Cytological studies on two varieties of *Ranunculus silerifolius* Lév. in Japan

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ABSTRACT

Ranunculus silerifolius var. *silerifolius* and var. *glaber* were cytologically examined. The karyotypes observed in var. *silerifolius* were classified into four different types, Matsuyama, Mugi, Otaru and Karatsu, as found in var. *glaber*. The F_1 plants between var. *silerifolius* and var. *glaber*, both of which have the same karyotype, showed the *silerifolius*-type in external morphology and their meioses were normal. The features of F_2 's were variable as seen in the field. Hence, it may be concluded that the external morphological variation occurred independently of the chromosomal variation, which played an prominent role in the intraspecific differentiation of *R. silerifolius*.

1. INTRODUCTION

It is well known that *Ranunculus silerifolius* Lév., which is abundantly seen as a common weed in paddyfields in Japan, is variable in external morphology. This taxon is sometimes classified into two different varieties (Ohwi 1975), var. *silerifolius* (Yamakitsunenobotan in Japanese name) and var. *glaber* H. Boiss. (Kitsunenobotan in Japanese). The former, var. *silerifolius*, is easily distinguishable in possessing thickly plagiotropic hairs on its stems and petioles from the latter, var. *glaber*, having no hair or a few (Fig. 1). In the field, however, the plants with intermediate features in various degrees are found. In addition *R. silerifolius* is also variable in karyotype (Fujishima and Kurita 1974; Okada and Tamura 1977). Hence, it follows that to make clear cytologically the intraspecific structure of *R. silerifolius* is one of the best methods of clarifying the courses in speciation of *R. silerifolius* and its allied species. The present study was carried out for this purpose.

2. MATERIALS AND METHODS

As shown in Table 1, 179 individuals of the typical var. *silerifolius* and 103 of the typical var. *glaber* were collected from 26 different localities for the identification of the karyotype. Crosses were made reciprocally between the plants from two varieties with the same karyotype.

Fig. 1. External morphology of the stems of *Ranunculus silerifolius* var. *silerifolius* (A), var. *glaber* (C) and their F_1 (B).

The karyotypic analysis was made with cytological preparations by the technique as described previously (Fujishima 1980). The position of the centromere was expressed by the value of the arm ratio according to Levan *et al.* (1964). Meiotic chromosomes were observed in PMCs. Anthers were fixed in 3:1 mixture of absolute ethanol and acetic acid for 12–24 hr, and successively were squashed in 0.1% acetic orcein after being stained with Feulgen's method.

3. RESULTS

1. Karyotypes of *Ranunculus silerifolius* var. *silerifolius* and var. *glaber*

(1) Karyotypes of var. *silerifolius*

As shown in Table 1, four different karyotypes were distinguished.

Matsuyama-type: The 16 somatic chromosomes of an individual collected from Mt. Ishizuchi are arranged in the order of chromosome lengths in Fig. 2A. Out of those chromosomes, both of the largest and the smallest pairs were submetacentric and the second one metacentric. The six chromosomes from the third to the fifth pair were submetacentric or metacentric, and the seventh one subterminal. The sixth pair was terminal and possessed a secondary constriction within the proximal region of their short arm. From these facts, this karyotype was identified as the Matsuyama-type which was defined by Fujishima and Kurita (1974).

Mugi-type: Out of the 16 chromosomes of an individual from Kofu, the second largest and the smallest pairs were subterminal, and the second smallest pair submedian (Fig. 2B). Hence, this karyotype was identified as the Mugi-type.

Otaru-type: Fig. 2C shows the karyotype of an individual from Mochigase. Out of the 16 chromosomes, the second largest pair was subterminal,

Table 1. *Localities and karyotypes of Ranunculus silerifolius var. silerifolius and var. glaber*

Variety	Locality	No. of individuals	Karyotype	Habitat
<i>var. silerifolius</i>				
	*Yaku T., Yakushima Isl., Kagoshima Pref.	19	K	paddyfield at the foot of mountains
	Toyotama T., Tsushima Isl., Nagasaki Pref.	13	K	ditch at the montane area
	Kake T., Hiroshima Pref.	8	K	moist pathside at the montane area
		6	O	
	Nakayama T., Tottori Pref.	11	K	paddyfield on a hill
	Komenono, Matsuyama C., Ehime Pref.	10	Ma	moist pathside at the montane area
	Misaka Pass, Kuma T., Ehime Pref.	15	Ma	moist grassy field on upland (ca. 760m alt.)
	Mt. Ishizuchi, Ehime Pref.	24	Ma	moist pathside abutting on the forest (ca. 1980 m alt.)
	Mt. Mitokusan, Tottori Pref.	5	Mu	moist grassy field (ca. 700m alt.)
	*Saji V., Tottori Pref.	8	Mu	paddyfield at the montane area
	*Mochigase T., Tottori Pref.	2	Mu	paddyfield at the montane area
		3	O	
	Kokufu T., Tottori Pref.	10	Mu	moist paddyfield at the montane area
	Mt. Daisen, Tottori Pref.	4	Mu	moist pathside abutting on the forest (ca. 890 m alt.)
		6	O	
	*Aoya T., Tottori Pref.	10	O	paddyfield on a hill
	Misasa T., Tottori Pref.	10	Mu	paddyfield at the montane area
		1	O	
	Hatano T., Sadogashima Isl., Niigata Pref.	2	O	paddyfield
	Noheji T., Aomori Pref.	10	O	moist pathside along a river at the montane area
	Towada C., Aomori Pref.	2	O	paddyfield

Table 1. (Continued)

Variety	Locality	No. of individuals	Karyotype	Habitat
<i>var. glaber</i>				
	Kunigami V., Okinawa Pref.	5	K	paddyfield at the seaside
	*Yaku T., Yakushima Isl., Kagoshima Pref.	2	K	paddyfield at the foot of mountains
	Kagoshima C., Kagoshima Pref.	10	K	paddyfield at lowland
	Kurashiki C., Okayama Pref.	5	K	paddyfield at lowland
	Nawa T., Tottori Pref.	9	K	paddyfield at the seaside
	Matsuyama C., Ehime Pref.	10	Ma	paddyfield at lowland
	Imabari C., Ehime Pref.	10	Ma	paddyfield at lowland
	Toyonaka T., Kagawa Pref.	10	Mu	paddyfield at lowland
	*Saji V., Tottori Pref.	8	Mu	paddyfield at the montane area
	*Mochigase T., Tottori Pref.	7 2	Mu O	paddyfield at the montane area
	*Aoya T., Tottori Pref.	5	O	paddyfield on a hill
	Kahoku T., Kochi Pref.	10	O	paddyfield at the montane area
	Hayama V., Kochi Pref.	10	O	paddyfield at the montane area

*, showing to grow with the other taxon. Ma, Matsuyama-type. Mu, Mugi-type. O, Otaru-type. K, Karatsu-type.

Fig. 2. Mitotic metaphase chromosomes, $2n=16$. A-D: *Ranunculus silerifolius* var. *glaber*. E-H: var. *glaber*. A and E: Matsuyama-type. B and F: Mugi-type. C and G: Otaru-type. D and H: Karatsu-type. Bar= $5\mu\text{m}$.

and the sixth pair, which carried a secondary constriction within the distal region of the short arm, was terminal. Thus the karyotype was identified as the Otaru-type.

Karatsu-type: Fig. 2D shows the karyotype of an individual from Yakushima. This karyotype was identified as the Karatsu-type from the facts that the subterminal pair possessed a secondary constriction within the proximal region of the short arm, and that the second smallest pair and two large pairs

Table 2. *Characteristics of Ranunculus silerifolius* var. *silerifolius* collected from Mt. Ishizuchi, of var. *glaber* from Matsuyama and of their F_1 's

Character	var. <i>silerifolius</i>	F_1	var. <i>glaber</i>
Stem length (cm)	23.3 $\pm$ 4.23*	15.6 $\pm$ 1.26	45.0 $\pm$ 6.33
Shape of leaf	ovate	ovate	oval
Number of plagiotropic hairs	30.4 $\pm$ 3.5	23.8 $\pm$ 4.1	4.3 $\pm$ 1.4
Diameter of flower (cm)	1.0 $\pm$ 0.05	1.2 $\pm$ 0.04	1.7 $\pm$ 0.05
Number of achenes	32.0 $\pm$ 1.5	40.3 $\pm$ 1.8	71.2 $\pm$ 3.2
Shape of acinum	globose	globose	globose
Seed fertility (%)	94.9 $\pm$ 1.3	92.5 $\pm$ 2.6	98.7 $\pm$ 0.7
Flowering season	late June to early Aug.	late June to early Aug.	Middle April to June

* standard error

Fig. 3. External morphology of the parental plants and their F_1 with the Matsuyama-karyotype. A: *Ranunculus silerifolius* var. *silerifolius* collected from Mt. Ishizuchi. B: the F_1 . C: var. *glaber* from Matsuyama. Scale=10 cm.

were metacentric.

Out of the examined 179 individuals of *R. silerifolius* var. *silerifolius*, 49 showed the Matsuyama-type, 39 the Mugi-type, 40 the Otaru-type and 51 the Karatsu-type.

(2) Karyotypes of var. *glaber*

As shown in the karyotypes of var. *silerifolius*, the karyotypes of the 103 individuals of *R. silerifolius* var. *glaber* also could be classified into four different types; Matsuyama, Mugi, Otaru and Karatsu. These results are shown in Table 1 and in Fig. 2E, F, G and H.

2. Artificial hybrids between var. *silerifolius* and var. *glaber*

The F_1 plants were easily produced.

(1) F_1 plants produced from the parents with Matsuyama-karyotype

One of the parents was var. *silerifolius* collected from Mt. Ishizuchi, and

Fig. 4. Chromosomes in the mitotic metaphase (A, C, E, G) and meiotic metaphase I (B, D, F, H) of the F_1 's between *Ranunculus silerifolius* var. *silerifolius* and var. *glaber*. A and B: Matsuyama-type, $2n=16$ and 8_{II} respectively. C and D: Mugi-type, $2n=16$ and 8_{II} . E and F: Otaru-type, $2n=16$ and 8_{II} . G and H: Karatsu-type, $2n=16$ and 8_{II} . Bar= $10\ \mu\text{m}$.

the other var. *glaber* from Matsuyama.

a. Morphology

Table 2 shows main characteristics of the F_1 (Fig. 3B) and the parents (Fig. 3A and C). All the reciprocal F_1 's examined were the var. *silerifolius*-type in many characters.

b. Cytological observations

The karyotypes (Figs. 4A and 5A) of the F_1 's were identified as the Matsuyama-type as found in the parental plants, and any other karyotype was not found. The meiotic divisions (Fig. 4B) of PMCs of the F_1 's were regular and stable, and the chromosome configuration at diakinesis to metaphase I showed patterns similar to those of the parental taxa. However, the chiasma frequencies per PMC of the reciprocal hybrids were lower than those of the parents (Table 3).

The pollen fertility and the frequency of normal achenes were 98.5% and 92.9% in average, respectively.

Fig. 5. Mitotic metaphase chromosomes of the F_1 's, $2n=16$. A: Matsuyama-type. B: Mugi-type. C: Otaru-type. D: Karatsu-type. Bar= $5\mu m$.

Table 3. Number of chiasmata (Xta) at diakinesis in PMCs of the parental taxa, var. *silerifolius* and var. *glaber*, and their F_1 's

Karyotype and Taxon (Locality)	No. of cells	Xta range	Mean of Xta per PMC	Mean of Xta per bivalent
Matsuyama-type				
var. <i>silerifolius</i> (Mt. Ishizuchi)	26	18~26	21.5	2.7
var. <i>silerifolius</i> × var. <i>glaber</i>	13	15~23	17.5	2.2
var. <i>glaber</i> × var. <i>silerifolius</i>	10	15~24	17.9	2.2
var. <i>glaber</i> (Matsuyama C.)	10	20~25	22.7	2.8
Mugi-type				
var. <i>silerifolius</i> (Kofu T.)	10	21~27	23.4	2.9
var. <i>silerifolius</i> × var. <i>glaber</i>	14	14~21	17.8	2.2
var. <i>glaber</i> (Toyonaka T.)	10	18~27	22.5	2.8
Otaru-type				
var. <i>silerifolius</i> (Noheji T.)	10	17~23	19.9	2.5
var. <i>silerifolius</i> × var. <i>glaber</i>	21	16~21	18.4	2.3
var. <i>glaber</i> (Towada C.)	12	16~22	19.6	2.4
Karatsu-type				
var. <i>silerifolius</i> (Nakayama T.)	14	14~20	17.3	2.2
var. <i>silerifolius</i> × var. <i>glaber</i>	10	16~21	17.5	2.2
var. <i>glabdr</i> (Kurashiki C.)	14	16~20	18.2	2.3

(2) F_1 plants produced from the parents with the other karyotypes

The F_1 's between var. *silerifolius* and var. *glaber* were produced from the parents with the Mugi-, Otaru, and Karatsu-karyotypes, respectively. All the F_1 's had the same karyotypes with the respective parents (Fig. 5B, C and D), being similar to var. *silerifolius* in external morphology. The meioses of their

Fig. 6. Number of plagiotropic hairs per 25 mm², at 5 cm above the ground, on the stems of the F_2 's between *Ranunculus silerifolius* var. *silerifolius* (Mugi-type) and var. *glaber* (Mugi-type).

PMCs were regular and stable (Fig. 4D and F and H . and Table 3)

(3) F_2 plants

F_2 generation was produced from the self-pollination of the respective F_1 's with the Matsuyama- or with Mugi-karyotype. The F_2 's obtained were variable in stem length, radical leaf length and number of achenes per syncarp from the *silerifolius*-type to the *glaber*-type, though the transgressive segregation was suggested in a partial character (Fig. 6).

4. DISCUSSION

Kurita (1955) has mentioned that the karyotype of *R. ternatus* var. *glaber* (syn. of *R. silerifolius* var. *glaber*) collected from Matsuyama, Ehime Pref., was exactly similar to that of *R. ternatus* var. *quelpaertensis* (syn. of *R. silerifolius* var. *silerifolius*) from the Onogahara-Heights, Ehime Pref. Subsequently, Okada (1981) has mentioned that the F_1 's between the same cytotypes collected from different localities also showed regular pairings (8_{II}) in MI and high pollen fertilities as well as the parental plants, although he has not stated their external morphology. Then he has made a proposal that there is no reproductive isolation among the plants showing the same karyotype, even in the case that they are isolated geographically mutually.

In the present observations, it was found that the karyotypes of var. *silerifolius* were classified into four different types as those of var. *glaber*,

and that the F_1 's between the two varieties with the same karyotype were regular in meiosis and showed high frequency in number of normal achenes. In addition, the artificial F_2 's showed continuous variation in many characters from the *silerifolius*-type to the *glaber*-type as observed among the individuals in the field. These facts indicate that the external morphological variations in this species occurred independently of the chromosome variations. Thus, it is assumed that there is no or little reproductive isolation between var. *silerifolius* and var. *glaber* with the same karyotype, and that the morphological differences between the two varieties are caused by several genes which do not play a significant role in the introspecific differentiation of *R. silerifolius*. In other words, the morphological classification of the two varieties in *R. silerifolius* is not biologically significant.

On the other hand, the reproductive isolation has been recognized at various levels among the individuals showing different karyotype (Okada 1981), even if in the same variety (Fujishima, unpubl.). Thus, it may be concluded that the chromosomal variations in diploid level play an prominent role in the intraspecific differentiation of *R. silerifolius*, and that the pattern of cytotypical differentiation within this species strongly suggests the catastrophic selection as a factor in speciation (Lewis 1962).

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