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Cytogeography of *Artemisia keiskeana* (Asteraceae: Anthemidae)

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Artermisia keiskeana Miquel belonging to the section *Abrotanum* Besser is a long-lived perennial herb propagating vegetatively by stout rhizomes as well as sexually by seed production. The sterile shoots are decumbent with a tuft of rosulate leaves at the tip. The flowering stems of 30–80 cm high are branched and often silky above. It has a wide distribution throughout Japan, Korea, northern China and southeastern Russia (Kitamura 1940, Volkova and Boyko 1986). It occurs in sunny and half-shady dry places on hills and low elevations in the mountains. The chromosome number of this species has been reported to be $n=9$ and $2n=18$ by Shimotomai (1946, 1947: Hiroshima, Japan), Suzuka (1950, 1952, 1958: Aomori and Kagawa, Japan), Arano (1946: Tokyo and Saitama, Japan), Lee (1967: Korea), Nishikawa (1984: Hokkaido, Japan) and Volkova and Boyko (1986: Vladivostok, Russia). In addition the occurrences of B chromosomes in a proportion of individuals in a population and triploids of this species have been reported by Masumori (1961, 1969).

A detailed cytological study may be able to reveal the structure and dynamics of the populations, the mechanisms of the maintenance of various cytotypes and the intraspecific relationship occurring over the wide geographical areas. In addition a detailed knowledge about the differences of ploidy level and habitat between species in relation to their present geographical range in *Artemisia* producing abundant pollen grains for wind pollination may be able to give a clue to specify the species as one of indicators of the expansion of open habitats in the Quarternary history of flora and vegetation for pollen analysis of the sediments. Because polyploidy has played a major role in the speciation of the genus *Artemisia* and the polyploids with larger pollen grains have frequently occupied different habitats from those of diploids.

In this paper we report the cytological characteristics for plants in 11 natural populations and the mitotic and meiotic behavior of B chromosomes in Japanese *A. keiskeana.*

Materials and methods

Original 11 collection localities of *Artemisia keiskeana* Miquel investigated are given in Table 6. Plants were dug from natural populations and raised in pots in an experimental garden at Yamaguchi University. For the somatic karyotype analyses and chromosome counts, root tips were pretreated with 0.002 M 8-hydroxyquinoline solution at 20°C for 70–90 min. They were fixed in 45% acetic acid solution at 5°C for 20 min and stained with 1% aceto-orcein solution following hydrolysis in a 1 : 1 mixture of N HCl and 45% acetic acid at 60°C for 20 sec, and squashed under a cover glass. Since the number of B chromosomes in each cell is not

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constant in root of each plant, chromosome number determination was made on 25 cells/5 roots/plant. For diploid karyotype analyses five cells containing well spread chromosomes were selected and used for measurements. Photographs of chromosome were magnified ×5600, and the lengths of the arms were measured using a scale with 0.5 mm unit. Chromosomes are designated following nomenclature recommended by Levan *et al.* (1964). For the observation of mitotic and meiotic chromosome behaviors, the same fixatives was used but no pretreatment or hybrolysis was given. The length of peduncle was measured on 100 samples by the slide calipers.

Results

Polymorphic karyotypes

Diploid cytotype (Fig. 1-A): The size of metaphase chromosome is 8.64 μm to 5.48 μm in the present study, ranging continuously in different pairs (Table 1). The haploid chromosomes are composed of 7 median and 2 submedian chromosomes. Two submedian chromosomes have a very small satellite at the end of short arm (Fig. 2).

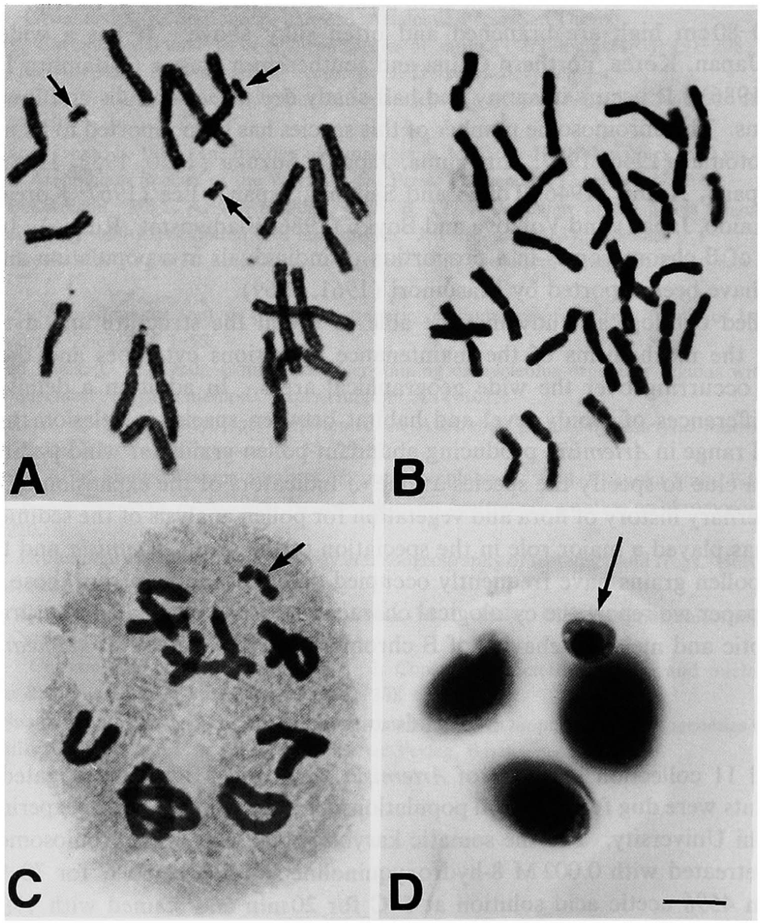


Fig. 1. Mitotic and meiotic chromosomes in *Artemisia keiskeana* A: diploid somatic cell with 2n=18+3Bs (arrowed), B: triploid somatic cell with 2n=27, C: chromosome pairing of 9 II+B III at diakinesis. Arrow indicates the chain trivalent of B chromosomes. D: additional microcyte (arrowed) formation at tetrad stage in a plant containing B chromosome. Scale=5 μm.

Table 1. Chromosome measurements of somatic metaphase in *Artemisia keiskeana*

Chromosome	Short arm (μm) mean (S.E., S.D.)		Long arm (μm) mean (S.E., S.D.)		Total length (μm)	Arm ratio	Chromosome mophology
1	4.06 (0.08, 0.27)	+	4.58 (0.10, 0.31)	=	8.64	1.13	m
2	3.37 (0.08, 0.26)	+	4.89 (0.11, 0.35)	=	8.26	1.45	m
3	3.52 (0.07, 0.21)	+	3.93 (0.08, 0.25)	=	7.45	1.12	m
4	3.12 (0.05, 0.17)	+	4.11 (0.06, 0.27)	=	7.23	1.32	m
5*	2.67 (0.09, 0.29)	+	4.55 (0.09, 0.28)	=	7.22	1.70	sm
6	2.83 (0.09, 0.30)	+	4.30 (0.15, 0.47)	=	7.13	1.52	m
7	2.82 (0.06, 0.20)	+	3.66 (0.09, 0.27)	=	6.48	1.30	m
8	2.78 (0.06, 0.18)	+	3.23 (0.11, 0.35)	=	6.01	1.16	m
9*	1.41 (0.07, 0.21)	+	4.07 (0.07, 0.22)	=	5.48	2.89	sm
B1	1.22	+	1.44	=	2.66	1.18	m
B2*	0.64	+	0.64	=	1.28	1.00	m
B3	0.39	+	0.63	=	1.02	1.62	m

*satellited chromosome

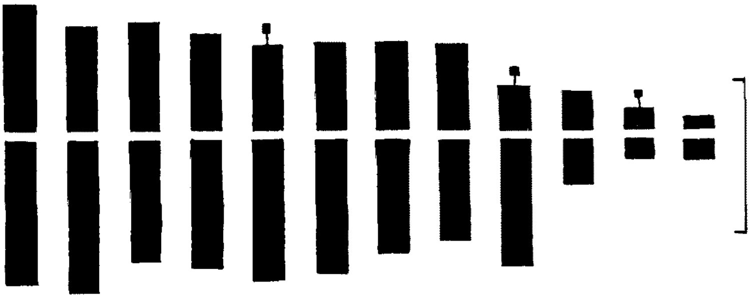


Fig. 2. The idiogram of mititic haploid karyotype with B chromosomes in *Artemisia keiskeana*. Scale = 5 μm.

There is no karyotypic difference between the regular diploid complement with and without B chromosomes.

Triploid cytotype (Fig. 1-B): The size of metaphase chromosome is 6.8 μm to 3.6 μm in the present study. The karyotype consists of three chromosome sets. Each set consists of 7 median and 2 submedian chromosomes. Two submedian chromosomes have a very small satellite at the end of short arm. It is karyotypically an auto-triploid.

The karyotypes of diploid and triploid are fundamentally consistent with those of previous reports (Masumori 1961, 1969, Arano 1964). The difference in length of karyotypes among these may be due to the different pretreatments of roots.

B chromosomes (Fig. 1-A): B chromosomes of this species are small (2.66–1.02 μm) and somewhat variable in appearence. The number of B chromosome varied from zero to ten both within and between plants. Table 2 shows a part of variation in number of B chromosomes within a plant at Locality 4. Although the ranges in number of B chromosomes are different from plant to plant, these data clearely show the cell to cell and root to root variation in B chromosome number within a plant. B chromosomes are approximately one second to fifth the size of the smallest autosome and appear to be entirely euchromatic. They are almost metacentric (arm ratio 1.00–1.64). It is, however, extremely difficult to prove whether a given B chromosome is homologous with others in the different somatic cells, roots or plants, or not. Then the measurements of three B chromosomes (the longest, satellited and the shortest) in Table 1 are give by the bare value just for the comparisons to the autosome lengths. Table 3

Table 2. Mosaicism of B chromosome number within a plant in *Artemisia keiskeana*

Plant	Root number	Number of examined cells	Number of B chromosome per cell									
			0	1	2	3	4	5	6	7	8	9
24	1	6	5	1								
	2	5	4	1								
	3	11	7	2	2							
	4	7	2	4		1						
	5	7		1	3	3						
28	1	6	3	3								
	2	6	4	2								
	3	6		1	5							
	4	5		1	2		2					
	5	7		1	1	3	2					
	6	7		1	2	2	1	1				
	7	15		2	4	4	3	2				
37	1	6		1	3	2						
	2	7		3	2	2						
	3	5			2	1	2					
	4	9	1		4	2	1	1				
	5	6		1		3	1	1				
	6	6				2	3	1				
	7	10			1	6	2	1				
	8	8			1	1	3	2	1			
	9	6				1	1	2	1	1		
	10	5					2	2		1		
	11	13					3	2	3	5		
	12	5						2	2	1		
	13	7						2	4	1		
	14	6						2	3		1	
	15	5						2	1	1	1	
	16	8		1		1	1	2	1	1		1

Table 3. Comparison in length and morphology of 4B chromosomes within a cell in *Artemisia keiskeana*

Plant	Short arm (μm)		Long arm (μm)		Total length (μm)	Relative length	Arm ratio
1	0.96	+	1.06	=	2.02	1.58	1.10
	0.57	+	0.88	=	1.45	1.13	1.54
	0.71	+	0.71	=	1.42	1.11	1.00
	0.64*	+	0.64	=	1.28	1.00	1.00
2	1.04	+	1.12	=	2.16	1.34	1.08
	0.67	+	0.97	=	1.64	1.02	1.45
	0.67	+	0.95	=	1.62	1.01	1.42
	0.67	+	0.94	=	1.61	1.00	1.40
3	1.19	+	1.24	=	2.43	1.52	1.04
	0.94	+	1.24	=	2.18	1.36	1.32
	0.90	+	0.90	=	1.80	1.13	1.00
	0.68	+	0.92	=	1.60	1.00	1.35

*satellited chromosome. Relative length is caluculated as 1.00 for the shortest in each cell. These measurements indicate that B chromosomes are slightly different from one another in morphology, except three chromosomes in Plant 2.

gives the measurements of the three cells having 4B chromosomes in different plants. B chromosomes are different slightly from each other in their length and centromeric position. One of them has a satellite at the end of one arm.

Male meiosis and pollen formation of triploid

In the PMCs of triploid the meiotic configurations are as follow: trivalents are comparatively few (1–3) in number as compared with those of the bivalents (6–9) and univalents (4–7). In spite of their irregular meiosis containing trivalents or univalents, the fairly regular pollen grain formation has been observed.

Mitotic and male meiotic behavior of B chromosome

In male mitosis B chromosome is euchromatic in nature and shows similar condensation and decondensation behavior as autosomes. At prometaphase B chromosomes seem to behave same as autosomes and they are on the equational plate at metaphase. Some of B chromosomes divide normally but others undergo non-disjunction and drift to poles at random during mitotic anaphase. The variable segregation patterns of B chromosomes such as 1 : 0, 2 : 2, 3 : 0 and 3 : 1 have been observed at anaphase.

In male meiosis B chromosomes are either unpaired or formed bivalent or multivalent (Fig. 1-C) in the PMCs containing more than two B chromosomes. B chromosomes show no pairing affinity with autosomes of the genome. At first metaphase B chromosomes may be on the equational plate or towards one pole. In the latter case it moves undivided to one pole and is then included in one interphase nucleus. Where it is placed on the metaphase plate, it may occasionally lag at anaphase when it may be lost in the cytoplasm, or even divide or misdivide. The chromosome lagging or non-disjunction seems to lead to the formation of micronucleus and microcyte (Fig. 1-D) at tetrad stage. In 189 (14.2%) of the total 1335 tetrads examined, the micronucleus and microcyte formation have been observed (Table 4).

Morphology and cell size in triploid and plant with B chromosome

The length of guared cells of stomata in triploid is significantly longer than that in diploid, although the range of their length in triploid (29–57 μm) overlaps that in diploid (23–40 μm) (Table 5). The length of peduncle in triploid (range 6–13 mm, mean 8.93±2.53 mm) is also singificantly longer than that in diploid (range 2–6 mm, mean 4.03±0.93 mm) ($t=18.18$; $p \ll 0.01$). We could not detect any other significant phenotypical differences between diploids and triploids.

There is no obvious phenotypical consequences on plant which carry B chromosomes, too.

Table 4. Micronucleus or microcyte formation at tetrad stage in plants containing B chromosome of *Artemisia keiskeana*

Floret number	No. examined	No. tetrads with micro-n	No. tetrads with micro-c	No. tetrads with micro-n & c (%)
1	61	1	0	1 (1.6)
2	160	4	1	5 (3.1)
3	125	8	4	12 (9.6)
4	51	4	1	5 (9.8)
5	110	7	5	12 (10.9)
6	160	13	7	20 (12.5)
7	98	7	7	14 (14.3)
8	68	5	5	10 (14.7)
9	134	13	11	24 (17.9)
10	146	17	14	31 (21.2)
11	162	20	17	37 (22.8)
12	60	10	8	18 (30.0)
Total	1335	109	80	189 (14.2)

Table 5. Comparison in length of the guard cell of stomata in diploids (5 plants) and triploids (4 plants)

Plant number (ploidy)	No. cells measured	Range (μm)	Mean±S.D. (μm)
1 (2x)	289	23–36	29.9±2.50
2 (2x)	289	25–37	31.5±2.34
3 (2x)	261	26–40	31.9±2.39
4 (2x)	256	25–40	32.2±2.70
5 (2x)	256	28–38	32.9±2.40
6 (3x)	400	32–45	36.8±2.22
7 (3x)	289	29–46	38.0±2.58
8 (3x)	400	36–52	44.7±9.89
9 (3x)	289	38–57	45.8±3.22

$(t6-5=21.26;\ p \ll 0.01)$

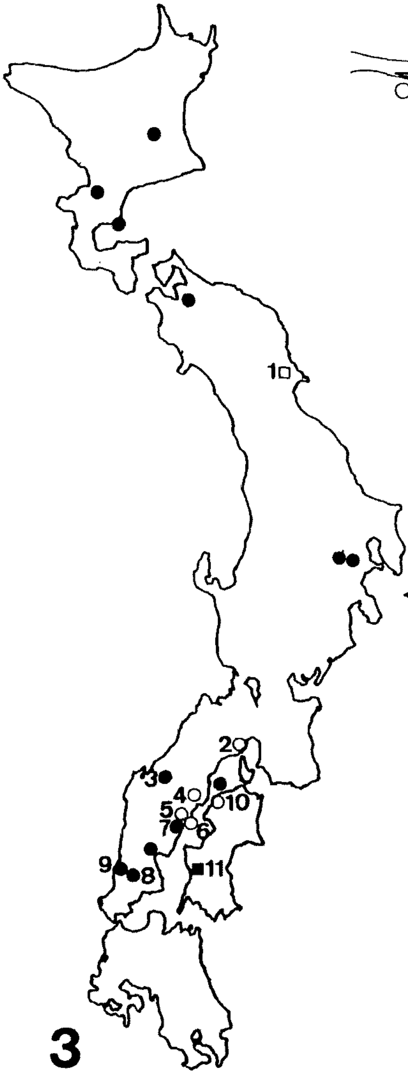
Geographical distribution of cytotypes

Chromosome counts are made on 391 accessions from 11 populations. These data have been collected over a period of ten years in different localities throughout the Japan Archipelago. Results of the observations are summarized in Table 6 and Fig. 3. Cell to cell variation of B chromosome within a plant make it impossible to assign a fixed number of B chromosome as typical of a give individual, and therefore data collected on the distribution of plant with B chromosome in Table 6 are expressed as proportion of plants with B chromosome, at least, in one cell. The order in which localities are listed is roughly that of their distribution from north to south. Of the 11 localities, 85 accessions from 4 populations (Localities 3, 7, 8 and 9) have the chromosome number $2n=18$ only. In the remaining 7 populations, standard diploids are found growing togather with triploids and/or diploids containing B chromosome. Fourteen accessions (3.5%) from 2 populations (Localities 1 and 11) have the chromosome number $2n=27$. These two populations are distantly isolated from each other. In the 6 populations, 80 accessions carry a variable number of B chromosomes over and above the regular diploid complement. Overall, 20.5% of accessions carry B chromosomes. Three close proximate populations (Localities 4, 5 and 6) contain the fairly high proportion of plants with B chromosomes. These frequencies are 58.9% at Locality 4, 55.2% at Locality 5, and 31.0% at Locality 6, respectively. The number of B chromosome also vary with a wide range in these

Table 6. Chromosome number determinations in *Artemisia keiskeana*

Locality	I	II (%)	III (%)	IV (%)
1. Iwate Pref., Nishiiwai-gun, Hanaizumi-cho, Mt. Takakurayama	63	52 (82.5)	2 (3.2) range 0– 1	9 (14.3)
2. Hyogo Pref., Kobe-city, kande-cho, Mt. Mekko	17	15 (88.2)	2 (11.8) range 0– 2	
3. Okayama Pref., Maniwa-gun, Yatsuka-mura, Mt. Hiruzen	28	28 (100)		
4. Okayama Pref., Kibi-gun, Takamatsu-cho, Myomi	56	23 (41.1)	33 (58.9) range 0– 9	
5. Hiroshima Pref., Hukayasu-gun, Kannabe-cho, Mitani (sites 1 & 2)	58	26 (44.8)	32 (55.2) range 0– 6	
6. Hiroshima Pref., Hukuyama-city Ashida-cho, Shimoyuchi	29	20 (69.0)	9 (31.0) range 0–10	
7. Hiroshima Pref., Mihara-city, Mara	14	14 (100)		
8. Yamaguchi Pref., Abu-gun, Ato-cho, Mt. Tokusagamine	15	15 (100)		
9. Yamaguchi Pref., Abu-gun, Susa-cho, Susa	28	28 (100)		
10. Kagawa Pref., Takamatsu-city, Yashima, Dainoue	30	28 (93.3)	2 (6.7) range 0– 8	
11. Ehime Pref., Matsuyama-city, Setokaze-toge (sites 1 & 2)	53	48 (90.6)		5 (9.4)
Total	391	297 (76.0)	80 (20.5)	14 (3.5)

I: Number of plants examined, II: Number of standard diploids, III: Number of diploids containing B chromosome, IV: Number of triploids with $2n=27$.



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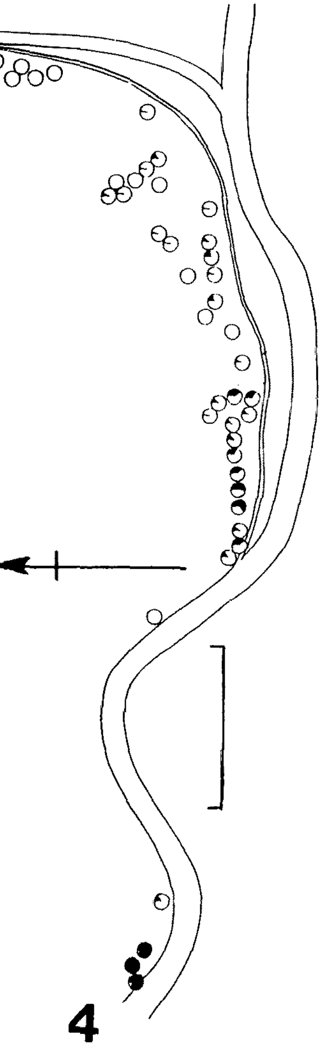

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Fig. 3. Localities of populations of *Artemisia keiskeana* of known cytotypes in Japan. Number refers to locality corresponding to the locality number in Table 6. Solid circles denote diploid populations without B chromosomes, open circles denote diploid populations with B chromosomes, solid squere denotes the mixed population of triploids and diploids with and without B chromosomes, open squere denote the mixed population of triploids and diploids with and aithout B chromosomes.

Fig. 4. Spatial distribution of *Artemisia keiskeana* with and without B chromosomes along the forest margin at site 1 of Locality 5. Proportion of circles darkened indicates the proportion of cells containing B chromosomes. Scale = 10 m.

populations. Fig. 4 shows the spatial distribution of plants with or without B chromosomes at site 1 of Locality 5. From a glance at the distribution map, it seems very likely that the distribution of plants with B chromosome is not random since they tend to grow close together.

Discussion

Fig. 3 displays the distribution of population of *Artemisia keiskeana* of known chromosome number, incorporating our data and those of previous Japanese reports. It shows that the diploids are widely distributed in the Japan Archipelago. Korean an Russian reports also confirmed the wide occurence of diploid throughout the ranges of the species. A mode of chromosomal variation in this species is characterized by the occurrence of triploid and B chromosome. For the limited samples of eastern end of the species range and for single count based on the previous reports, we can refer only to the preliminary comment on the tendancy of the occurrences of different cytotypes in relation to the geographical location of the population. Triploids are restricted two populations distantly isolated from each other. Since we could not find any aneuploid derivatives or hybrids with diploid in these natural populations, the failure of seed propagation in triploid has been suggested in spite of their fairly regular pollen formation. They seem to have originated spontaneously and to be maintained vegetatively in the field. Although an increase in length of the guared cell and peduncle has been observed, there is no evidence that triploids are far superior in fitness to diploids in these fields. Polyploidy has played a major role in the speciation of the genus *Artemisia* and the polyploids occupy different habitats from those of diploids in several other species in this genus (Taylor *et al.* 1964, Eates 1968). Tetraploids *A. indica* Willd. (2n=34, Japanese name Yomogi in Hara 1984) and *A. japonica* Thunb. (2n=36) occur over the wide geographical ranges same as *A. keiskeana* in the Japan Archipelago. The former species is a successful colonizer or weed occupying more waste or disturbed places. *Artemisia keiskeana* associates with the more natural and stable vegetation, and seems not to have formidable colonizing ability such as *A. indica.* Then the significance of polyploidy in *A. keiskeana* should not be overemphasized at present.

B chromosomes are found over a wide geographical range of Japan. These B chromosomes, however, are mitotically unstable and vary in number in different cells within and between root tips. The unequall B chromosome segregation and non-disjunction at mitotic anaphase may result in a variability in number of B chromosome in different cells. The degree and pattern of B chromosome mosaicism in this species suggests that they eliminate at various stages in the developments. Presumably elimination must occur by non-disjunction and loss of chromatids into the cyloplasm and by microcyte formation in meiosis. A question that must, at present, remain unanswered is why the B chromosome has become so widely distributed and has occurred in large proportion of individuals of three populations around the boundary of Okayama and Hiroshima Prefectures, in spite of their eliminations both in mitosis and meiosis. The tendency of non-random distribution of plants with B chromosome in their respective localities may, at least in part, be due to asexual reproduction. It is uncertain at present whether B chromosome has any selective advantage, or make any contribution to the fitness of the individuals with them, or not.

B chromosomes are generaly less frequent in polyploids than in diploids (Darlington 1956). In *Artemisia keiskeana* we also have not found any B chromosome in triploids.

In the genus *Artemisia* B chromosomes have been reported in several other species (Bakshi and Kaul 1984, Stahevitch and Wajtas 1988). All these B chromosomes may be irregular as are those of *A. keiskeana.*

The origin of B chromosome in these species is unknown at present.

Summary

A total of 391 accessions from 11 populations of Japanese *Artemisia keiskeana* were cytologically examined. Twenty-one percent of these accessions carried B chromosomes in addition to the regular diploid complement and 3.5% were triploids. The frequency of plant with B chromosomes ranged from 0% to 59% among 11 populations. B chromosomes were

1/2–1/5 in length as compared with the smallest autosome and were euchromatic in nature. They were ususally mitotically unstable and varied in number from zero to ten both within and between plants. This variation in number of B chromosomes within a plant may be caused by the non-disjunction and unequall segregation during mitotic anaphase. These B chromosomes seemed not to have any phenotypic consequences on plant. Neverthless B chromosomes have been found in 6 examined populations distantly isolated from each other and have occurred in large proportion of the individuals of three populations around the boundary of Okayama and Hiroshima Prefectures. The non-random spatial distribution of plants with or without B chromosome in these natural populations may, at least in part, be due to asexual reproduction. Triploids were found in 2 populations distantly isolated from each other. They are karyotypically auto-triploids. They have occurred spontaneously and are maintained asexually in natual populations.

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