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Paper for the lecture of the Society's Award 2012 Yoshikane Iwatsubo: *Rumex acetosa* (Polygonaceae) Chromosomal variations occurring in Toyama Prefecture, Central Japan

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Abstract

Following a thorough review of chromosome studies on the number, karyotype, and mitotic and meiotic chromosomal behaviors of *Rumex acetosa*, data collected on *R. acetosa* specimens in Toyama Prefecture, central Japan, are reported in this paper.

A total of 1,640 individual *R. acetosa* specimens were collected from 17 localities in Toyama Prefecture. Nearly all individuals were diploid, with either $2n = 14 = 12+2X$ (1,296 individuals, 79.02%) or $2n = 15 = 12+X+Y_1Y_2$ (321 individuals, 19.57%). Seventeen individuals, constituting 1.04% of all plants examined, were triploid (nine individuals with $2n = 21 = 18+3X$ and eight individuals with $2n = 22 = 18+2X+Y_1Y_2$). Four individuals were tetraploid (two individuals with $2n = 28 = 24+4X$ and two individuals with $2n = 29 = 24+3X+Y_1Y_2$), and two individuals were aneuploid, with chromosome constitutions of $2n = 15 = 13+2X$ and $2n = 16 = 13+2X+Y_2$. Among the nine diploid karyotype forms classified by Ono (1935), AB was the most frequently (29.4%), followed by AD (20.1%), AA (15.7%), BB (11.3%), AC (9.6%), BD (8.6%), CD (2.5%), DD (1.8%), and CC (1.0%). No supernumerary segments (SS) were found on the A1 chromosomes of the individuals identified in the study.

The frequency of SS5 chromosome occurrence in each locality of normal diploid plant A5 chromosomes was between 7.6% and 44.0%, with a mean frequency of 24.5%. These results indicate that SS5 chromosomes are prevalent, and that about one fourth of A5 chromosomes in Toyama Prefecture contain SS5 chromosomes. The frequency of SS6 chromosome occurrence in each locality of normal diploid plant A6 chromosomes was between 23.7% and 64.4%, with a mean frequency of 47.7%. These results indicate that SS6 chromosomes are also prevalent, and that nearly half of *R. acetosa* A6 chromosomes in Toyama Prefecture are composed of SS6 chromosomes.

Key words : chromosome variation, Japan, Polygonaceae, *Rumex acetosa*, sex chromosome, Toyama Prefecture

Introduction

Rumex acetosa L. (Polygonaceae) is a well-known dioecious perennial with sex chromosomes that was originally distributed throughout the temperate zones of the Eurasian continent (Fig. 1), and was subsequently naturalized in the Americas. In Japan, this species occurs on the main islands of Hokkaido, Honshu, Shikoku, and Kyushu, as well as on most of the small islands surrounding these islands. On Hokkaido, the most northern of the four main islands, however, its distribution is limited to the southwestern region.

Most *R. acetosa* individuals are diploid with chromosome complements composed of six pairs of autosomes. Each autosomal set described in

this study is expressed as occurring on chromosomes A1 through A6, and different sex chromosomes are expressed as follows: XX in plants with pistillate flowers (female plants) and $X Y_1 Y_2$ in plants with staminate flowers (male plants) (Kihara and Ono 1923a, 1923b, 1925; Kuroki 1976) (Fig. 2). In addition to ordinary individuals with normal diploid chromosome complements, a small minority of *R. acetosa* individuals in Japan was found to be triploid. Of these triploid individuals, two chromosome forms were observed, and were divided between different sex chromosome constitutions as follows: $2n = 21 = 18+3X$ (Ono and Shimotomai 1928; Ono 1928, 1930, 1932; Kuroki 1976) and $2n = 22 = 18+2X+Y_1Y_2$ (Ono and Shimotomai

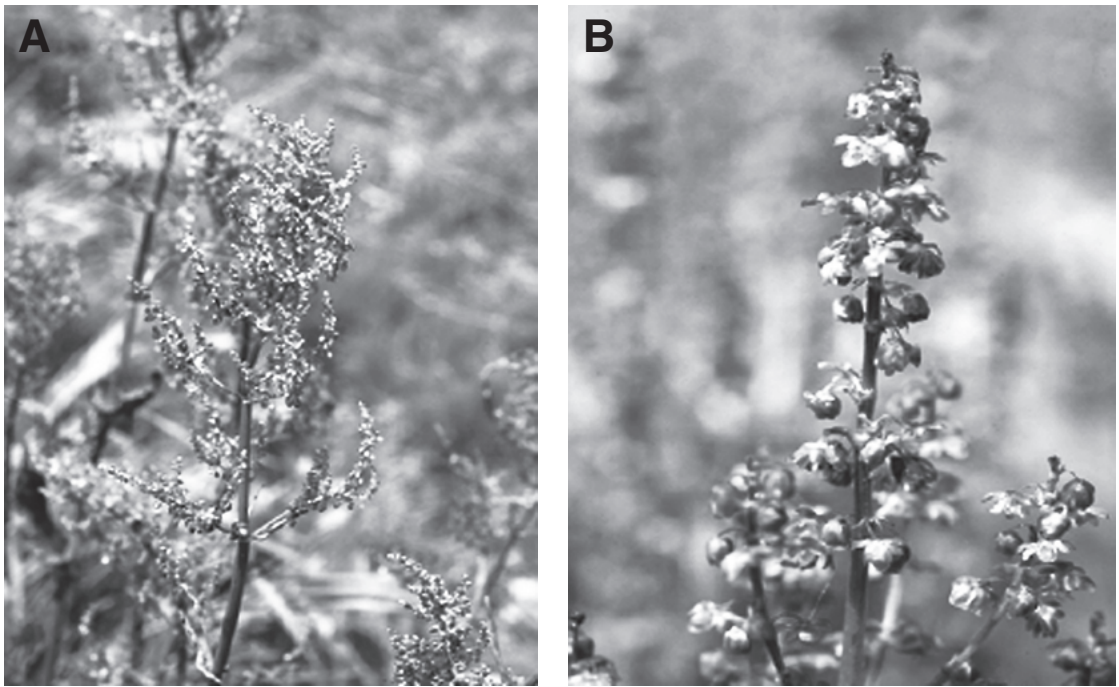


Fig. 1. Inflorescences of *R. acetosa*. A. Female inflorescence. B. Male inflorescence.

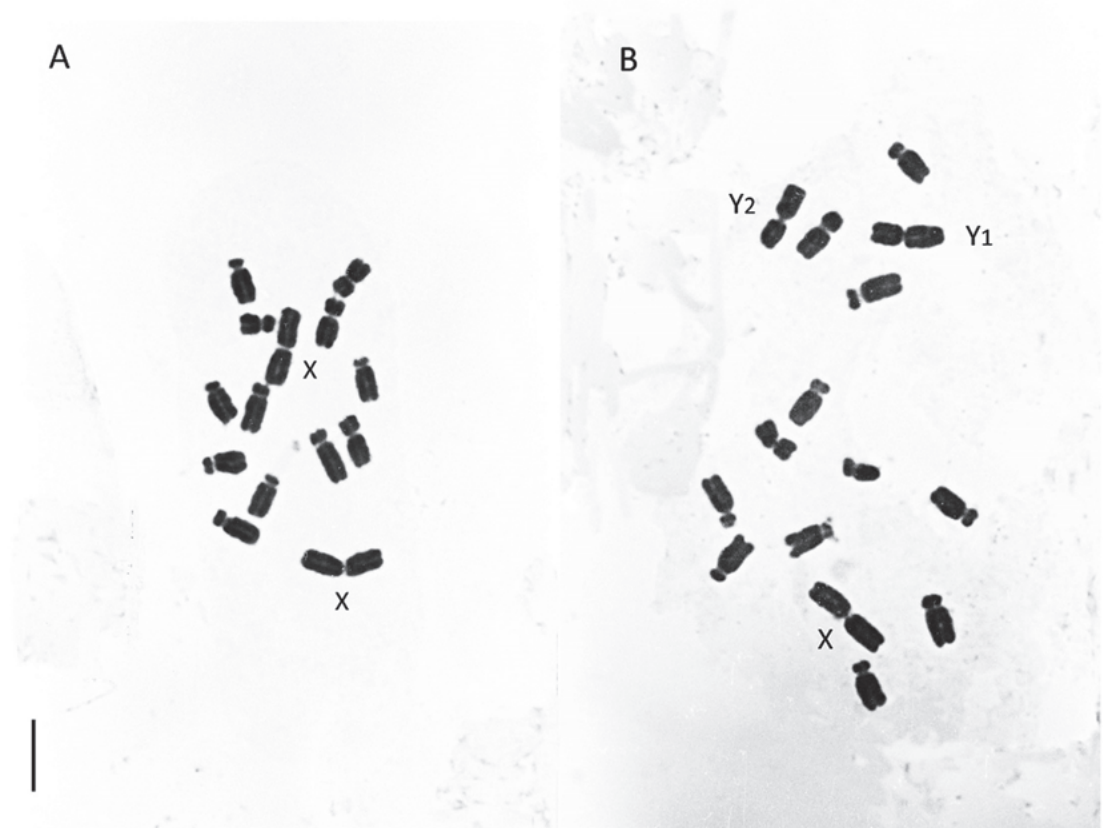


Fig. 2. Somatic metaphase chromosomes of *R. acetosa*. A. Female plant ($2n = 14 = 12 + 2X$). B. Male plant ($2n = 15 = 12 + X + Y_1Y_2$). Bar: $5\mu\text{m}$.

1928; Ono 1928, 1930,1932; Kihara and Yamamoto 1931; Takenaka 1931; Kuroki and Kurita 1970a, 1971a; Kurita and Kuroki 1972; Kuroki 1976). Moreover, the following two rare tetraploid chromosome forms were found among natural populations in Japan: $2n = 28 = 24+4X$ (Ono 1932; Kuroki 1976) and $2n = 29 = 24+3X+Y_1Y_2$ (Ono and Shimotomai 1928; Ono 1930,1932; Kurita et al. 1971). Several forms of aneuploid *R. acetosa* were also found among natural populations in Japan, with the following chromosome constitutions: $2n = 21 = 18+2X+Y$ (Ono 1935); $2n = 21 = 18+X+2Y$ (Ono 1932); $2n = 22 = 17+2X+3Y$ (Ono 1930); $2n = 27 = 24+3X$ (Kurita et al. 1971; Kuroki 1976); $2n = 29 = 24+2X+3Y$ (Ono 1932), and others. In addition, an uncommon mixoploid mutant was found, which included the following two different chromosome constitutions: $2n = 14 = 12+2X$ and $2n = 16 = 14+2X$ (Kuroki and Kurita 1973).

Sex determination in *R. acetosa* was shown by Ono (1935) to have an X:A balance system. Within this system, which was initially identified in *Drosophila* (Bridges 1921), the Y chromosome has no role in sex determination. Hence, the sex of each *R. acetosa* individual is determined by the balance between X chromosomes and autosomal sets.

Previous karyotype studies of *R. acetosa* from Great Britain, Japan, and Poland revealed its karyotype to be variable based upon the following: 1) the centric positions of its two Y chromosomes (Y_1 and Y_2) are polymorphic (Zuk 1969, as *R. thyrsiflorus* Fingerh.; Kuroki and Kurita 1969a, 1970b; Wilby and Parker 1986,1987a); 2) satellites on the short arm of A3 chromosomes are polymorphic in size (Kuroki and Kurita 1969b, 1971b; Kuroki 1976); 3) within the six pairs of autosomes, A1 and A6 chromosomes sometimes carry heterochromatic segments (also known as supernumerary segments (SS)) in European *R. acetosa* (Zuk 1969, as *R. thyrsiflorus*; Parker and Wilby 1988), whereas A1, A5, and A6 chromosomes sometimes have SS in Japanese *R. acetosa* (Kuroki 1976). Among the polymorphic autosomes of A1, A5, and A6 chromosomes found in Japanese *R. acetosa*, both A5 chromosomes with SS (SS5) and A6 chromosomes with SS (SS6) are widely

distributed (Ono 1935; Kuroki 1976); however, the distribution of A1 chromosomes with SS (SS1) in Japan is unclear.

The karyotypes of *R. acetosa* occurring in Japan were first reported by Kihara and Yamamoto (1931) and Yamamoto (1933), and were classified into eight forms (types I to VIII) by Yamamoto (1938). Ono (1935) classified the haploid autosomal set of Japanese *R. acetosa* into four forms: A (composed of six i-type chromosomes), B (composed of five i-type chromosomes and one v-type chromosome), C (composed of 5 i-type chromosomes and one j-type chromosome), and D (composed of 4 i-type chromosomes, one v-type chromosome, and one j-type chromosome). These forms were based on combinations of the two chromosome forms found in A5 and A6 chromosomes (Fig. 3). Ono (1935) then classified and expressed the karyotype forms of normal diploid *R. acetosa* occurring in Japan as: AA, AB, AC, AD, BB, BC, BD, CC, CD, and DD. Within these 10 forms, AD and BC are the same form (Fig. 4); thus, BC is not used in this study to express the karyotype forms of natural *R. acetosa*, and will not be used in further karyotypic studies of this species, except in the case of plants that are born artificially by cross-fertilization between plants with BB karyotype forms and plants with CC karyotype forms.

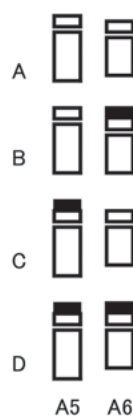


Fig. 3. Diagrams showing the four forms of haploid autosomal sets of *R. acetosa*, as classified by Ono (1935). The four forms (A, B, C, D) are shown only on autosomes 5 (A5) and 6 (A6). The black areas represent the supernumerary segments (SS) composed of heterochromatin.

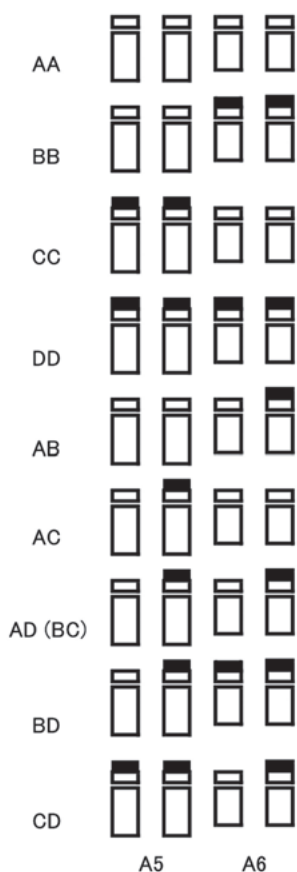


Fig. 4. Diagrams of the nine forms of diploid chromosomal sets of *R. acetosa* (Ono, 1935). Solid black areas show supernumerary segments (SS).

In this species, the two Y chromosomes, along with the SS (Kurita and Kuroki 1975; Kuroki 1987) found on the short arms of some A1, A2, and A6 chromosomes, are composed of heterochromatin (Vana 1972; Kurita and Kuroki 1971a, 1975; Kuroki 1987; Wilby and Parker 1986, 1988; Parker and Clark 1991). The two Y chromosomes found in the chromosome complements of diploid male plants undergo a longer deoxyribonucleic acid (DNA) replication process than the other chromosome complements (Kusanagi 1963), generally showing late replication (Zuk 1969, in *R. thyrsiflorus*). In addition to Y chromosomes and the SS of autosomes, satellite bodies of A3 chromosomes have been shown to contain C-bands (Yonezawa et al. 1978), indicating that the satellite bodies of *R. acetosa* are also composed of heterochromatin. A size varia-

tion in the SS of A6 chromosomes was also detected in Japan (Kurita and Kuroki 1971a). Kuroki (1987) used fluorescence analysis of *R. acetosa* karyotypes to conclude that the karyotype variation found within this species was mainly caused by the addition or deletion of heterochromatin.

In Toyama Prefecture, which is located near the Sea of Japan in central Honshu, *R. acetosa* commonly grows on the plains and along roadsides and paths. This species is prevalent in almost all areas, excluding the alpine and subalpine flora of the Hida Mountains (the northern portion of the Japan Alps), situated in the eastern region of Toyama Prefecture. *Rumex acetosa* is an attractive plant for studying karyotype variations, due to its two forms of A1, A5, and A6 chromosomes, polymorphic Y chromosomes (Y_1 and Y_2) in centric positions, chromosome mutations, and genome mutations. This paper reports on karyotypic variations of *R. acetosa*, along with this species' meiotic chromosome behaviors, based on data collected from natural populations throughout Toyama Prefecture.

Materials and methods

A total of 1,640 mature plants were collected from 17 localities throughout the distribution area in Toyama Prefecture (Table 1). On average, 96 individuals per population were used for this study. In each locality, a population sample of over 50 diploid plants was achieved.

All collected plants were grown in clay or plastic pots at the experimental garden of the University of Toyama. Each individual plant's chromosome count was examined in somatic cells with fully spread chromosomes from root tip meristem cells.

Approximately 1.5 cm of root tip was excised from each potted plant, before being pretreated in a 1.9 mM 8-hydroxyquinoline solution at room temperature for one hour, and subsequently stored at 5°C for 15 hours. Root tips were fixed in a mixture of glacial acetic acid and absolute ethyl alcohol (1:3) at room temperature for one hour, macerated in 1N hydrochloric acid at 60°C for ten minutes, and then washed in tap water. They were stained and

Table 1. Collection localities, chromosome constitutions, and numbers of *R. acetosa* individuals collected in Toyama Prefecture, central Japan

Ploidy level	Number of plants examined	Diploid (2x) ♀ (2n=12+XX) ♂ (2n=12+XXY)	Hyperdiploid (Aneuploid) 2n=13+XX 2n=13+XXY	Triploid (3x) 2n=18+XXX 2n=18+XXY	Tetraploid (4x) 2n=24+XXXX 2n=24+XXXXY
Asahi-machi, Shimoniikawa-gun	53	41	-	-	-
Nyuzen-machi, Shimoniikawa-gun	51	44 (3)	-	1	-
Kakuma, Uozu City	62	48 (1)	-	1	-
Tateyama-machi, Nakaniikawa-gun	66	48 (12)	-	1	-
Hamakurosaki, Toyama City	51	40	-	-	-
Hiyodoriijima, Toyama City	51	45	-	-	-
Funato, Toyama City	53	42 (1)	-	-	1
Shinjo-ginza, Toyama City	297	238* (1)	1	-	-
Shimosasahara, Yatsuomachi, Toyama City	163	120 (2)	-	2	1
Fuchumachi-Rengeji, Toyama City	314	257* (1)	-	1	1
Uwano, Imizu City	59	46	-	-	-
Jokoji, Takaoka City	119	95	-	1	2
Mera, Himi City	56	41 (1)	-	1	-
Taya, Nanto City	64	50 (2)	-	1	1
Kagoto, Nanto City	60	45 (5)	-	-	-
Togamura, Nanto City	59	43	-	-	1
Hakkoda, Oyabe City	62	53 (9)	-	-	-
Total (%)	1,640	1,296 (79.02%)	1 (0.06%)	9 (0.55%)	2 (0.12%)
		321 (19.57%)	1 (0.06%)	8 (0.49%)	2 (0.12%)

Numbers enclosed in parentheses are the number of individuals with B-chromosomes.

*: one plant had an autosome reciprocal translocation.

***: three plants had an autosome reciprocal translocation.

squashed in 1% lacto-propionic orcein. Fully spread nuclei with prophase and metaphase chromosomes were used for the study.

Chromosome counts and constitutions were examined in either prometaphase, metaphase, or both. Chromocenters were examined in the nuclei of two female plants from each of AA, AB, AC, AD, BB, BD, CC, CD, and DD forms. Karyotypes were examined in metaphase with fully spread chromosomes. Metaphase chromosome forms were expressed following the conventions of Levan et al. (1964), in which chromosome form was identified by the centromeric position. Karyotype classification of diploid *R. acetosa* was expressed following the conventions of Ono (1935).

For the study of meiotic chromosome behavior, young staminate flower buds were fixed and preserved in Newcomer's fluid until use. Flower buds were macerated with the same procedure as with root tips, and were stained and squashed in one drop of 1.5% lacto-propionic orcein. Pollen mother cells (PMCs) from the anthers of young staminate flowers were used to study meiotic chromosome behavior.

Results and discussion

Chromosome Constitution

A total of 1,640 individual *R. acetosa* specimens were collected from 17 localities in Toyama Prefecture (Table 1). Nearly all individuals were diploid, with either $2n = 14 = 12+XX$ (1,296 individuals, 79.02%) or $2n = 15 = 12+X+YY$ (321 individuals, 19.57%) (Fig. 2). Seventeen individuals, constituting 1.04% of all plants examined, were triploid (nine individuals with $2n = 21 = 18+3X$ and eight individuals with $2n = 22 = 18+2X+Y_1Y_2$) (Fig. 5). Four individuals were tetraploid (two individuals with $2n = 28 = 24+4X$ and two individuals with $2n = 29 = 24+3X+Y_1Y_2$) (Fig. 6), and two individuals were aneuploid, with chromosome constitutions of $2n = 15 = 13+2X$ and $2n = 16 = 13+2X+Y_2$ (Fig. 7). In normal diploid plants, the ratio of plants with $2n = 14 = 12+2X$ pistillate flowers (female plants) to plants with $2n = 15 = 12+X+Y_1Y_2$ staminate flowers (male plants) was about 4:1 (Table 1). Triploid plants with $2n = 21 = 18+3X$ were considered to be produced by fusion between an unreduced gamete ($n = 14 = 12+2X$) from a female diploid plant and

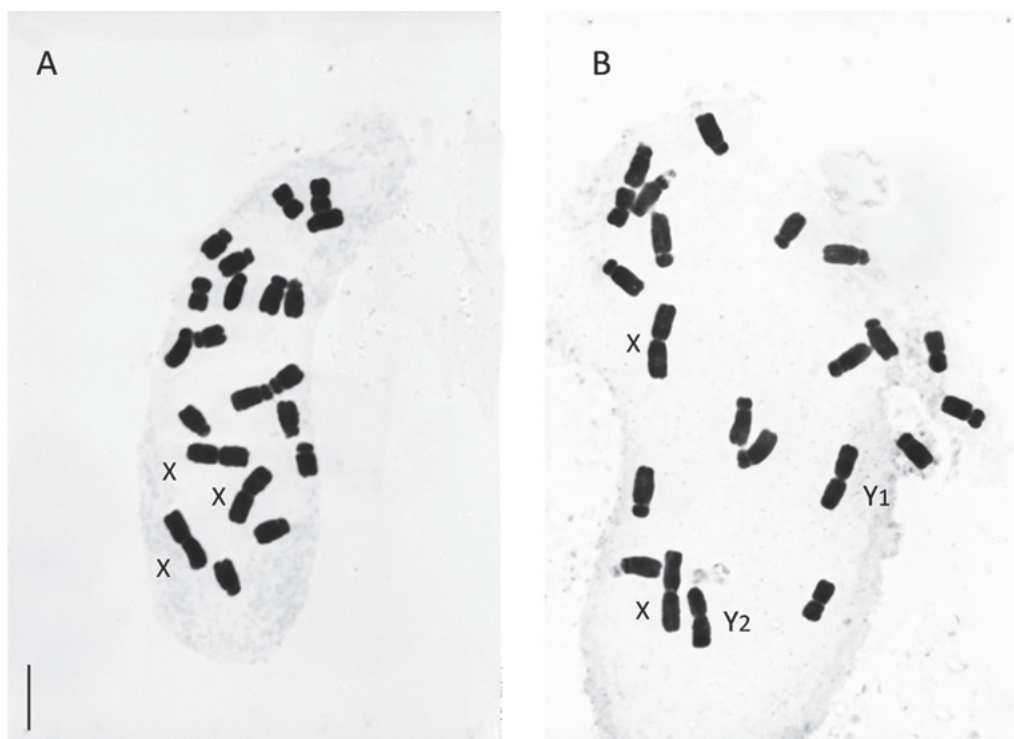


Fig. 5. Two karyotype forms of triploid *R. acetosa*. A. $2n = 21 = 18+3X$. B. $2n = 22 = 18+2X+Y_1Y_2$. Bar: 5 μ m.

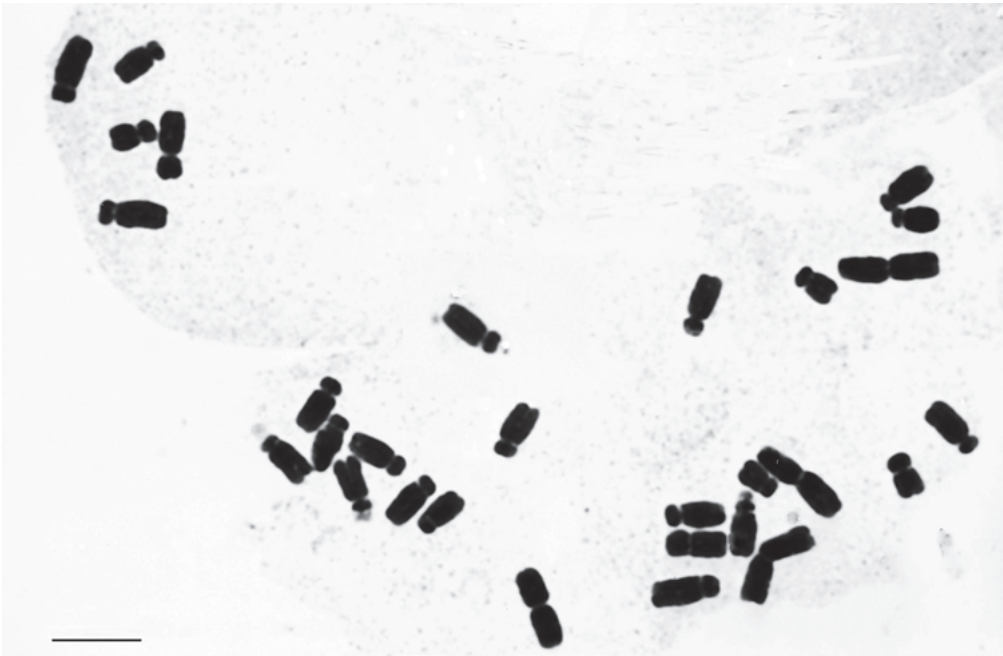


Fig. 6. Metaphase chromosome of tetraploid *R. acetosa* with $2n = 29 = 24+3X+Y_1Y_2$. Bar: 5 μ m.

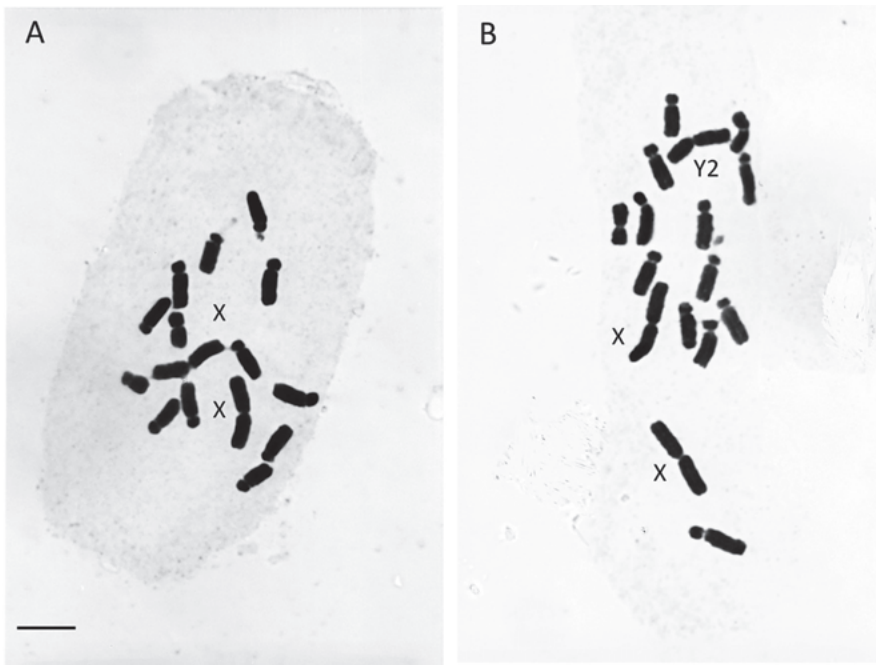


Fig. 7. Two forms of aneuploid *R. acetosa* found in (A) Shinjo-ginza, Toyama City and (B) Fuchumachi-Rengeji, Toyama City. A. $2n = 15 = 13+2X$. B. $2n = 16 = 13+2X+Y_2$. Bar: 5 μ m.

a normal haploid gamete ($n = 7 = 6+X$) from a male diploid plant (Ono 1928,1935). Triploid plants with $2n = 22 = 18+2X+ Y_1Y_2$ were considered to be produced by fusion between either a haploid gamete ($n = 7 = 6+X$) from a female diploid plant and an unreduced gamete ($2n = 15 = 12+X+Y_1Y_2$) from a male diploid plant, or alternatively by fusion of an unreduced gamete ($n = 14 = 12+2X$) from a female diploid plant and a normal haploid gamete ($n = 8 = 6+Y_1Y_2$) from a male diploid plant (Ono 1928,1935). Tetraploid plants with $2n = 29 = 24+3X+ Y_1Y_2$ were considered to be produced by fusion between an unreduced gamete ($n = 14 = 12+2X$) from a female diploid plant and an unreduced gamete ($n = 15 = 12+X+ Y_1Y_2$) from a male diploid plant (Ono 1928). However, the origin of tetraploid plants with $2n = 28 = 24+4X$ is unclear. Figure 8 shows the diploid cell and tetraploid cell in a female *R. acetosa*. It is possible that tetraploid plants with $2n = 28 = 24+4X$ may have been produced by the accidental polyploidization of diploid female plants in early stage embryos.

Karyotype

In the chromosome complement of male *R. acetosa* plants, the X chromosome is the longest, the Y_1 chromosome is the second longest, and the Y_2 chromosome is the third longest chromosome (Table 2). The centric positions of both the Y_1 and Y_2 chromosomes were variable (Fig. 9). The relative length of Y_1 chromosomes to the length of X chromosomes was nearly constant at about 83%, while the length of ordinary Y_2 chromosomes was also constant at about 75% of the length of X chromosomes (Table 2). These values correspond with the relative lengths of the X, Y_1 , and Y_2 chromosomes of British *R. acetosa* plants, as reported by Wilby and Parker (1986), as well as the relative lengths of Japanese *R. acetosa* plants, as reported by Kuroki and Kurita (1969a, 1970b).

In the six pairs of autosomes composed of the somatic chromosome complement in diploid plants, A1 and A2 chromosomes are subtelo-centric, and are similar to one another in both centromeric position and chromosome length. The similar appearance of these two chromo-

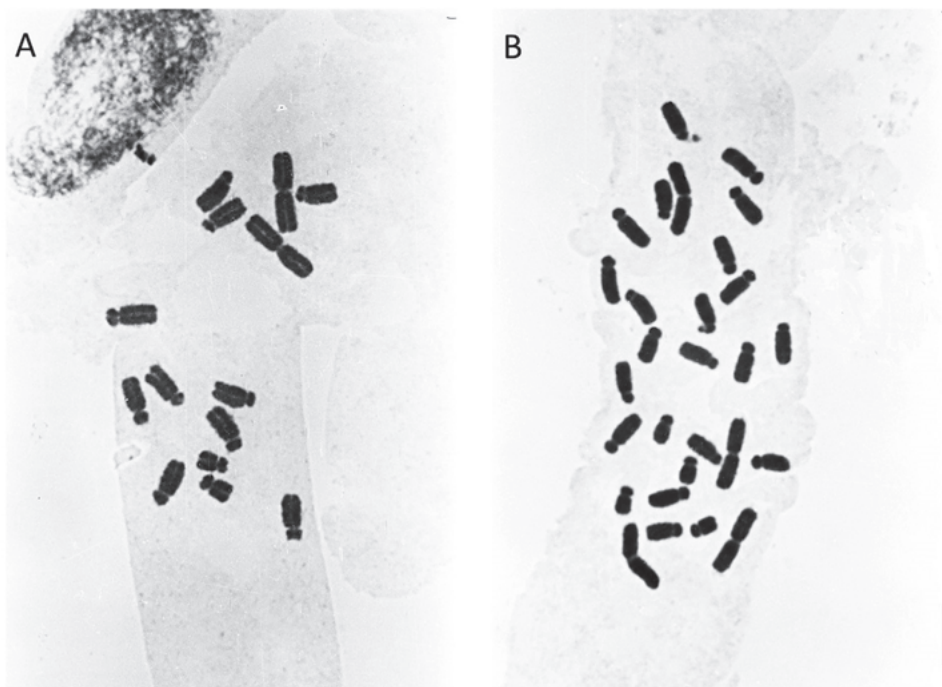


Fig. 8. A mixoploid *R. acetosa* individual with diploid cells (A: $2n = 14 = 12+2X$) and tetraploid cells (B: $2n = 28 = 24+4X$). Bar: 5 μ m.

somes often makes it difficult to distinguish one from the other when using conventional staining techniques, particularly during mid-metaphase. The A3 and A4 chromosomes are also subtelocentric and are similar to one another in length. However, the A3 chromosome is easily distinguished by the existence of a satellite on its short arm. Satellite bodies occurring in plants from Toyama Prefecture were polymorphic in size (Figs. 9 and 10), as reported by Kuroki and Kurita (1969b, 1971b) and Kuroki (1976). None of the plants used in this study

displayed SS in their A1 chromosomes.

Among the six pairs of autosomes, the A5 chromosome is the second shortest and the A6 chromosome is the shortest in the chromosome complement. These chromosomes exhibited polymorphism in the existence or absence of SS in *R. acetosa* plants occurring in Toyama Prefecture (Figs. 9 and 10).

In the chromosome complement of Japanese *R. acetosa*, A1, A5, and A6 chromosomes are known to be polymorphic for the existence or absence of SS composed of heterochromatin

Table 2. Chromosome lengths of male *R. acetosa*

Chromosomes	Short arm + Long arm (μm)	Total length (μm)	Arm ratio	Relative length	Form
X	3.59 + 3.70	7.29	1.03	1.00	M
Y ₁	-	6.07	polymorphic	0.83	-
Y ₂	-	5.46	polymorphic	0.75	-
A1	1.00 + 3.50	4.50	3.50	0.62	st
A2	0.86 + 3.31	4.17	3.85	0.57	st
A3	t-0.62 + 3.24	3.86	5.23	0.53	st
A4	0.78 + 3.19	3.97	4.09	0.55	st
A5	0.69 + 2.80	3.49	4.06	0.48	st
SS5	1.55 + 2.84	4.39	1.83	0.60	sm
A6	0.85 + 2.05	2.90	2.41	0.40	sm
SS6	1.55 + 2.04	3.59	1.32	0.49	m

Chromosome lengths represent the mean of 22 plants. Total length and arm ratio do not include satellite. t: satellite

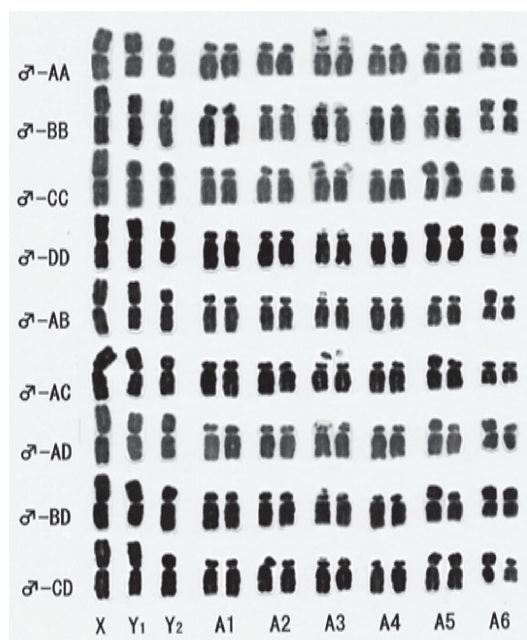


Fig. 9. Nine forms of diploid chromosomal sets of male *R. acetosa* found in Toyama Prefecture.

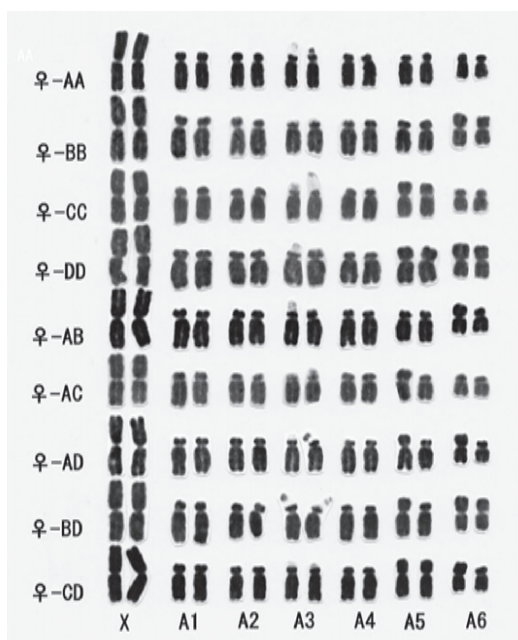


Fig. 10. Nine forms of diploid chromosomal sets of female *R. acetosa* found in Toyama Prefecture.

(Kuroki 1976, 1987). However, in plants occurring in Toyama Prefecture, some A5 and A6 chromosomes were shown to have SS. Thus, the karyotype forms occurring in this area could be expressed, following the conventions of Ono (1935), as AA, AB, AC, AD, BB, BD, CC, CD, and DD in both female and male plants (Figs. 9 and 10). Among these nine karyotype forms, AB was the most frequent (29.4%), followed by AD (20.1%), AA (15.7%), BB (11.3%), AC (9.6%), BD (8.6%), CD (2.5%), DD (1.8%), and CC (1.0%) (Table 3).

Behavior of Y Chromosomes and Supernumerary Segments in Mitosis and Meiosis

During prophase of somatic cell division, the two Y chromosomes and SS on the short arms of both A5 chromosomes (SS5 chromosomes) and A6 chromosomes (SS6 chromosomes) showed early condensation (Fig. 11), as reported by Kurita and Kuroki (1971b, 1975). During diplotene and diakinesis in meiosis of PMCs, the two Y chromosomes and SS of both SS5 and SS6 chromosomes showed early condensa-

tion (Fig. 12), in the same manner described during prophase in mitosis.

B Chromosomes of *R. acetosa* Occurring in Toyama Prefecture

B chromosomes were recorded as fragment chromosomes in *R. acetosa*, and were described as small and telocentric (Takenaka 1931; Kihara and Yamamoto 1931). Several forms of B chromosomes have since been found in Japanese *R. acetosa* (Ono 1935; Haga 1961; Kurita and Kuroki 1971b; Kuroki and Kurita 1972, 1973). In the present study, B chromosomes were detected in 48 (3%; 38 female diploid plants and 10 male diploid plants) of the 1,617 diploid plants examined (Table 1). B chromosomes found in this study were all small and telocentric (Fig. 13), similar to B chromosomes that were reported for the first time in Japanese *R. acetosa* by Takenaka (1931) and Kihara and Yamamoto (1931).

Chromocenters

In interphase nuclei, two Y chromosomes

Table 3. Karyotype forms of normal diploid *R. acetosa* and frequencies of SS5 chromosomes and SS6 chromosomes in 17 localities in Toyama Prefecture, central Japan

Collection locality	Number of normal diploid plants	Number of normal diploid plants and karyotype forms									SS5 Freq.	SS6 Freq.
		AA	AB	AC	AD	BB	BD	CC	CD	DD		
Asahi-machi, Shimonikawa-gun	53	6	18	7	10	6	5	0	1	0	0.226	0.481
Nyuzen-machi, Shimonikawa-gun	50	9	12	4	9	12	1	1	2	0	0.200	0.490
Kakuma, Uozu City	60	11	14	2	16	9	7	0	0	1	0.225	0.533
Tateyama-machi, Nakaniikawa-gun	65	13	19	4	11	14	1	1	1	1	0.169	0.485
Hamakurosaki, Toyama City	51	5	22	5	11	5	0	3	0	0	0.216	0.422
Hiyodorijima, Toyama City	51	6	7	5	15	8	5	1	2	2	0.343	0.529
Funato, Toyama City	52	2	14	4	9	10	10	1	0	2	0.279	0.644
Shinjo-ginza, Toyama City	295	27	74	29	76	32	41	0	9	7	0.302	0.541
Shimosasahara, Yatsuomachi, Toyama City	158	22	51	11	30	16	18	2	7	1	0.250	0.500
Fuchumachi-Rengeji, Toyama City	306	35	96	33	58	28	35	4	9	8	0.275	0.498
Uwano, Imizu City	59	14	23	3	6	13	0	0	0	0	0.076	0.466
Jokoji, Takaoka City	116	47	35	19	10	4	1	0	0	0	0.129	0.237
Mera, Himi City	55	8	18	4	11	6	4	0	2	2	0.245	0.500
Taya, Nanto City	61	14	23	4	9	5	2	1	3	0	0.189	0.402
Kagoto, Nanto City	60	18	12	9	13	4	2	2	0	0	0.233	0.308
Togamura, Nanto City	58	1	9	8	21	6	4	0	4	5	0.440	0.552
Hakkoda, Oyabe City	62	15	26	4	9	4	3	0	1	0	0.145	0.403
Total (%) of each karyotype form and total frequencies of SS5 and SS6	1612	253 (15.7%)	473 (29.5%)	155 (9.6%)	324 (20.1%)	182 (11.3%)	139 (8.6%)	16 (1.0%)	41 (2.5%)	29 (1.8%)	0.245	0.477

and SS on the short arms of A1, A5, and A6 chromosomes are known to appear as chromocenters (Kuroki and Kurita 1971b,1971c; Wilby and Parker 1988) (Fig. 14). Among female *R. acetosa* plants, the mean number of chromocenters in the interphase nuclei of each plant

of karyotype forms AA, AB, AC, AD, BB, BD, CC, CD, and DD was similar among female plants with the same karyotype form (Table 4). Moreover, each mean number of chromocenters for the nine karyotype forms was in correspondence with the total numbers of SS for each

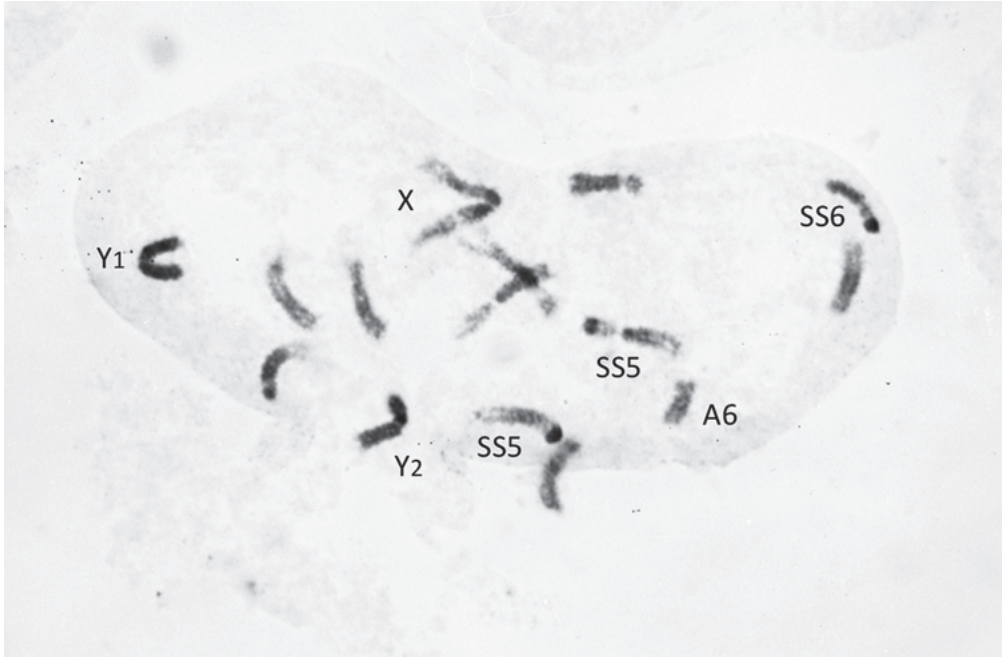


Fig. 11. Mitotic prophase in a male *R. acetosa* individual homozygous for SS5 and heterozygous for SS6 (CD form). Y chromosomes and supernumerary segments (SS) of SS5 and SS6 chromosomes show early condensation.

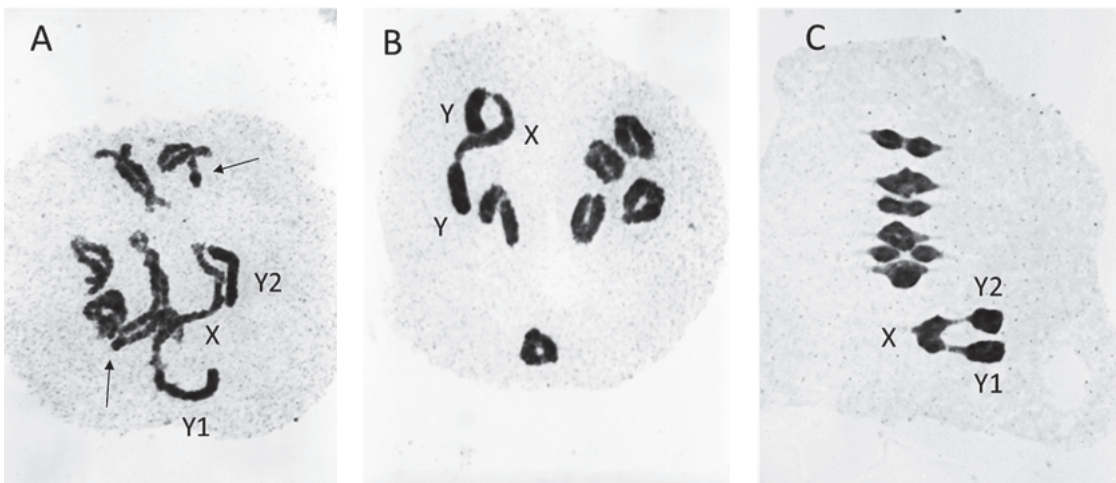


Fig. 12. Meiosis in a male *R. acetosa* individual with an AD chromosome form. A. Diplonema; B. Diakinesis; C. Metaphase I. This plant is heterozygous for SS5 and SS6. Arrows indicate supernumerary segments (SS) of SS5 and SS6. Y chromosomes and supernumerary segments (SS) of SS5 and SS6 show early condensation in (A) diplonema and (B) diakinesis.

karyotype form, as reported by Kuria and Kuroki (1975).

Distribution and Frequency of SS5 and SS6 Chromosomes in Toyama Prefecture

The distribution and frequency of SS5 and SS6 chromosomes in each locality were as follows:

SS5 Chromosomes: All populations exhibited SS5 chromosomes (A5 chromosomes with SS). The frequency of SS5 chromosomes among the total number of A5 chromosomes in normal diploid plants from each locality was between 7.6% and 44.0%, with a mean of 24.5%. This



Fig. 13. Mitotic metaphase in a female *R. acetosa* individual with B chromosomes. Arrows indicate B chromosomes.

distribution shows that SS5 chromosomes are prevalent, and that about one fourth of all *R. acetosa* A5 chromosomes in Toyama Prefecture were composed of SS5 chromosomes (Table 3).

SS6 Chromosomes: All populations also exhibited SS6 chromosomes (A6 chromosomes with SS). The frequency of SS6 chromosome among the total number of A6 chromosomes in normal diploid plants from each locality was between 23.7% and 64.4%, with a mean of 47.7%. This distribution shows that nearly half of all *R. acetosa* A6 chromosomes in Toyama Prefecture were composed of SS6 chromosomes (Table 3).

Kuroki and Kurita (1969c) and Kuroki (1976) investigated the frequency of autosomes with SS in populations of *R. acetosa* occurring in several localities in Japan. In their reports, SS1 and SS5 chromosomes were not differentiated from one another. However, the distributions of SS6 chromosomes in *R. acetosa* populations were detailed as follows: 1) 66 diploid plants (53 females and 13 males; 93.9%) in Kuroishi City, Aomori Prefecture, Tohoku District, Honshu; 2) 97 diploid plants (83 females and 14 males; 40.2%) in Naruto-machi, Miyagi Prefecture, Tohoku District, Honshu; 3) 100 diploid plants (79 females and 21 males; 71.5%) in Gero City, Gifu Prefecture, Chubu District, Honshu; 4) 96

Table 4. Numerical variation of chromocenters in interphase nuclei of root-tip cells of nine karyotype forms of female *R. acetosa*

Karyotype	Number of Cells	Number of Chromocenter										Total	Mean
		0	1	2	3	4	5	6	7	8	9		
♀-AA	1158	516	50	11	2							579	0.13
♀-AA	1076	515	20	2	1							538	0.05
♀-AB	1196	31	526	31	8	2						598	1.04
♀-AB	1316	64	480	88	21	5						658	1.12
♀-AC	1064	55	404	60	13							532	1.06
♀-AC	1110	153	292	97	11	2						555	0.95
♀-AD	1054	12	79	352	72	9	3					527	1.99
♀-AD	1036	27	102	270	74	33	9	3				518	2.04
♀-BB	1138	23	68	297	119	43	14	5				569	2.27
♀-BB	1082	16	76	304	100	36	7	2				541	2.17
♀-CC	1080	36	104	287	87	20	4	2				540	1.95
♀-CC	1046	20	91	311	73	21	6	1				523	2.01
♀-BD	1132	11	28	160	260	87	17	2	1			566	2.79
♀-BD	1036	12	19	102	226	94	43	15	4	3		518	3.15
♀-CD	1116	7	17	136	222	133	28	12	2	1		558	3.08
♀-CD	1164	24	41	155	250	87	21	4				582	2.71
♀-DD	1064	11	12	57	134	200	93	20	5			532	3.66
♀-DD	1116	1	5	38	136	218	112	38	5	4	1	558	3.99

diploid plants (55 females and 41 males; 31.8%) in Misaka, Tottori Prefecture, Chugoku District, Honshu; 5) 98 diploid plants (77 females and 21 males; 83.2%) in Yunadani, Matsuyama City, Ehime Prefecture, Shikoku; and 5) 99 diploid plants (62 females and 37 males; 41.9%) in Kaimon, Kagoshima Prefecture, Kyushu. These reports showed that the frequency of SS6 chromosomes in A6 chromosomes varied considerably between different areas of Japan.

The present study also revealed that the ratios of chromosome forms found in both A5 and A6 chromosomes vary considerably between different areas of Toyama Prefecture. Rough estimates show that, among *R. acetosa* populations in Toyama Prefecture, one fourth of A5 chromosomes contain SS5 chromosomes, and half of A6 chromosomes contain SS6 chromosomes.

Wilby and Parker (1987b) reported that, in England, SS1 chromosomes are distributed throughout *R. acetosa* populations in Britain at a low frequency, found to be present in an average of 4% of A1 chromosomes. In comparison, SS6 chromosomes are distributed throughout

the southern and western parts of Britain, but are absent from areas where the mean daily temperature in January is less than 4.4°C, showing clear environmental correlations.

Further karyotypic studies of Japanese *R. acetosa* are necessary to clarify the cytogeography of SS1, SS5, and SS6 chromosomes in Japan.

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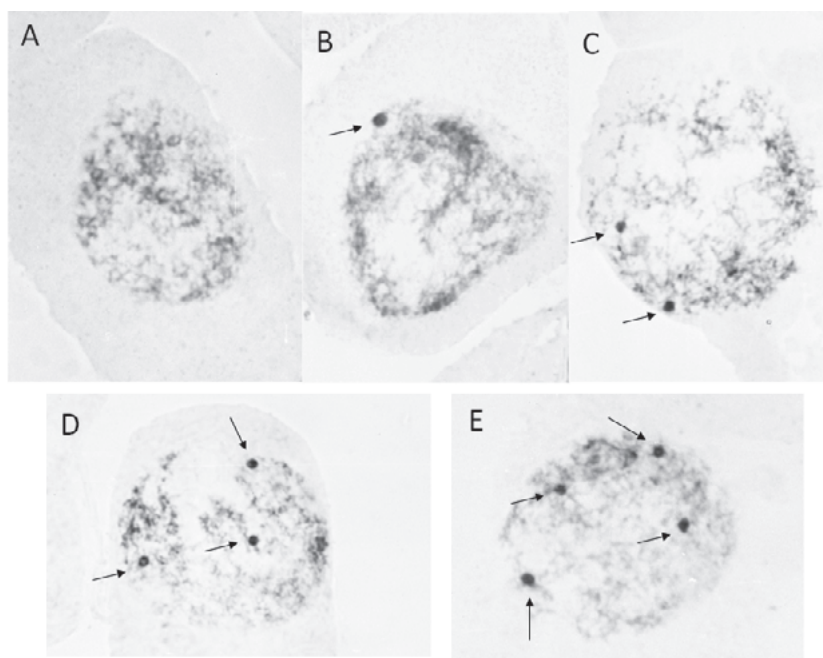


Fig. 14. Interphase nuclei of female *R. acetosa*.

A: interphase without chromocenter. B: interphase with one chromocenter. C: interphase with two chromocenters. D: interphase with three chromocenters. E: interphase with four chromocenters. Arrows indicate chromocenters.

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- 2012年度 植物地理・分類学会賞受賞記念講演記録**
岩坪美兼：富山県におけるスイバ（タデ科）の染色体変異
- スイバは、性染色体が分化した雌雄異株植物（雌株は $2n = 12+2X$, 雄株は $2n = 12+ X +Y_1Y_2$ ）である。Y染色体の動原体の位置と付随体のサイズは変異することが知られている。第1, 5, 6常染色体には、短腕に過剰分節（supernumerary segment）が存在するタイプと存在しないタイプの2型が存在するため、スイバの核型は多様である。
- 最初に、これまでの染色体研究について概説することで、スイバにおいて明らかにされた核型変異について紹介を行った。
- 次に、富山県産スイバの核型変異を明らかにすることを目的として、県内の17カ所より採集した1,640個体について染色体の観察を行い、その結果を報告した。1,640個体のうち、二倍体雌個体（ $2n = 12+2X$ ）は1296個体（79.07%）、二倍体雄個体（ $2n = 12+ X+Y_1Y_2$ ）は321個体（19.57%）であった。三倍体は17個体（1.2%）含まれており、 $2n = 18+XXX$ が9個体、 $2n = 18+XXYY$ が8個体であった。四倍体は $2n = 24+XXXX$ と $2n = 24+XXXYY$ がそれぞれ2個体ずつの4個体、それに $2n = 15 = 13+2X$ （1個体）と $2n = 16 = 13+2X+Y_2$ （1個体）の2個体の異数体が含まれていた。48個体（全観察個体の3%）にはB染色体が存在した。日本産スイバには過剰分節の有無により、第1常染色体（A1）、第5常染色体（A5）、第6常染色体（A6）に、それぞれに2型が知られている。観察を行った17カ所の二倍体個体における過剰分節をもった染色体の割合は、SS5（過剰分節をもつA5）が7.6%～44.0%、平均24.5%であった。SS6（過剰分節をもつA6）では23.7%～64.4%、平均47.7%であった。過剰分節をもつ第1常染色体（SS1）は観察されなかった。イギリスにおいてSS6は1月の1日の平均気温が4.4℃以下の地域のスイバには存在しないことが報告されている。スイバを対象とした細胞地理学的研究において、SS1, SS5, SS6染色体の国内での分布域を明らかにすることが望まれる。
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