 64.

Matsuura, H. & T. Suto (1935). Jour. Fac. Sci. Hokkaido Imp. Univ., ser. V (Bot.), 5 : 53~54.

Ohwi, J (1953). Flora of Japan 1120.

——— (1965). Ibid. rev. ed. 1281.

Shimizu, T. (1958). Acta Phytotax. Geobot. 17 : 86.

——— (1963). Jour. Fac. Tex. Sci. Technol., Shinshu Univ., No. 36, ser. A (Biol.),

No. 12 : 65.

———— (1965). Jour. Jap. Bot. 40 : 372.

———— & T. Aoki (1964). Jour. Geobot. 12 : 90～93 & 13 : 11～14.

Sugiura, T. (1942). Cytologia 12 : 422.

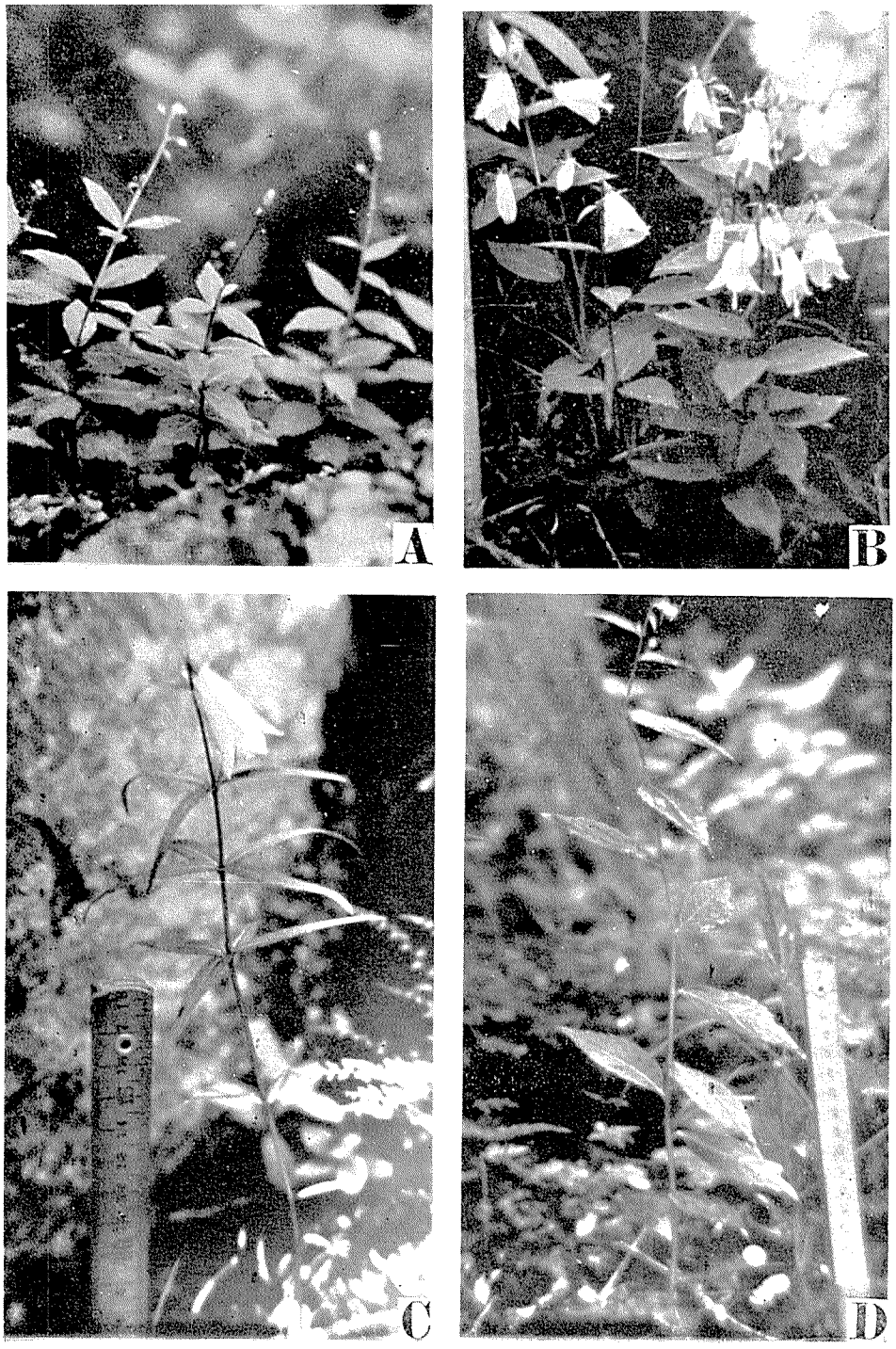
Plate I

Adenophora petrophila near the Jumoji Pass

A, B : Plants from the gravelly ridge

C, D : Plants from the limestone bluff

All photographs were taken on July 22, 1966.


A
B
C
D