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Biosystematic Study of *Sedum* L. Subgenus *Aizoon* (Crassulaceae) II. Chromosome Numbers of Japanese *Sedum aizoon* var. *aizoon*

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Chromosome numbers of 114 individuals from twelve populations of *Sedum aizoon* L. var. *aizoon* (Crassulaceae) are reported. They include 37 different chromosome numbers ranging from 2n=71 to 124. Although the chromosome number variation has been found in all populations examined, no correlation with geographical distribution could not be found. Various kinds of meiotic irregularities, i.e., multivalents, univalents, chromosome lagging, and polysporous "tetrad" formation have been found. These irregularities lead to the formation of gametes with various chromosome numbers. All aneuploid plants set seeds and seem to reproduce sexually. The extensive aneuploidy in var. *aizoon* seems to be caused by the unequal chromosome segregation in meiosis and the subsequent fertilization of gametes with various chromosome numbers.

Key words: Aneuploid — Irregular meiosis — Polyploid — Sexual reproduction — *Sedum aizoon* var. *aizoon*

Sedum aizoon L. (Crassulaceae) is a polymorphic species with many varieties. It has a wide distributional range from eastern Siberia to Japan. In Japan two varieties, *aizoon* and *floribundum* Nakai, are recognized (Ohba, 1982). The variety *aizoon* differs from var. *floribundum* in several morphological characters (Table 1). Their habitats are also distinct from each other, i.e., var. *aizoon* occurs in grassland while var. *floribundum* on rocks or gravelly slopes.

In var. *floribundum* geographical and infrapopulational variation in chromosome number has been reported (Amano, 1990). In var. *aizoon* Ogawa and Yuasa (1970) have reported 15 different chromosome numbers ranging from 2n=80 to 158. However, there are many unsolved problems such as whether any geographical pattern to variation in chromosome number is present or not, and whether the aneuploidy is found within a population or not.

The present paper provides data on chromosome numbers, meiotic behaviours, and pollen and seed fertilities, and clarifies the cause of the extensive chromosome number variation in *Sedum aizoon* var. *aizoon*.

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Table 1. Diagnostic characters of *Sedum aizoon* var. *aizoon* and var. *floribundum*

Characters	var. *aizoon*	var. *floribundum*
Serrations of leaves	10-15	3-10
Number of stems	one or a few	several to several dozen
Last year's stem	underground part remaining	died, rarely remaining
Shape of rhizome	elongated, not thickened	tuberous, thickened
Position of winter buds	on last year's stem	on rhizome or rarely last year's stems

Materials and Methods

The living material of *Sedum aizoon* var. *aizoon* was collected from 12 populations in Japan (Table 2). Cytological and sampling methods used were the same as those reported by Amano (1990). Voucher specimens were deposited in TI.

Table 2. Inter- and infrapopulational variation in chromosome number

Number	Localities	Number of individuals	Chromosome number*
1	Shiundai, Kushiro, Hokkaido	13	2n=96(1), 100(1), 103(3), 105(1), 106(1), 107(3), 108(1), 109(1), 111(1)
2	Shiranuka, Kushiro, Hokkaido	1	2n=103(1)
3	Toei, Urakawa, Urakawa, Hokkaido	1	2n=98(1)
4	Horokeshi, Biratori, Saru. Hokkaido	2	2n=96(1), 99(1)
5	Mt. Hakodate site A, Hakodate, Hokkaido	5	2n=71(1), 77(1), 80(2), 84(1),
6	Mt. Hakodate site F, Hakodate, Hokkaido	17	2n=72(3), 73(1), 74(2), 75(2), 76(2), 77(5), 78(2)
7	Iwaizumi, Shimohei, Iwate	2	2n=72(1), 86(1)
8	Higashinippara, Okutama, Nishitama, Tokyo	15	2n=72(1), 75(1), 77(1), 80(7), 84(1), 104(1)
9	Mt. Shakushi, Fujiyoshida, Yamanashi	19	2n=86(1), 93(1), 96(7), 102(3), 104(1), 106(2), 109(1), 112(1), 113(1), 124(1)
10	Nenba, Ashiwada, Minamitsuru, Yamanashi	1	2n=96(1)
11	Nobeyama, Minamimaki, Minamiaiki, Nagano	34	2n=88(1), 89(1), 92(1), 93(1), 94(1), 95(1), 96(2), 98(3), 100(1), 101(2), 102(2), 103(3), 104(5), 105(1), 106(2), 108(3), 109(2), 110(1), 116(1)
12	Mt. Tateshina, Chino, Nagano	4	2n=96(1), 101(1), 102(2)

* Numbers in parenses indicate the number of individuals examined.

Pollen mother cells and tetrads were stained by 2% aceto-carmine or 2% aceto-orcein. Cotton blue lacto-phenol solution (1%) was used to check the pollen stainability. Pollen stainability was estimated from samples of 200 grains.

The inflorescences of var. *aizoon* is a polychasium usually consisting of five main branches. The seed fertility was estimated on the terminal flowers of these branches from 28 plants of two populations.

Results

Variation in chromosome number

Somatic chromosome numbers of 114 individuals of *Sedum aizoon* var. *aizoon* were counted (Table 2) and some of the mitotic metaphase plates are shown in Fig. 1. They included 37 different chromosome numbers ranging from 2n=71 to 124. The chromosome numbers were constant through an individual without any exception. The chromosome numbers of 2n=71−78, 84, 86, 89, 92−95, 98−100, 103, 105, 107, 109, 113, and 124 are reported for the first time. Among them, the chromosome number 2n=96 was the most frequent (13 out of 114 individuals). No correlation could be found between chromosome number and geographical distribution (Fig. 2). However, the chromosome number was highly variable in all populations. The plants with different chromosome numbers were found side by side (Fig. 3).

Irregularities in microsporogenesis

Male meiosis was investigated in 19 individuals with 2n=82 to 116. All indivi-

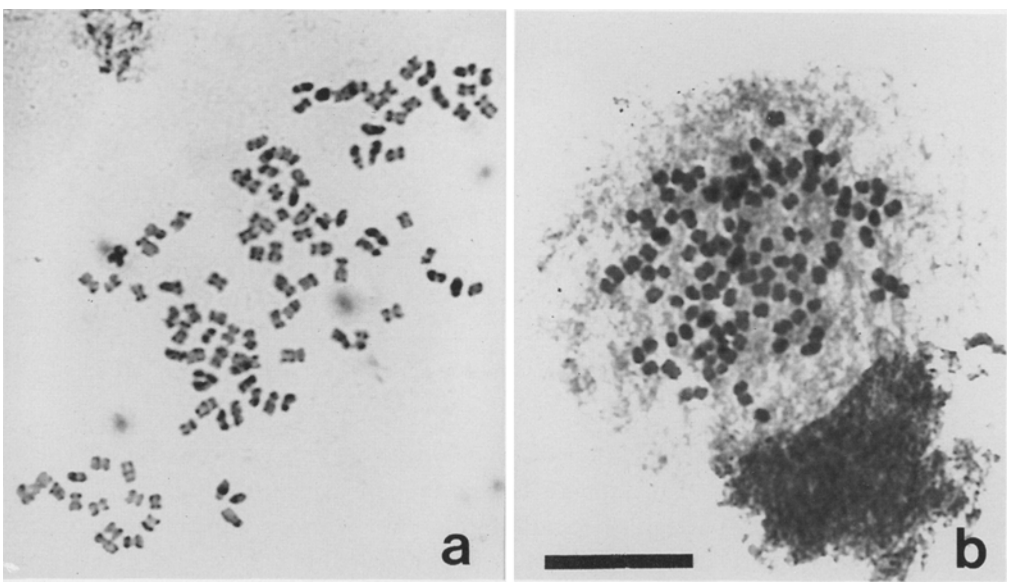

Fig. 1. Somatic chromosomes of *Sedum aizoon* var. *aizoon*. **a**, (2n=106), No. 22 Population 9. **b**, (2n=112), No. 38 Population 9. Bar indicates 10 μm.

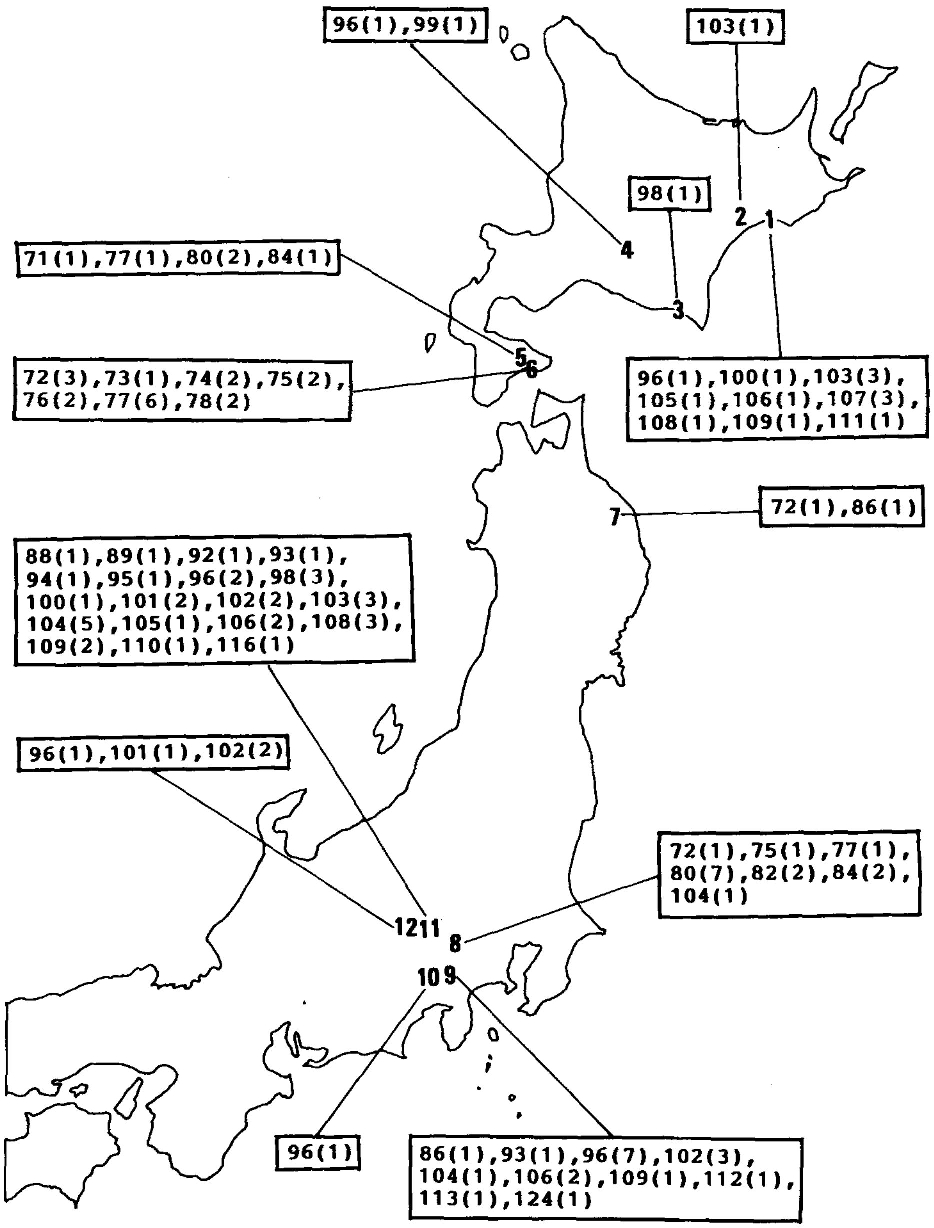

Fig. 2. Geographical variation of chromosome numbers in *Sedum aizoon* var. *aizoon*.

duals examined showed various kinds of irregularities: 1) univalent and multivalent formation at diakinesis and metaphase I (Fig. 4a); 2) asynapsis (Fig. 4b); 3) chromosome lagging; and 4) irregular spindle formation. Various numbers and sizes of microspores were observed at the "tetrad" stage (Figs. 4c, 4d).

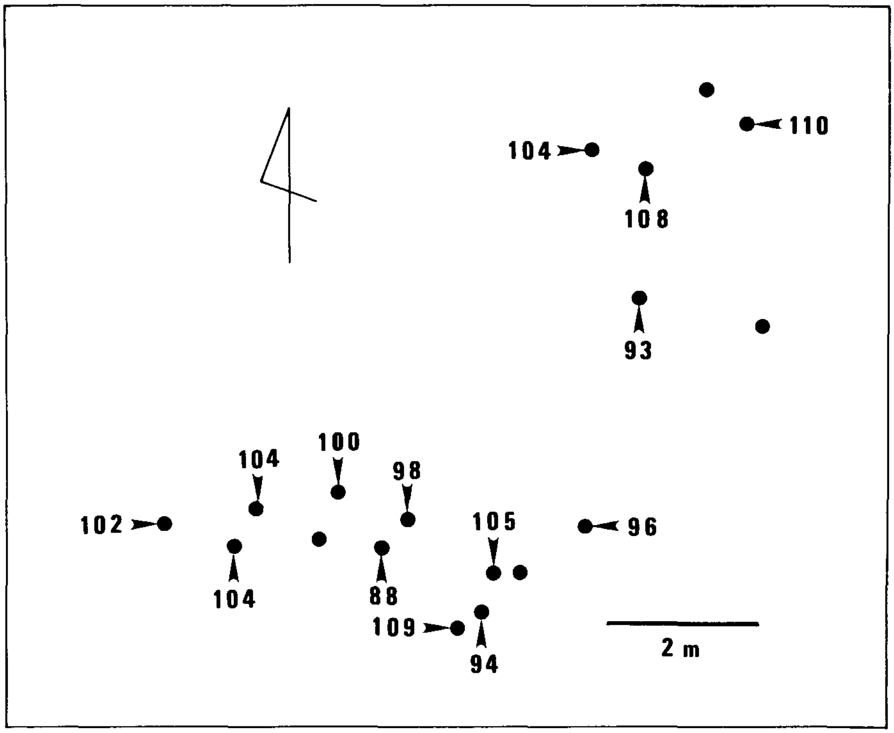

Fig. 3. Distribution of individuals and their chromosome numbers in a part of the Population 11 (Minamimaki).

Pollen size and stainability

Fig. 5 shows the variation in diameter of the pollen grains. The pollen grains are easily divided into two classes by their size (small, 3–12 μm in diameter; and large, 16–34 μm). Most of the large pollen grains were stained by cotton blue, but small ones were not.

All individuals examined produced various kinds of abnormal "tetrads" (11.5–48.5%) with many normal tetrads. The most frequent types of abnormal "tetrads" were different in different individuals. Most are included in one of two types. One consists of four large microspores and a small one (4+1), and the other, only three large ones (3+0). All individuals examined produced both types.

There was no correlation between pollen stainability and chromosome numbers (Fig. 6). The proportion of stained pollen grains differs among the populations. The Nippara (Population 8) and the Minamimaki (Population 11) populations showed rather high pollen stainability, while the Mt. Hakodate (Populations 5 and 6), the Mt. Shakushi (Population 9) and the Tateshina (Population 12) populations showed rather low stainability.

Seed fertility

The seed fertility varied from 11.4% to 42.9% in Population 9 (Table 3) and

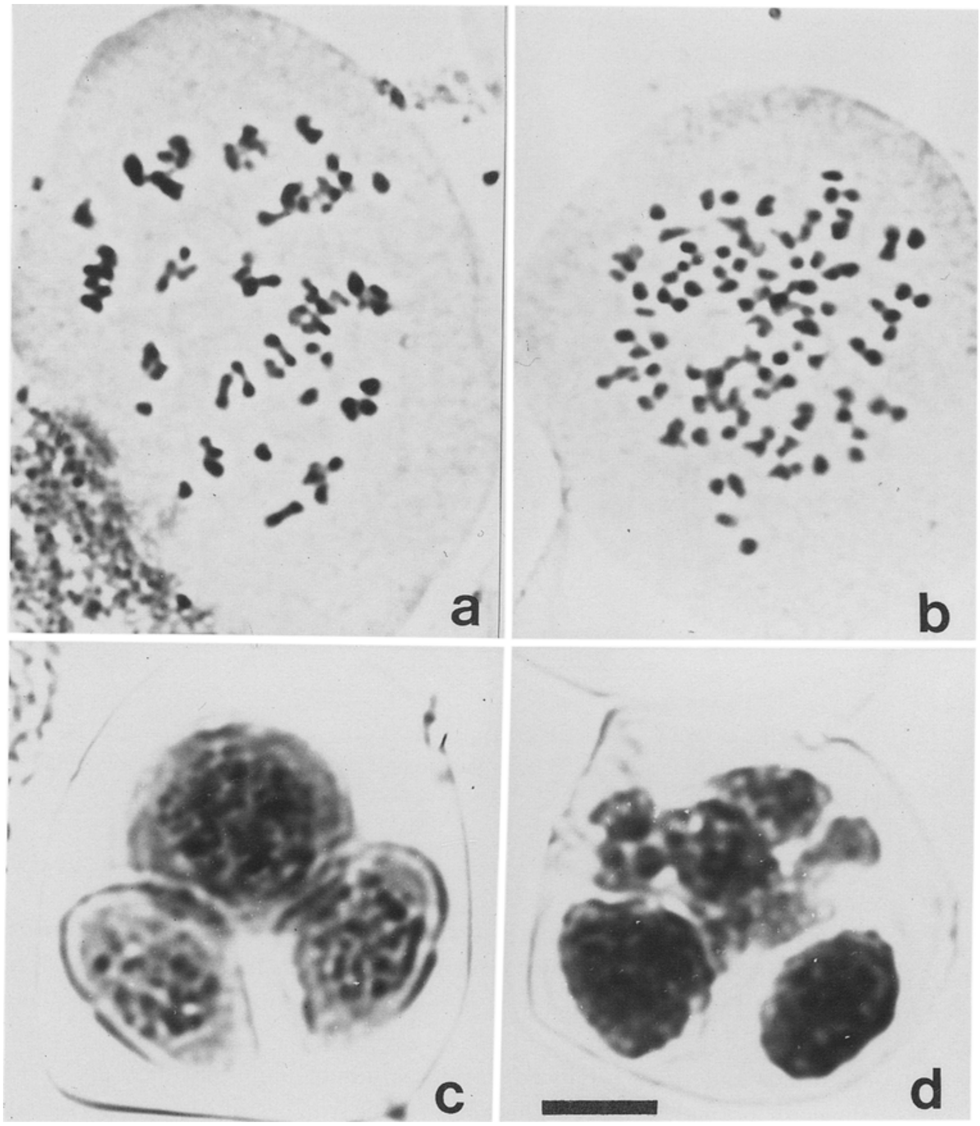

Fig. 4. Meiotic irregularities in *Sedum aizoon* var. *aizoon* (2n=104) (a, b). **a**, Occurrence of multivalents and univalents at diakinesis stage. **b**, Asynapsis. **c**, **d**, The tetrad stage of an individual of *Sedum aizoon* var. *aizoon* (2n=96). **c**, One large and two medium-sized microspores. **d**, Two medium-sized and six small microspores. Bar indicates 10 μm.

0.6% to 18.3% in Population 11 under natural conditions. The proportions were different among individuals, and even aneuploids set seeds.

Chromosome number variation of seedlings from a single mother plant

The chromosome numbers of eight seedlings derived from the single mother plant with 2n=86 collected from Mt. Shakushi (Population 9) were counted. The seedlings

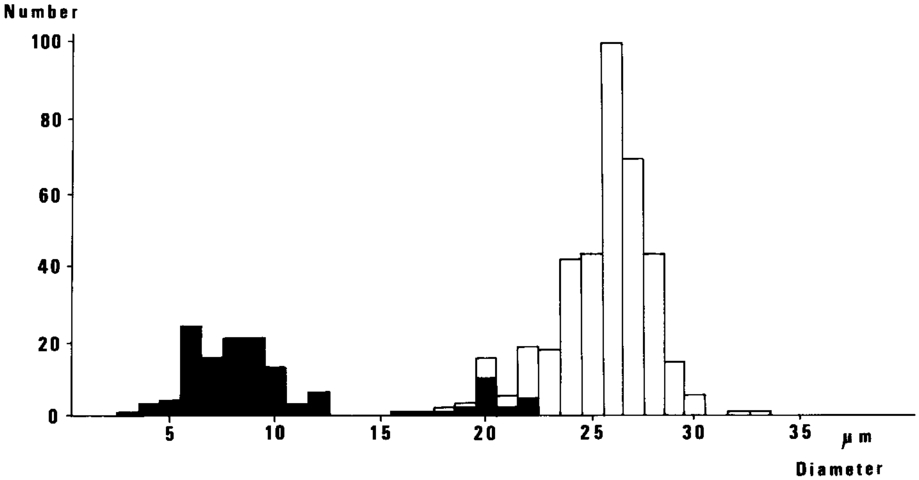

Fig. 5. Variation in pollen diameter of one individual (2n= ca. 121) from the Population 9. Open bars indicate the numbers of stained pollen grains by cotton blue lacto-phenol solution, and solid bars indicate those of unstained grains.

Table 3. Seed fertility in Population 9 (Mt. Shakushi)

Plant Number	Number of flowers	Number of carpels	Number of fertile seeds (total)	Percentage of fertile seeds	Chromosome number
1	5	25	79 (184)	42.9	2n=106
2-1	5	25	154 (494)	31.2	2n=104
3-1	4	20	84 (276)	30.4	2n=102
4	4	19	47 (175)	26.9	2n=113
5	5	29	130 (501)	25.9	2n=96
3-2	4	20	80 (314)	25.5	2n=102
6	5	33	115 (467)	24.6	2n=96
7	4	20	64 (266)	24.1	2n=96
8	6	29	101 (419)	24.1	2n=102
3-3	4	20	74 (338)	21.9	2n=102
9	5	30	51 (239)	21.4	2n=96
3-4	4	20	72 (345)	20.8	2n=102
10	4	20	50 (222)	18.1	2n=105
11	4	20	47 (265)	17.9	2n=109
12	6	32	123 (700)	17.6	2n=96
13	5	30	36 (290)	12.4	2n=86
2-2	2	10	19 (166)	11.4	2n=104

Data in Plant 2 were taken from two different inflorescences, and data in Plant 3 were taken from four different inflorescences.

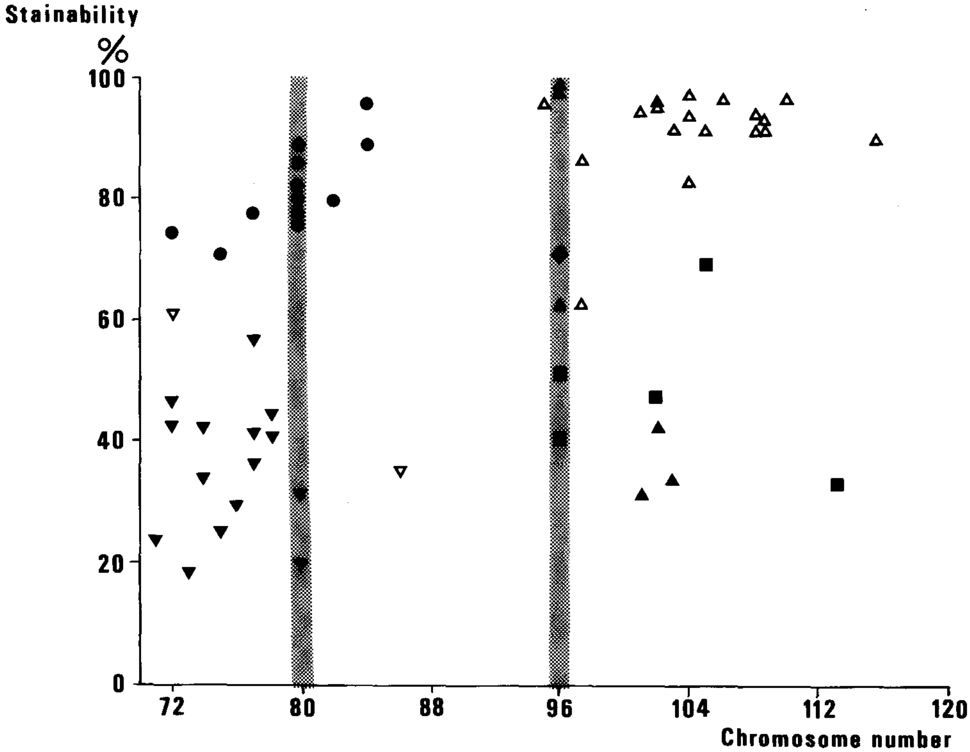

Fig. 6. Correlation between proportion of stained pollen and chromosome numbers. The chromosome numbers of 2n=80 and 96 are euploidal numbers (10x and 12x based on x=8). Different marks indicate different localities. Closed circle, Nippara; Open inverse triangle, Iwaizumi; Closed inverse triangle, Mt. Hakodate; Open triangle, Minamimaki; Closed square, Mt. Shakushi; Closed triangle, Mt. Tateshina; Closed diamond, Nenba.

obtained from open pollinated seeds had chromosome numbers of 2n=86, 96, 98 (three seedlings), 102, 106, and 110.

Discussion

The somatic chromosome numbers of *Sedum aizoon* var. *aizoon* are extremely variable. They are almost serial from 2n=71 to 113. The basic chromosome number for the subgenus *Aizoon* is eight (Amano and Ohba, 1990), hence var. *aizoon* seems to include decaploids (2n=80), dodecaploids (2n=96), tetradecaploids (2n=112), and various kinds of aneuploids. Lower polyploids such as 4x, 6x and 8x are not found in this variety, in contrast they are frequently found in var. *floribundum* (Amano, 1990). Given the morphological resemblance between var. *aizoon* and var. *floribundum* it is clear that they are closely related. Some of differences between them are quantitative (number of serration on leaves, plant height, number of leaves on flowering stems, etc.) and the mean values of these characters are larger in var. *aizoon* than in var. *floribundum*, so that var. *aizoon* appears to be a large-sized var. *floribundum*. Other characters indicate that var. *aizoon* is a specialized form. The lack of lower ploidy levels than octaploid indicates that var. *aizoon* has an allopolyploid origin or the lower polyploid ancestors have become extinct in Japan. In comparable individuals of the same ploidy levels of var. *aizoon* and var. *floribundum*, they are distinguishable by the morphological differences and classified into the two varieties, hence it is not plausible to suggest that var. *aizoon* was directly derived from higher polyploids of var. *floribundum*.

In var. *floribundum* there are several "morphotypes" with their own combination of morphological characters, ploidy levels and geographical distribution (Amano, 1990). In contrast var. *aizoon* is monotypic.

The 12 populations examined were chosen to cover the geographical range of var. *aizoon* in Japan. No correlation was found between geographical distribution and chromosome number. In contrast, in the var. *floribundum* tetraploids occurred in the eastern parts of Honshu, octaploids in Hokkaido and the other polyploids including 10x and 12x mainly in the western parts of Honshu, Shikoku, and Kyushu (Amano, 1990, 1991).

In var. *aizoon* chromosome numbers vary within all populations examined (Table 2). This is a characteristic of var. *aizoon*. The chromosome numbers within a single population vary almost continuously. Plants whose chromosome numbers are one and a half or twice that of other individuals in the same population were not found. This indicates that infrapopulational chromosomal variation is not due to the union of unreduced gametes or somatic chromosome doubling. This inference is possible in the case of var. *floribundum* (Amano, 1991).

Extraordinary infrapopulational chromosome number variations have been reported in *Poa* species (Nygren, 1950; Löve, 1952; Tateoka, 1980), *Kalimeris yomena* Kitam. (Shindo, 1966, 1967), *Cardamine pratensis* L. (Lövkvist, 1956) and *Claytonia virginica* L. (Rothwell, 1959; Lewis *et al.*, 1967; Lewis and Semple, 1977).

In *Poa irrigata* all individuals examined also set seeds and the chromosome numbers of seedings differed from that of the parent plant in 15 out of 19 strains (Löve, 1952). He considered that such chromosome number variation was caused in two different ways: one is due to "the uneven somatic divisions of extra chromosomes", and another is due to "the irregularities in the apomictic behaviour of mother plants making normal sexual seed formation". Rothwell (1959) supposed that in *Claytonia virginica* infrapopulational variation of chromosome numbers is due to various kinds of irregularities in meiosis. He pointed out the possibility that abnormalities in meiosis such as nondisjunction and chromosome lagging leads to chromosome number variation within a population. He also considered the union of reduced and unreduced gametes to be a cause of their chromosome number variation. Lövkvist (1956) considered that the chromosome number variation in *Cardamine pratensis* was caused by the segregation of aneuploids after hybridization between populations with different chromosome numbers. In the case of var. *aizoon* the type of chromosome number variation is similar to that of *Claytonia virginica*.

In *Claytonia virginica*, meiotic irregularities were found in aneuploids, however most euploids did not show any irregularities (Rothwell, 1959). In contrast, in var. *aizoon* all plants show various kinds of irregularities even in the putative euploids based on chromosome number. It suggests that the putative euploids seem to have unbalanced chromosome constitutions rather than "euploidal" constitutions, because their meiotic behaviour is very irregular. The meiotic irregularities in *Poa arctica* and *P. pratensis* (Nygren, 1950) were similar to those in var. *aizoon*, but these *Poa* species are predominantly apomictic.

It is of interest that extensive infrapopulational chromosome number variation has always been found in the high polyploid states in several taxa (Nygren, 1959; Lövkvist, 1956; Shindo, 1966, 1967; Amano, 1991). There might be some compensations for the detrimental effects of unbalanced chromosome constitution in higher polyploids.

Various kinds of irregularities in meiosis lead to the formation of gametes with different chromosome numbers. Various kinds of irregular "tetrads" were observed in var. *aizoon*. The small microspores contained one or a few blocks of chromatin. The existence of "tetrads" having one or more small microspores suggests the formation of pollen grains with different chromosome numbers within a single "tetrad". Fig. 4C shows unreduced and reduced microspore formation.

All plants examined set seeds. The seedlings derived from the single mother plant had various chromosome numbers. Variety *aizoon* has no effective method for vegetative propagation; seed formation is essential for reproduction. In addition very few seeds set when their inflorescences are bagged (Amano, unpublished), thus it seems that var. *aizoon* is a sexual outbreeder.

In var. *aizoon* the extensive infrapopulational chromosome number variation is most possibly caused by unequal chromosome segregation in meiosis and the subsequent fertilization of gametes with various chromosome numbers. But we cannot exclude completely the possibility that this is caused by the combination of the irregular behaviour of B chromosomes and several levels of polyploidy. The autosomes are very small and hence may not be distinguished from B chromosomes by their size.

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