Taxonomic relationship between Japanese Potentilla anemonefolia and Himalayan P. sundaica (Rosaceae)

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Naohiro Naruhashi[1], Takayuki Nishikawa[1,2] and Yoshikane Iwatsubo[1] : **Taxonomic relationship between Japanese *Potentilla anemonefolia* and Himalayan *P. sundaica* (Rosaceae)**

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Abstract

Japanese *Potentilla anemonefolia* and Himalayan *P. sundaica* which are sometimes thought to be conspecific by some taxonomists were compared in morphology, phenology, dry matter allocation, karyotype and meiotic chromosome behavior. The former is diploid, with larger leaves, with larger self-incompatible flowers, having considerably vegetative reproduction, while the latter is tetraploid, with smaller hairy leaves, smaller self-compatible flowers and having less vegetative reproduction. The analysis of the artificial F_1 hybrid showed slightly differentiated genomes in the two species. As a result of the present investigation, we conclude that the two taxa can be regarded as different species.

Key words : chromosome, artificial hybrid, *Potentilla, Potentilla anemonefolia, Potentilla sundaica.*

Lehmann (1853 a, b) described the plant named O-hebi-ichigo (Japanese name) which is somewhat common in or around paddy fields in Japan as *Potentilla anemonefolia*.

However, many Japanese taxonomists have considered that *P. kleiniana* (described by Wight and Arnott in 1834) is earlier name than *P. anemonefolia* for that plant. Professor Kalkman (1968) in Leiden, Netherland, treated *P. kleiniana* and *P. sundaica* as conspecific taxa and he adopted *P. sundaica* as a correct name. Recently Kitagawa (1980), Momiyama (1982) and Ono et al. (1989) use *P. sundaica* var. *robusta* as the plant name, because Japanese plants are larger than *P. sundaica* itself. Until now adopted scientific names for O-hebi-ichigo and their authors are listed in Table 1.

Since we, the present authors consider *P. kleiniana* and *P. sundaica* as conspecific taxa depending on literatures and herbarium specimens, taxonomic relationship between *P. anemonefolia* and *P. sundaica* needs a critical examination.

As a member of the Nepal-Japan Botanical Expedition to Nepal from June 24 to September 25, 1988, Naruhashi, one of the authors, was able to observe *P. sundaica* in several places and collected seeds of the plant at Tashi Gaun 2,200 m alt., Sankhuwa Sabha Distr., Koshi Zone, E. Nepal (Voucher specimen : M. Suzuki, N. Naruhashi, N. Kurosaki, Y. Kadota, M. N. Subedi, M. Minaki, S. Noshiro & H. Ikeda, Jun. 13, 1988, no. 8810327).

Artificial F_1 hybrids between *P. anemonefolia* and *P. sundaica* were produced and their chromosome behavior in PMC were examined. Present paper deals with the comparison of morphology, phenology, dry matter allocation, chromosome number in the two species and genome analysis of PMC in the F_1 hybrids.

Materials and methods

Living plants for materials were used in the Observating and Training Garden for Nature, Toyama University. *Potentilla sundaica* originated from Nepalese seeds collected in Tashi Gaun, 1988 and *P. anemonefolia* was collected at Kureha, Toyama City and multiplied at Toyama University. Floral organs, such as petal, calyx and epicalyx, were optionally selected one from each flower. Characters examined in this study are length, width and area of petal, length and

Table 1. Adopted scientific names as Japanese O-hebi-ichigo and their authors

Scientific name	Author
Potentilla anemonefolia Lehm.	Lehmann（1853 a, b）; Lehmann（1856）; Handel-Mazzetti（1933）; Naruhashi（2001）
P. anemonefolia Lehm. var. *kleiniana*（Wight et Arn.）Kitag.	Kitagawa（1979）
P. gelida C.A.Mey	Gray（1856）
P. inclinata Vill. var. *laxa* Franch. et Sav.	Franchet and Savatier（1873）
P. kleiniana Wight et Arn.*	Miquel（1867）; Franchet and Savatier（1873）; Makino（1910）; Koidzumi（1913）; Ohwi（1965）; Okuyama（1977）; Ohwi and Kitagawa（1983）
P. kleiniana Wight et Arn. var. *robusta*（Th.Wolf）Kitag.	Franchet and Savatier（1875）; Kitagawa（1979）
P. kleiniana Wight et Arn. ssp. *anemonefolia*（Lehm.）Murata	Murata（1961, 1965）
P. reptans L.	Gray（1859）; Franchet and Savatier（1875）
P. reptans L. var. *trifoliata* A.Gray ex Miq.	Miquel（1867）
P. sundaica（Blume）Kuntze**	Kalkman（1968）
P. sundaica（Blume）Kuntze var. *robusta*（Th.Wolf）Kitag.	Kitagawa（1980）; Momiyama（1982）; Ono et al.（1989）
P. wallichiana Delile ex Lehm.	Maximowicz（1873）; Maekawa et al.（1961）
P. wallichiana Lehm. var. *anemonefolia*（Lehm.）Nakai	Nakai（1916）
P. wallichiana Lehm. var. *robusta* Franch. et Sav. ex Th.Wolf	Wolf（1908）

* This plant is described by Wight and Arnott（1831, 1834）.
** This plant is described in 1826 by Blume as *Fragaria sundaica*（Blume 1826）.

width of calyx, length and width of epicalyx, number of stamens, pistils and achenes, dry weight of achenes, area of leaf and hair length on leaf.

For the comparison of reproductive system, self-incompatibility tests were done in green house, i.e., wrapping flower bud by paper bag, emasculation, pollination with pollen grains of same individual and other individual in the species, again wrapping, and finally maturing seeds in the open.

Fertility of pistils by counting the number of fertile pistils and achenes per flower in both species was also examined. The flower used was chosen on the basis of anthesis order in an inflorescence from early blooming to late blooming.

In the present investigation of seasonal growth cycles and dry matter economy, sampling materials of two species were carried out throughout the year from February in 1991 to January in 1992 in the above mentioned garden. For the biomass allocation study, sequential harvests of mature individuals were made in monthly intervals throughout the year. Each time one individual of both of the two species was collected. The plant was cut and separated into its component organs, dried in an oven for at least 48 h at 75℃ and weighed.

The two plants of *P. anemonefolia* and *P. sundaica* were crossed reciprocally. In the reciprocal crossing, the cross between *P. sundaica*（♀）and *P. anemonefolia*（♂）yielded enough seeds as open pollinated flowers in the maternal plants. The seeds sown were normally grown into mature plants. The reverse crosses done using the nine flowers of *P. anemonefolia* had no seed in all the flowers.

Methods for the karyotype and meiotic chromosome behavior are as follows.

Root tips collected from potted plants were pretreated in a 2 mM 8–hydroxyquinoline solution for 1 h at room temperature, and subsequently held at 5℃ for 15 h. The root tips were fixed in a glacial acetic acid and absolute ethanol mixture（1 : 3）for 1 h, soaked in 1 N HCl at room temperature (ca. 25℃) for a few hours, and subsequently macerated in 1 N HCl at 60℃ for 10 min. After being immersed in tap water, they were stained in 1.5 % lacto-propionic orcein, and the ordinary squash technique was applied for the examination of somatic chromosomes.

Meiotic chromosome pairings were investigated in pollen mother cells (PMCs). For the studies, young flower buds were fixed in Newcomer's fluid at 17℃ for 3 h and macerated with the same procedure as for the root tips. The anthers were stained and squashed in 1.5% lacto-propionic orcein. Chromosome pairing was examined in the PMCs at first meiotic metaphase stage. In F_1 hybrid plants, the pollen grains stained with lacto-propionic orcein were also examined under light microscope to determine the percentage of stained pollen grains as a possible indicator of pollen fertility.

Results

Morphology

Both species show almost the same habit and morphological characteristics. Stems are first decumbent, thereafter ascending at flowering. Rosette leaves are palmately (pedately) 5–foliolate (rarely 3 or 7) with obovate leaflets. The diffferent points between the two species are in degree of hairiness (i.e., plants are almost glabrous except for floral organs in *P. anemonefolia*, while plants are pilose, especially long white hairs on stems and petioles in *P. sundaica*), in the form of inflorescences (compact inflorescences with very short peduncles in *P. sundaica* against somewhat spreading inflorescences (compound dichasium) in *P. anemonefolia*), and in the size of petals (small petals in *P. sundaica*, 4.0 mm in length, 2.7 mm in width on average, against the large petals of *P. anemonefolia*, 7.2 mm in length, 6.9 mm in width on average) (Fig. 1).

Morphological comparison of characters between the two species is shown in Table 2 and Fig. 2. In *P. anemonefolia* the size of almost all floral organs and leaf area are larger than those of *P. sundaica*. However, width of calyx shows almost the same value, and the values of lengh/ width ratio of petal (L/Wp) and hair length on leaf are converse. In summary there are many differences in many morphological characters of both species.

Result of self-incompatibility test is shown in Table 3. Fruit setting ratio was 0% under self-

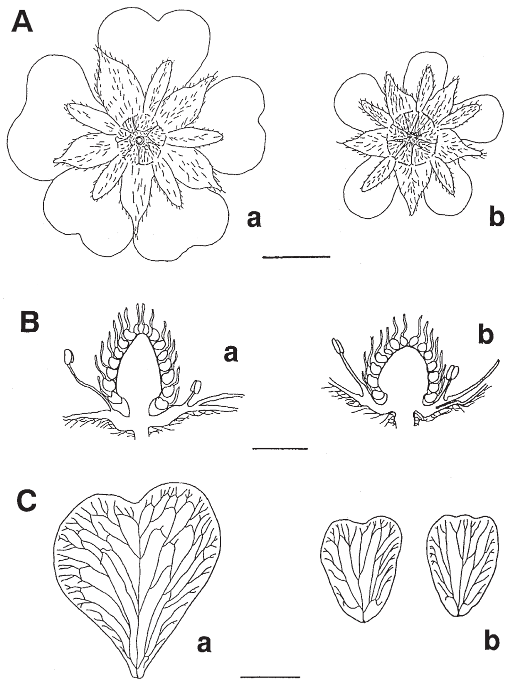

Fig. 1. Comparison of flowers of *Potentilla anemonefolia* (a) and *P. sundaica* (b).
A: Back view of flower (scale bar = 3 mm). B: Longitudinal section of flower (scale bar = 3 mm). C : Petal (scale bar = 2 mm).

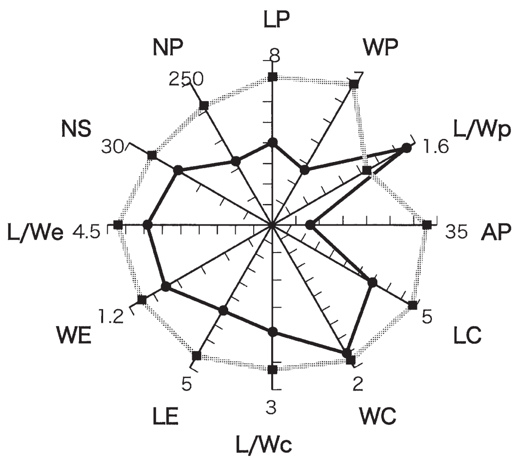

Fig. 2. Radar charts of morphological characters in *Potentilla anemonefolia* (solid square with gray line) and *P. sundaica* (solid circle with black line). LP : Length of petal. WP : Width of petal. L/Wp : Length/Width ratio of petal. AP : Area pf petal. LC : Length of calyx. WC : Width of calyx. L/Wc : Length/Width ratio of calyx. LE : Length of epicalyx. WE : Width of epicalyx. L/We : Length/Width ratio of epicalyx. NS : Number of stamens. NP : Number of pistils.

Table 2. Morphological comparison between *Potentilla anemonefolia* and *P. sundaica*

	Symbol	*P. anemonefolia* Mean±S.D.	Range	(n)	*P. sundaica* Mean±S.D.	Range	(n)
Length of petal (mm)	LP	7.2±1.0	5.6−8.9	(66)	4.0±0.4	3.2−4.8	(68)
Width of petal (mm)	WP	6.9±1.1	5.2−8.6	(66)	2.7±0.4	1.9−3.5	(68)
Lenght/Width rario of petal	L/Wp	1.06±0.06	0.92−1.17	(66)	1.50±0.11	1.30−1.78	(68)
Area of petal (cm^2)	AP	32.8±6.8	22.5−45.1	(66)	8.0±1.8	5.8−13.1	(68)
Lengh of calyx (mm)	LC	4.9±0.7	3.6−6.0	(66)	3.5±0.5	2.6−4.5	(68)
Width of calyx (mm)	WC	1.9±0.2	1.5−2.3	(66)	1.8±0.3	1.3−2.4	(68)
Length/Width ratio of calyx	L/Wc	2.64±0.29	2.15−3.49	(66)	1.95±0.29	1.54−2.87	(68)
Length of epicalyx (mm)	LE	4.6±1.3	2.4−6.9	(66)	3.0±0.6	1.9−4.4	(68)
Width of epicalyx (mm)	WE	1.1±0.3	0.5−1.7	(66)	0.9±0.2	0.6−1.5	(68)
Length/Width ratio of epicalyx	L/We	4.22±0.46	3.07−5.48	(66)	3.42±0.41	2.71−4.73	(68)
Number of stamens per flower	NS	25.3±4.7	20−35	(66)	19.9±0.4	18−21	(68)
Number of pistils per flower	NP	208.6±75.1	100−345	(66)	112.0±18.7	81−144	(68)
Number of achens per flower		86.6±19.2	41−115	(44)	93.9±24.6	42−136	(34)
Dry weight of achenes (mg)		0.077*		(300)	0.092*		(300)
Area of leaf (cm^2)		25.6±9.4	9.1−43.7	(22)	10.8±2.1	9.6−15.7	(28)
Hair length on leaf		1.2±0.2	1.0−2.0	(39)	2.1±0.3	1.4−2.8	(39)

* From total weight.

pollination and 90% under cross-pollination in *P. anemonefolia*, while 92.5% under self-pollination and 75% under cross-pollination in *P. sundaica*. This makes it evident that *P. anemonefolia* is self-incompatible and *P. sundaica* is self-compatible.

Table 3. Self-incompatibility of *Potentilla anemonefolia* and *P. sundaica*

	P. anemonefolia	*P. sundaica*
Self-pollination		
Number of flowers	13	40
Number of fruits	0	37
Fruit setting ratio (%)	0	92.5
Cross-pollination		
Number of flowers	11	24
Number of fruits	10	18
Fruit setting ratio (%)	90.0	75.0

The result of the fertility test in the two species is shown in Table 4. Fertility of pistils in *P. sundaica* is 84.1% in average and in uniformity. On the contrary, fertility of pistils in *P. anemonefolia* is lower than those of *P. sundaica*. Furthermore fertility in early blooming flower by anthesis order is less than in later blooming flower. This might depend on the effect of self-incompatibility of the plant, because of less chance of outbreeding.

Phenology

The two species are very similar because both have a rosette of evergreen leaves in winter, ascending flowering stems in spring, sprouting roots from node of flowering stem and they are without stolons in comparison to other Japanese *Potentilla* (Sato and Naruhashi 1978). The

Table 4. Fertility of pistils in *Potentilla anemonefolia* and *P. sundaica*

Anthesis order*	*P. anemonefolia* Fruits/Flower Mean±S.D.	Pistils/Flower Mean±S.D.	Fruits/Pistils ratio Mean	*P. sundaica* Fruits/Flower Mean±S.D.	Pistils/Flower Mean±S.D.	Fruits/Pistils ratio Mean
1	94.1±22.9	287.4±35.3	32.7	109.7±23.5	127.3±15.7	86.2
2	96.3±17.2	252.9±28.0	38.1	96.0±26.3	113.3±20.9	84.7
3	87.4±15.6	161.8±6.1	54.0	85.2±19.6	115.1±8.5	74.0
4	79.8±14.4	118.1±10.5	67.6	83.4±22.2	91.3±4.3	91.3
Mean			48.1			84.1

* Anthesis order is according to number of flower in the sequence of blooming flowers in an inflorescence.

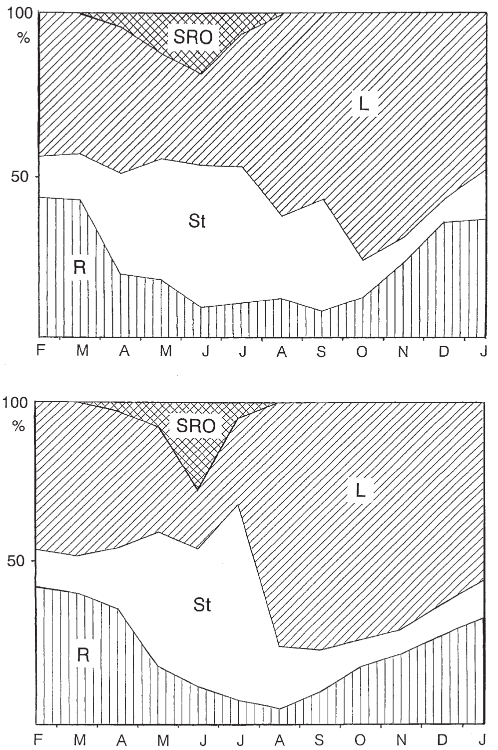

Fig. 3. Proportional distributions of dry matter into various organs in *Potentilla anemonefolia* (upper) and *P. sundaica* (lower). SRO : Sexual reproductive organ (containing peduncle, bud, flower and fruit). L : Leaf. St : Stem. R : Root. Horizontal axis : Month.

plants initiate to expand the stem and leaves in early April and flowers are borne on the apex of stem in middle to late of May. Stretching of flowering stems in *P. sundaica* stops about 10 days earlier than *P. anemonefolia*. And the average length in *P. sundaica* is about 20 cm shorter than in *P. anemonefolia*. The fruits mature in middle of June, but some remain immature until July. Usually from nodes of basal part of inflorescence of *P. anemonefolia* adventitious roots occur, but in *P. sundaica* rooting is rare. In August leaves on flowering stem without rooting from node decay and fall off. New radical leaves occur from September and some of them keep until next spring.

Dry matter allocation

Seasonal changes in the partitioning of dry matter into various component organs of two *Potentilla* species are illustrated in Fig. 3.

The seasonal changes in the ratio of dry weight of the root to total individual biomass in two species are similar, i.e., increasing from October and the maximum in February. The minimum ratio (8.7% in *P. anemonefolia* and 7.5% in *P. sundaica*) is from June to September. The seasonal changes in the ratio of dry weight of the stem to total individual biomass in two species are nearly similar, i.e., increasing from April and the maximum ratio (46.2% in *P. anemonefolia* and 62.4% in *P. sundaica*) in June or July and decreasing from August. The minimum ratio (9.4% in *P. anemonefolia* and 8.2% in *P. sundaica*) is in October to December. Different point is that *P. sundaica* showed rapid increase from April, higher ratio (60.3%) in July, rapid decrease between July and August, and gradual decrease from August to November while *P. anemonefolia* showed the maximum ratio (44.0%) in June, decrease between July and August, and again increase in September (34.1%). The seasonal changes in the ratio of dry weight of the leaf to total individual biomass in the two species are also similar, i.e., decreasing from April, the minimum ratio in June, increasing between July and October, and the maximum ratio 75.7% in October in *P. anemonefolia* and 77.4–70.9% in September to November in *P. sundaica*. The ratio of dry weight of the sexual reproductive organs which contain peduncle, bud, flower and fruit, shows 4.2% (April), 12.1% (May), 18.3% (June) and 6.0% (July) in *P. anemonefolia*, while 3.0% (April), 7.7% (May), 28.0% (June) and 5.2% (July) in *P. sundaica*. The maximum ratio in both species is in June.

Karyotypes and meiotic chromosome behaviors

The karyotype and meiotic chromosome behavior of each in *P. anemonefolia, P. sundaica* and their artificial F_1 hybrid plants were as follows :

Potentilla anemonefolia (2n=14, Fig. 4 A and D)

The 14 chromosomes at somatic metaphase ranged from 1.1–1.6 μm in length and 1.0–2.3 in arm ratio (Table 5). They were divided into two groups : three metacentric chromosome pairs, and four submetacentric chromosome pairs. In the seven chromosome pairs, the longest one submetacentric pair had a satellite on the short

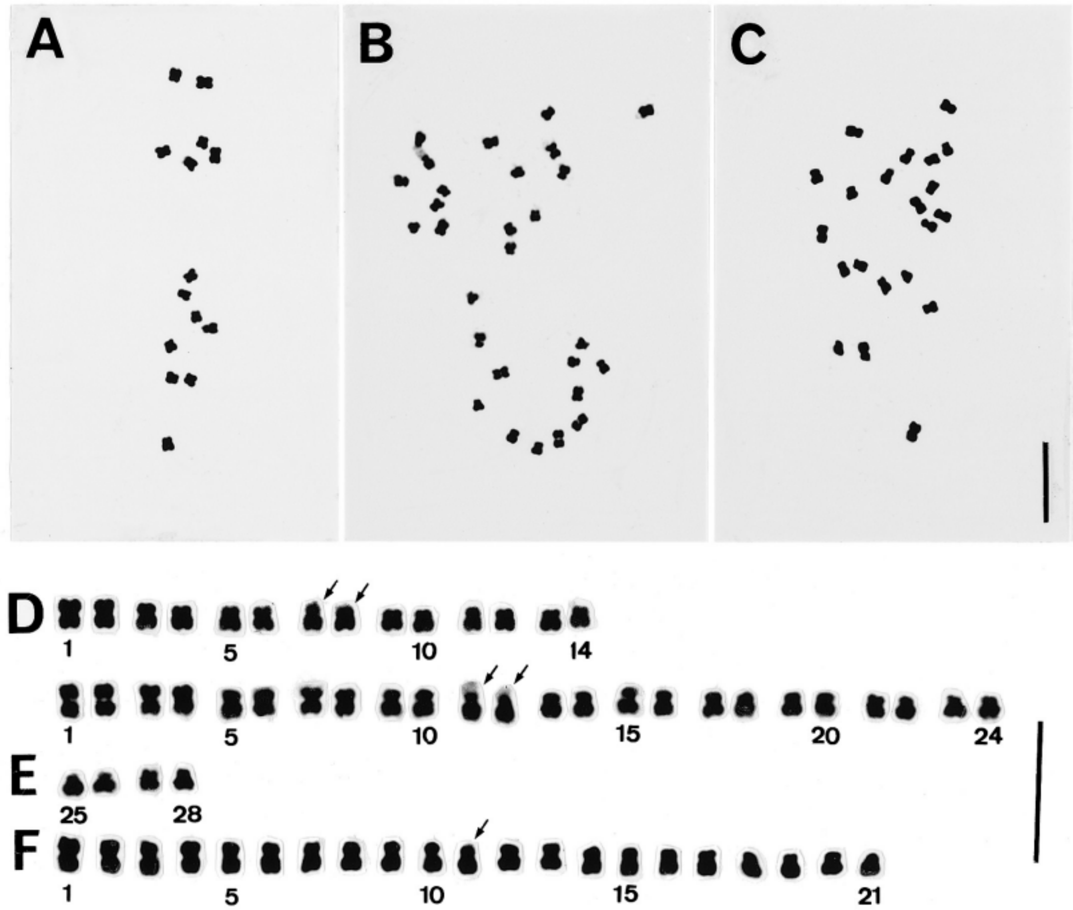

Fig. 4. Somatic metaphase chromosomes (A, B, C) and karyotypes (D, E, F) of *Potentilla anemonefolia, P. sundaica* and the F_1 hybrid. A and D : *P. anemonefolia*. B and E : *P. sundaica*. C and F : F_1 hybrid. Arrows indicate satellite chromosomes. Bars represent 7 μm.

arm. The somatic chromosome complement was formulated as 2n=14=6 m+6 sm+2 ^{t}sm. Meiotic chromosomes were examined in the 431 PMCs at first metaphase. Most of them (96.5%) showed seven bivalents (Fig. 5 A, Table 8).

Potentilla sundaica (2n=28, Fig. 4 B and E)

The 28 chromosomes at somatic metaphase ranged from 0.9–1.7 μm in length and 1.0–2.3 in arm ratio (Table 6). They were classified into two groups : eight metacentric chromosome pairs and six submetacentric chromosome pairs. In the six submetacentric pairs, the longest one pair had a satellite on the short arm. The somatic chromosome complement of this plant was formulated as 2n=28=16 m+10 sm+2 ^{t}sm. Meiotic chromosomes were examined in the first metaphase of 202 PMCs. Most of them (95%) showed 14 bivalents (Fig. 5 B, Table 8).

F_1 hybrids of *P. sundaica* (♀) × *P. anemonefolia* (♂) (2n=21, Fig. 4 C, D, E and F)

The F_1 hybrid plants were triploids with 2n=21 chromosomes, as expected. The 21 chromosomes at somatic metaphase ranged from 0.9–1.8 μm in length and 1.0–2.8 in arm ratio (Table 7). They were divided into two groups : 13 metacentric chromosomes and eight submetacentric chromosomes. The longest one submetacentric chromosome had a satellite on the short arm. The somatic chromosome complement was thus formulated as 2n=21=13 m+7 sm+1 ^{t}sm. Chromosome pairing was examined in 703 PMCs. They had

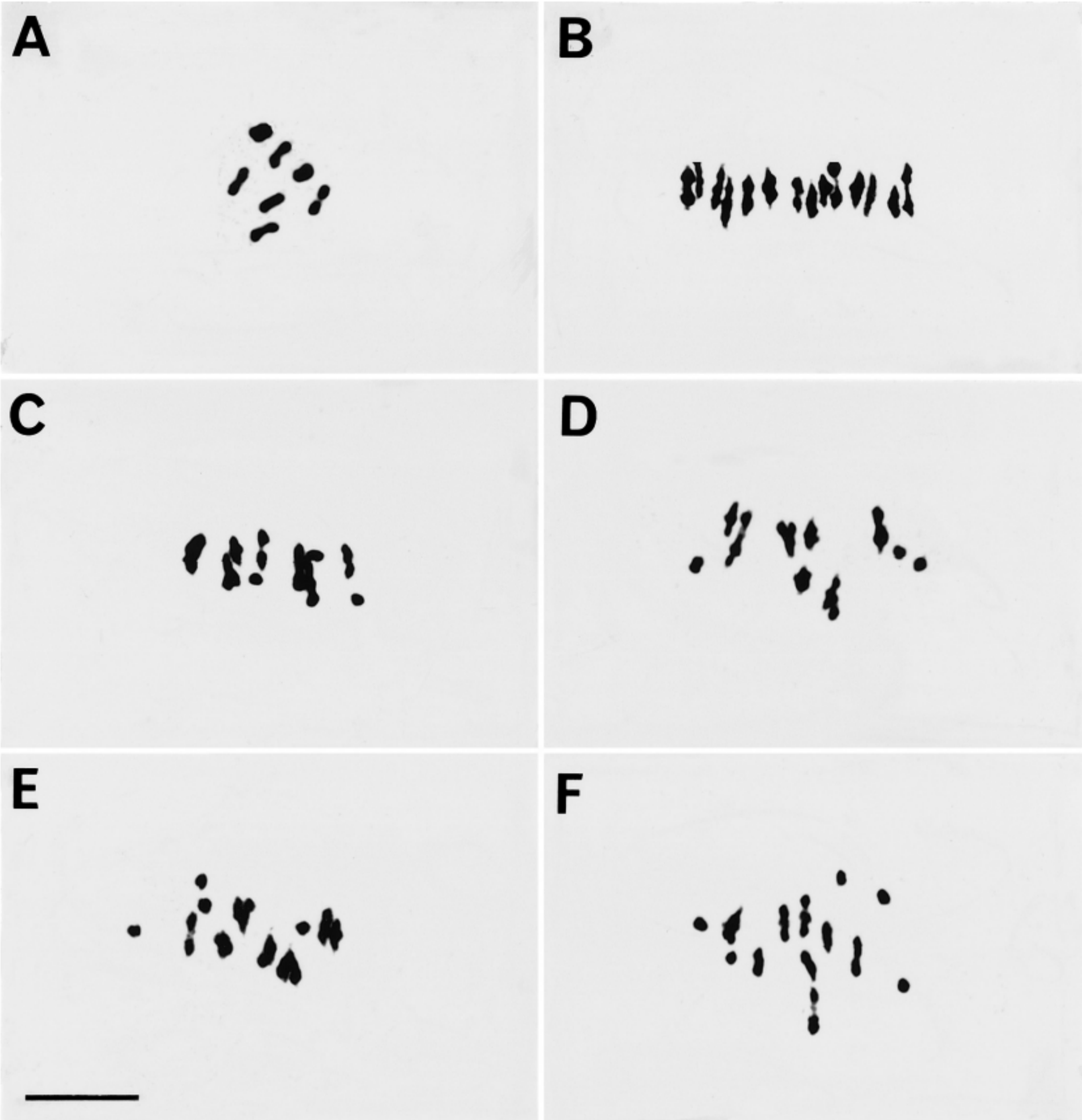

Fig. 5. Chromosome pairing at first metaphase in PMCs of *Potentilla anemonefolia, P. sundaica* and the F_1 hybrid. A : 7Ⅱ in *P. anemonefolia*. B : 14Ⅱ in *P. sundaica*. C : 5Ⅲ+2Ⅱ+2Ⅰ, D : 4Ⅲ+3Ⅱ+3Ⅰ, E : 3Ⅲ+4Ⅱ+4Ⅰ, and F : 2Ⅲ+5Ⅱ+5Ⅰ in F_1 hybrid. Bar represents 10 μm.

various numbers of univalents, bivalents and trivalents ranging from 1 to 7, 1 to 7 and 1 to 6, respectively. The most frequent form of chromosome association was 3Ⅲ+4Ⅱ+4Ⅰ (27.9%), followed by 4Ⅲ+3Ⅱ+3Ⅰ (25.0%), 5Ⅲ+2Ⅱ+2Ⅰ (16.1%), 2Ⅲ+5Ⅱ+5Ⅰ (13.4%), 6Ⅲ+1Ⅱ+1Ⅰ (9.2%), 1Ⅲ+6Ⅱ+6Ⅰ (6.1%), and 7Ⅱ+7Ⅰ (2.3%) (Fig. 5 C, D, E and F, Table 8). The mean number of associations per cell was 3.52Ⅲ+3.34Ⅱ+3.34Ⅰ. The F_1 hybrids had undeveloped and abortive pollen grains.

In somatic cells, the F_1 hybrids had one satellite chromosome, although each of the parent plants had one pair of satellite chromosomes. The absence of secondary constriction in a satellite chromosome, suggesting the suppression of its nucleous organizer activity, was already known for natural hybrids in *Potentilla* (Iwatsubo and Naruhashi 1992 a, b). This phenomenon, termed as differential amphiplasty (cf.

Table 5. Measurements at somatic metaphase chromosomes of *Potentilla anemonefolia*

No.	Length（μm）	Total（μm）	Arm ratio	Form
1	0.8+0.8	1.6	1.0	M
2	0.8+0.8	1.6	1.0	M
3	0.6+0.8	1.4	1.3	m
4	0.6+0.8	1.4	1.3	m
5	0.5+0.8	1.3	1.6	m
6	0.5+0.8	1.3	1.6	m
7	t−0.4+0.9	1.3	2.3	sm
8	t−0.4+0.9	1.3	2.3	sm
9	0.4+0.8	1.2	2.0	sm
10	0.4+0.8	1.2	2.0	sm
11	0.4+0.8	1.2	2.0	sm
12	0.4+0.7	1.1	1.8	sm
13	0.4+0.7	1.1	1.8	sm
14	0.4+0.7	1.1	1.8	sm

t： satellite.

Table 6. Measurements at somatic metaphase chromosomes of *Potentilla sundaica*

No.	Length（μm）	Total（μm）	Arm ratio	Form
1	0.8+0.9	1.7	1.1	m
2	0.8+0.8	1.6	1.0	M
3	0.8+0.8	1.6	1.0	M
4	0.8+0.8	1.6	1.0	M
5	0.7+0.8	1.5	1.1	m
6	0.7+0.8	1.5	1.1	m
7	0.7+0.8	1.5	1.1	m
8	0.7+0.8	1.5	1.1	m
9	0.6+0.8	1.4	1.3	m
10	0.5+0.8	1.3	1.6	m
11	t−0.4+0.9	1.3	2.3	sm
12	t−0.4+0.9	1.3	2.3	sm
13	0.5+0.7	1.2	1.4	m
14	0.5+0.7	1.2	1.4	m
15	0.5+0.7	1.2	1.4	m
16	0.5+0.7	1.2	1.4	m
17	0.5+0.7	1.2	1.4	m
18	0.5+0.7	1.2	1.4	m
19	0.4+0.7	1.1	1.8	sm
20	0.4+0.7	1.1	1.8	sm
21	0.3+0.7	1.0	2.3	sm
22	0.3+0.7	1.0	2.3	sm
23	0.3+0.6	0.9	2.0	sm
24	0.3+0.6	0.9	2.0	sm
25	0.3+0.6	0.9	2.0	sm
26	0.3+0.6	0.9	2.0	sm
27	0.3+0.6	0.9	2.0	sm
28	0.3+0.6	0.9	2.0	sm

t： satellite.

Table 7. Measurements at somatic metaphase chromosomes of the F_1 hybrid

Chromosome pair	Length (μm)	Total (μm)	Arm ratio	Form
1	0.9+0.9	1.8	1.0	M
2	0.9+0.9	1.8	1.0	M
3	0.8+1.0	1.8	1.3	m
4	0.8+0.9	1.7	1.1	m
5	0.7+0.9	1.6	1.3	m
6	0.7+0.9	1.6	1.3	m
7	0.7+0.9	1.6	1.3	m
8	0.7+0.8	1.5	1.1	m
9	0.6+0.9	1.5	1.5	m
10	0.6+0.9	1.5	1.5	m
11	t−0.4+1.1	1.5	2.8	sm
12	0.6+0.8	1.4	1.3	m
13	0.6+0.8	1.4	1.3	m
14	0.6+0.8	1.4	1.3	m
15	0.5+0.9	1.4	1.8	sm
16	0.4+0.8	1.2	2.0	sm
17	0.4+0.8	1.2	2.0	sm
18	0.3+0.7	1.0	2.3	sm
19	0.3+0.7	1.0	2.3	sm
20	0.3+0.6	0.9	2.0	sm
21	0.3+0.6	0.9	2.0	sm

t: satellite.

Table 8. Chromosome associations at the first metaphase in pollen mother cells of *Potentilla anemonefolia*, *P. sundaica* and their F_1 hybrid

Taxa / configurations	No. of PMCs				Frequency (%)
P. anemonefolia					
7Ⅱ		416			96.5
6Ⅱ+2Ⅰ		15			3.5
P. sundaica					
14Ⅱ		192			95.0
13Ⅱ+2Ⅰ		8			4.0
12Ⅱ+4Ⅰ		2			1.0
F_1-hybrid					
	①*	②*	③*	total	
6Ⅲ+1Ⅱ+1Ⅰ	16	12	37	65	9.2
5Ⅲ+2Ⅱ+2Ⅰ	23	23	67	113	16.1
4Ⅲ+3Ⅱ+3Ⅰ	41	37	98	176	25.0
3Ⅲ+4Ⅱ+4Ⅰ	58	26	112	196	27.9
2Ⅲ+5Ⅱ+5Ⅰ	18	14	62	94	13.4
1Ⅲ+6Ⅱ+6Ⅰ	10	3	30	43	6.1
7Ⅱ+7Ⅰ	3	−	13	16	2.3

*: Three individuals of the F_1 hybrid.

Navashin 1934), is found in the present F_1 hybrid plants.

All of the somatic chromosome complements of 2x *P. anemonefolia*, 4x *P. sundaica* and F_1 hybrid plants were composed of metacentric chromosomes and submetacentric chromosomes. Their karyotypic features are similar to one another, however, the karyotypic formula represented by 2n=28=18 m+8 sm+2 ^{t}sm of 4 x *P. sundaica* suggests that the 4 x plant was not produced by a simple autopolyploidization of 2 x *P. anemonefolia* with 2n=14=6 m+6 sm+2 ^{t}sm. The chromosome associations found in the F_1 hybrids suggest that the hybrid plants have either slightly differentiated three chromosome sets designated such as $A^1A^2A^3$ or two similar chromosome sets and one slightly differentiated chromosome set designated such as $A^1A^1A^2$. Along with the meiotic chromosome associations found in the F_1 hybrid plants, their karyotypic features suggest that 4 x *P. sundaica* is allopolyploid plant composed of slightly differentiated two chromosome sets.

Discussion

Many taxonomists have recognized the difference between *P. anemonefolia* and *P. sundaica*. Both species are distinguished into variety or subspecies rank by Franchet and Savatier (1873), Wolf (1908), Nakai (1916), Murata (1961, 1965) and Kitagawa (1979). On the contrary, Lehmann (1856), Handel-Mazzetti (1933) recognized them as different species.

According to our observations of the two plants, remarkable differences in their morphology and life cycle are existing. Some differences in the geographical distribution of both plants are also recognized, i.e., *P. anemonefolia* in China (N. W. China), Korea and Japan, *P. sundaica* in Himalaya to S. E. Asia including S. W. China. Chromosome numbers of both plants show polyploid relationship. Furthermore between two plants there are little differences in genome components depending on cytogenetical analysis.

Potentilla anemonefolia is self-incompatible and shows vegetative reproduction by rooting from nodes of flowering stem, while *P. sundaica* is self-compatible and is considerable reproductive in seed production representing high amount of sexual reproductive effort without remarkable vegetative reproduction.

Judging from the investigation of artificial hybid, tetraploid *P. sundaica* is not merely polyploidization of diploid *P. anemonefolia*, but *P. sundaica* is allotetraploid because of having slightly differentiated two chromosome sets.

On the basis of these results, the two plants can be regarded as different species. Therefore Japanese plant is identified as *P. anemonefolia*, while Himalayan plant is *P. sundaica* with synonym of *P. kleiniana*.

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鳴橋直弘[1]・西川孝行[1,2]・岩坪美兼[1]：日本のオヘビイチゴ（*Potentilla anemonefolia*）とヒマラヤのオヘビイチゴ（*P. sundaica*）の分類学的関係

日本のバラ科キジムシロ属オヘビイチゴとヒマラヤのオヘビイチゴが違うことは，これまで多くの研究者（Lehmann 1856； Wolf 1908； Handel–Mazzetti 1933；Murata 1961；Kitagawa 1980）が気付いている。しかし，それら２分類群の差の認識は研究者によって異なり，その結果は Table 1 に示したように変種，亜種，別種として表現されている。今回これらの２分類群を形態，生活，繁殖様式，染色体の比較，および両種の雑種の作出とゲノム分析をすることにより，総合的に両種は別種であるとの結論を得た。その理由は，形態，生活，繁殖様式，染色体数，核型に差があり，両種のゲノムに分化が起こっているからである。

それら２種の学名として，日本のオヘビイチゴは日本産の植物で記載された *Potentilla anemonefolia* Lehm. が正名と考えられ，ヒマラヤのオヘビイチゴはインドネシアから記載された *P. sundaica* (Blume) Kuntze が正名と考えられる。インドから記載された *P. kleiniana* Wight et Arn. は *P. sundaica* の異名と考えられる。

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