Karyotypes of tetraploid Rubus parvifolius and octoploid R. rugosus（Rosaceae）

Journal of Phytogeography and Taxonomy **52** : 185-190, 2004

Yoshikane Iwatsubo and Naohiro Naruhashi : **Karyotypes of tetraploid *Rubus parvifolius* and octoploid *R. rugosus* (Rosaceae)**

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Rubus, a large genus comprised of 250 sexual species and many apomicts (Mabberley 1997), is known to have a polyploid series, based on x=7, from diploid (2n=14) to 14-ploid (2n=98) (Fedorov 1969 ; Nybom 1986). In polyploid plants, karyotype analysis is a useful method, in order to investigate their genomic constitutions.

Rubus parvifolius L. has a polyploid series of diploid (2x) (Jinno 1958 a,b ; Iwatsubo and Naruhashi 1991, 1993, 1996, 1998 ; Naruhashi and Iwatsubo 1993), triploid (3x) (Naruhashi and Iwatsubo 1993 ; Iwatsubo and Naruhashi 1999) and tetraploid (4x) plants (Chen 1993). Diploid *R. parvifolius* is known to have seven bivalents in almost or every PMCs, showing their genomic constitution with two sets of homologous chromosomes (Iwatsubo and Naruhashi 1991, 1998 ; Naruhashi and Iwatsubo 1993). Triploid *R. parvifolius*, reported by Iwatsubo and Naruhashi (1999), was also had three basic sets of homologous chromosomes. The triploid plant seemed to arise in the progeny of diploid *R. parvifolius* by spontaneous origin from the sexual fusion between an unreduced diploid gamete and a reduced haploid gamete.

Two years ago, the authors received seeds of tetraploid *R. parvifolius* from USDA-ARS National Clonal Germplasm Repository, USA (Inventory ID : CRUB 197 : 000, Inventory Name : *R. crataegifolius* 96050). The seeds were collected from peddlers at Songhua Lake, near Harbin in the Yabuli region, China (Thompson et al. 1996). Plants raising from the seeds were estimated tetraploid *R. parvifolius* using nuclear DNA flow cytometry (Meng and Finn 2002). The tetraploid plant is attracting the attention of horticulturists because of its large fruits.

Rubus rugosus Sm. has three cytotypes of diploid (2x) with 2n=14 chromosomes (Malla et al. 1975, as n=7 ; Subramanian 1987), octoploid (8x) with 2n=56 chromosomes (Iwatsubo and Naruhashi 1992c) and quattuordecoploid (14x) with 2n=98 chromosomes (Nybom 1986). Octoploid *R. rugosus* grown from seeds collected from Godawari, Kathmandu Valley, Lalitpur, Nepal are preserved in the experimental garden in the Toyama University.

This study shows the karyotypes of tetraploid *R. parvifolius* and octoploid *R. rugosus*.

Materials and methods

Tetraploid *Rubus parvifolius* and octoploid *R. rugosus* preserved in the experimental garden in the Toyama University were used for the study. The actively growing root tips of the two plants were collected and soaked in 2mM 8-hydroxyquinoline for an hour at 25℃ and subsequently kept at 6℃ for 15 hr. After fixation in a mixture of acetic acid and ethyl alcohol (1 : 3) for 1.5 h, the root tips were soaked in 1 N HCl for a few hours, hydrolyzed in 1 N HCl at 60℃ for 10 min. and immersed in tap water. The meristems of the root tips were stained in a drop of 1.5% lacto-propionic orcein on the slide glass and ordinary squash technique were applied in preparation. Arm ratio of each chromosome was calculated as length of long arm / length of short arm. Chromosome form was expressed utilizing the nomenclature of Levan et al. (1964).

Results and discussion

Tetraploid *Rubus parvifolius*

As shown in Fig. 1, this plant had 2n=28 chromosomes. The length of the metaphase chromosomes had a range of 1.0 μm to 1.8 μm, and the arm ratio varied from 1.0 to 4.5 (Table 1). They were classified into three groups: 17 metacentric chromosomes, 7 submetacentric chromosomes and 4 subtelocentric chromosomes. The two subtelocentric chromosomes had satellites on the short arms.

Diploid *R. parvifolius* is known to have one pair of satellited subtelocentric chromosomes (Iwatsubo and Naruhashi 1991, 1993, 1996), and triploid *R. parvifolius* has three satellited subtelocentric chromosomes (Iwatsubo and Naruhashi 1999). The karyotypes of diploid and triploid *R. parvifolius* show that the one set of chromosomes has one satellited subtelocentric chromosome. However, the tetraploid *R. parvifolius* had one pair satellited subtelocentric chromosomes, instead of four satellited chromosomes expected in its chromosome complement. Chen (1993) also reported karyotype of tetraploid *R. parvifolius*. Judging from the picture in its paper, the karyotype by Chen (1993) is based on prometaphase chromosomes. Chen (1993) did not observe satellites in any chromosomes. The satellites of tetraploid *R. parvifolius* are very small. Thus the tiny satellites at prometaphase may be unclear.

Absence of a secondary constitution of a nucleolus organizer region, a phenomenon termed differential amphiplasty, was first found in hybrid plants in *Crepis* (cf. Navashin 1934). This phenomenon is known in the hybrid plants of Rosaceae as the following genera : *Duchesnea* (Naruhashi and Iwatsubo 1991), *Potentilla* (Iwatsubo and Naruhashi 1992 a, b), *Rosa* (Akasaka et al. 2002) and *Rubus* (Iwatsubo and Naruhashi 1998), suggesting that the activity of the nucleolus organizer region was suppressed in the hybrid condition.

In the tetraploid *R. parvifolius*, the secondary constrictions of two subtelocentric chromosomes were not found, which suggests that the two chromosome sets with disappeared secondary constrictions and the two chromosome sets with secondary constrictions are slightly differentiated each other, or the genomes of this tetaploid plant are structured as a dipliod plant by its chromosomal diploidization. Thus, the plant can be considered as an allotetraploid.

Octoploid *Rubus rugosus*

As shown Fig. 2, this plant had small chromo-

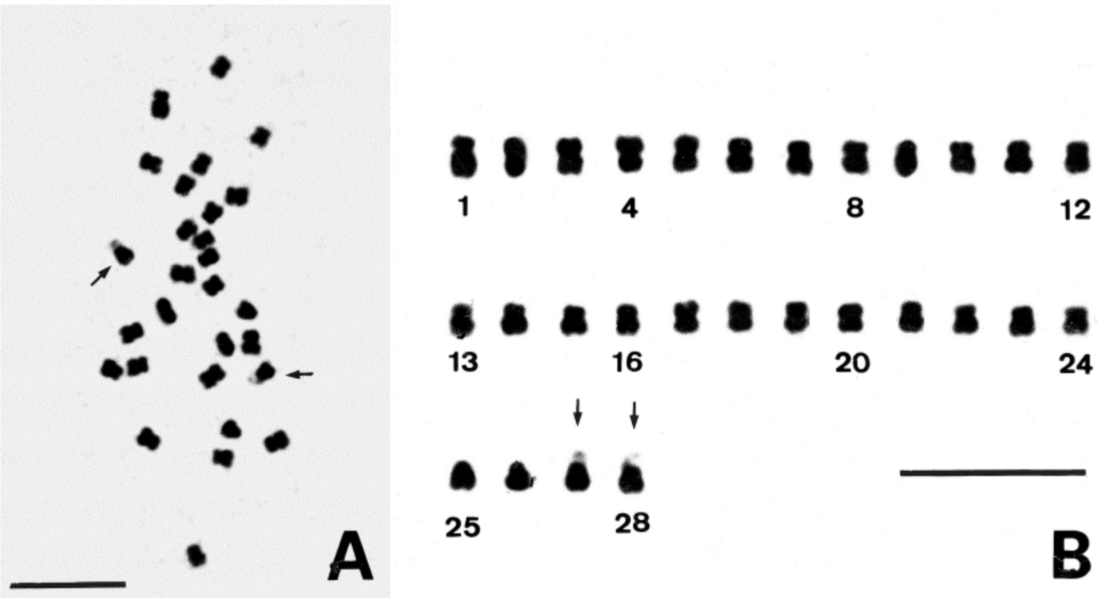

Fig. 1. Somatic metaphase chromosomes (A) and karyotype (B) of tetraploid *Rubus parvifolius*. Arrows indicate satellite chromosomes. Bar = 7 μm.

Table 1. Measurements of somatic metaphase chromosomes in tetraploid *Rubus parvifolius*

No.	Length (μm)	Total (μm)	Arm Ratio	Form
1	0.6 + 1.2	1.8	2.0	sm
2	0.7 + 1.1	1.8	1.6	m
3	0.8 + 0.9	1.7	1.3	m
4	0.8 + 0.8	1.6	1.0	M
5	0.8 + 0.8	1.6	1.0	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 + 0.9	1.5	1.5	m
10	0.6 + 0.9	1.5	1.5	m
11	0.6 + 0.9	1.5	1.5	m
12	0.6 + 0.9	1.5	1.5	m
13	0.5 + 0.9	1.4	1.8	sm
14	0.5 + 0.9	1.4	1.8	sm
15	0.5 + 0.8	1.3	1.6	m
16	0.5 + 0.8	1.3	1.6	m
17	0.6 + 0.6	1.2	1.0	M
18	0.6 + 0.6	1.2	1.0	M
19	0.6 + 0.6	1.2	1.0	M
20	0.6 + 0.6	1.2	1.0	M
21	0.4 + 0.8	1.2	2.0	sm
22	0.4 + 0.8	1.2	2.0	sm
23	0.4 + 0.8	1.2	2.0	sm
24	0.4 + 0.8	1.2	2.0	sm
25	0.2 + 0.9	1.1	4.5	st
26	0.2 + 0.8	1.0	4.0	st
27	t-0.2 + 0.8	1.0	4.0	st
28	t-0.2 + 0.8	1.0	4.0	st

t : satellite.

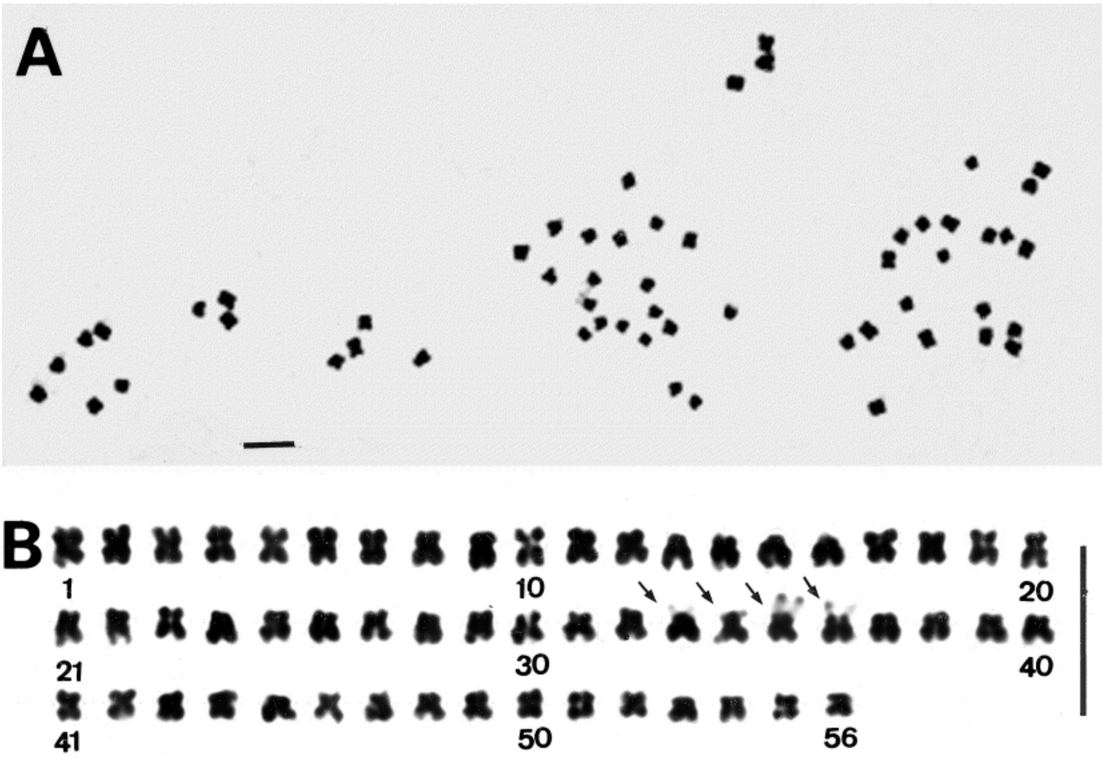

Fig. 2. Somatic metaphase chromosomes (A) and karyotype (B) of octoploid *Rubus rugosus*. Arrows indicate satellite chromosomes. Bar = 7 μm.

somes, ranging in length from 0.8 μm to 1.6 μm, with a range in arm ratio from 1.0 to 3.7 (Table 2). The metaphase chromosomes were divided into three groups ; 28 metacentric chromosomes, 24 submetacentric chromosomes, and 4 subtelocentric chromosomes. Among the submetacentric ones 4 chromosomes had satellites on their short arms. Thus the somatic chromosome complement was formulated as 2n=56= 28m + 20sm + 4^{t}sm + 4st. As a result, the octoploid *R. rugosus* seems to be allooctoploid, because each of the number of three forms in the chromosome complement is

Table 2. Measurements of somatic metaphase chromosomes in octoploid *Rubus rugosus*

No.	Length (μm)	Total (μm)	Arm Ratio	Form
1	0.8 + 0.8	1.6	1.0	M
2	0.7 + 0.9	1.6	1.3	m
3	0.7 + 0.8	1.5	1.1	m
4	0.7 + 0.8	1.5	1.1	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	0.6 + 0.9	1.5	1.5	m
9	0.5 + 1.0	1.5	2.0	sm
10	0.5 + 1.0	1.5	2.0	sm
11	0.6 + 0.8	1.4	1.3	m
12	0.6 + 0.8	1.4	1.3	m
13	0.3 + 1.1	1.4	3.7	st
14	0.3 + 1.1	1.4	3.7	st
15	0.3 + 1.1	1.4	3.7	st
16	0.3 + 1.0	1.3	3.3	st
17	0.6 + 0.7	1.3	1.2	m
18	0.6 + 0.7	1.3	1.2	m
19	0.4 + 0.8	1.2	2.0	sm
20	0.4 + 0.8	1.2	2.0	sm
21	0.4 + 0.9	1.3	2.3	sm
22	0.4 + 0.9	1.3	2.3	sm
23	0.4 + 0.8	1.2	2.0	sm
24	0.4 + 0.8	1.2	2.0	sm
25	0.4 + 0.8	1.2	2.0	sm
26	0.4 + 0.8	1.2	2.0	sm
27	0.4 + 0.8	1.2	2.0	sm
28	0.4 + 0.8	1.2	2.0	sm
29	0.4 + 0.8	1.2	2.0	sm
30	0.4 + 0.8	1.2	2.0	sm
31	0.4 + 0.8	1.2	2.0	sm
32	0.4 + 0.8	1.2	2.0	sm
33	t-0.4 + 0.8	1.2	2.0	sm
34	t-0.4 + 0.8	1.2	2.0	sm
35	t-0.4 + 0.8	1.2	2.0	sm
36	t-0.3 + 0.8	1.1	2.7	sm
37	0.4 + 0.7	1.1	1.8	sm
38	0.4 + 0.7	1.1	1.8	sm
39	0.4 + 0.7	1.1	1.8	sm
40	0.4 + 0.7	1.1	1.8	sm
41	0.5 + 0.5	1.0	1.0	M
42	0.5 + 0.5	1.0	1.0	M
43	0.5 + 0.5	1.0	1.0	M
44	0.5 + 0.5	1.0	1.0	M
45	0.4 + 0.6	1.0	1.5	m
46	0.4 + 0.6	1.0	1.5	m
47	0.4 + 0.6	1.0	1.5	m
48	0.4 + 0.6	1.0	1.5	m
49	0.5 + 0.5	1.0	1.0	M
50	0.5 + 0.5	1.0	1.0	M
51	0.5 + 0.5	1.0	1.0	M
52	0.5 + 0.5	1.0	1.0	M
53	0.4 + 0.6	1.0	1.5	m
54	0.4 + 0.6	1.0	1.5	m
55	0.4 + 0.4	0.8	1.0	M
56	0.4 + 0.4	0.8	1.0	M

t : satellite.

multiples of 4 and not 8. Cytogenetic proof for the genome constitution of octoploid *R. rugosus*, gained from the study of hybrid plant between diploid *R. rugosus* and octoploid *R. rugosus*, is awaited.

We are grateful to Dr. Maxine M. Thompson and USDA-ARS National Clonal Germplasm Repository for providing plant material.

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(Received June 23, 2004; accepted November 5, 2004)

岩坪美兼・鳴橋直弘：四倍体ナワシロイチゴと八倍体キイチゴ *Rubus rugosus* の核型

ナワシロイチゴは，日本では二倍体が一般的であり，まれに三倍体が存在する。しかし，中国からは四倍体のナワシロイチゴが報告されている（Chen 1993）。この四倍体ナワシロイチゴは，果実が大きく，農学的にも注目されている。この度，中国産のナワシロイチゴの種子を得ることができたので，その種子を発芽させて核型の観察を行った。その結果，日本の二倍体ナワシロイチゴでは 2 本，三倍体では 3 本の付随体をもつ染色体が存在するのに対して，中国産の四倍体ナワシロイチゴには付随体をもつ染色体が 2 本しか見られなかった。この観察結果から，この四倍体ナワシロイチゴは，互いに幾分異なったゲノムを二組ずつ持つ異質四倍体であり，一対の仁形成が抑制される differential amphiplasty 現象を生じているか，または細胞学的二倍体化によって，付随体染色体を 1 組だけ持つようになったかのいずれかであろうと推測された。

Rubus rugosus には，二倍体型，八倍体型および十四倍体型が知られている。ネパール産八倍体の核型を分析したところ中期染色体の長さは 0.8-1.6 µm であり，腕比は 1.0-3.7 であった。この植物の体細胞中期の染色体組は，中部動原体型染色体，次中部動原体型染色体，それに次端部動原体型染色体から構成されており，4 本の次中部動原体型染色体の短腕には付随体が観察された。核型式は 2n=56=28m + 20sm + 4^{t}sm + 4st であった。いずれの型の染色体も 4 の倍数からなることから，この植物は異質八倍体であることが示唆された。

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