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Ploidy, geographical distribution and morphological differentiation of *Parasenecio auriculata* (Senecioneae; Asteraceae) in Japan

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Abstract *Parasenecio auriculata* is a woodland perennial herb widely distributed in Northeastern Asia, constituted by a poorly understood polyploidy with a diploid ($2n = 2x = 60$) and a tetraploid ($2n = 4x = 120$). In this study, for a better understanding of the polyploid evolution, cytogeography and morphological variation were analyzed in Japanese *P. auriculata*, including two varieties; var. *bulbifera* endemic to central Hokkaido and var. *kamtschatica* widely distributed in northern Honshu and Hokkaido. The occurrence of two polyploid levels was reconfirmed. While var. *bulbifera* is predominantly tetraploid, var. *kamtschatica* is comprised of diploid and tetraploid. Morphological variation among 22 quantitative characteristics is continuous and not distinctive among cytotypes or varieties, but plant size tended to be larger in the order, diploid of var. *kamtschatica*, var. *bulbifera*, and tetraploid of var. *kamtschatica*. The cytotype distribution showed a conspicuous geographical pattern. Besides var. *bulbifera* endemic to the central Hokkaido, the diploid of var. *kamtschatica* is mainly found in Southern Hokkaido, and the tetraploid has a disjunct distribution in eastern and northern Hokkaido and northern Honshu. Such a geographical pattern is possibly attributable to the differentiation of climatic preference among cytotypes and varieties, and may have been established in association with the climatic cline along the Japanese archipelago.

Key words Climate · Cytogeography · Morphological variation · *Parasenecio auriculata* · Polyploidy

Introduction

Polyploidization has been essential events for the evolution of flowering plants. This event is typically associated with

the morphological differentiation of a polyploid (Otte and Whitton 2000) and potentially contributes to the acquisition of new genetic and/or physiological features (Levin 1983). Such evolutionary novelty is expected to result in enhanced competitive ability or ecological tolerance compared to the diploid progenitor(s). Consequently, polyploids tend to show a distinct or peripheral distribution compared to that of the parental diploid along climatic or environmental gradients (Levin 2002; Richards 1997). In this regard, the geographical distribution of polyploid expected to reflect their ecological preferences, and cytogeographical investigations can provide basic information on the evolutionary dynamics and ecological differentiation of polyploid.

Parasenecio auriculata (DC.) J.R. Grant (Senecioneae; Asteraceae) is a perennial herb, widely distributed in Northeastern Asia (Aleutians, Kamchatka, Sakhalin, Kurils, NE China, Korea, and the northern part of Japan). This species is distinguishable from other congeneric species by several morphological characteristics such as narrow panicles, auriculated petioles and reniform leaves (Koyama 1966). Kitamura (1942) recognized three varieties in this species, two of which are distributed in Japan (Koyama 1995). One variety, *P. auriculata* var. *bulbifera* (Koidz.) H. Koyama (referred to here as “var. *bulbifera*”) is endemic to central Hokkaido and characterized by bulbils at leaf axils. The other variety, *P. auriculata* var. *kamtschatica* (Maxim.) H. Koyama (referred to as “var. *kamtschatica*”), has robust stems, larger auriculates and larger heads compared to the type variety. This variety is widely distributed in Hokkaido and the northern part of Honshu, as well as Sakhalin, Kurils, Kamchatka and Korea, representing a typical geographical range of boreal elements in Japan (Koyama et al. 1971).

In *Parasenecio auriculata*, the occurrence of two polyploid races was indicated by previous studies (summarized in Table 1), with $2n = 60$ (reported from var. *auriculata*, var. *bulbifera* and var. *kamtschatica*) and $2n = 120$ (found at var. *bulbifera* and var. *kamtschatica*). Assuming $x = 30$ in the genus (Robinson et al. 1997), the cytotypes with $2n = 60$ and 120 are diploid and tetraploid, respectively, while the $2n = 118$ cytotype was also reported in var. *bulbifera*. Despite the cytological observations, little is known about

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Table 1. Previous reports of chromosome number in *Parasenecio auriculata*

Taxa ^a /locality	Chromosome No.	Source
<i>P. auriculata</i> var. <i>auriculata</i>		
Russia		
Sakhalin	60	Sokolovskaya (1966)
<i>P. auriculata</i> var. <i>bulbifera</i>		
Japan/Hokkaido		
Yukomanbetsu	120	Nishikawa (1982)
Yukomanbetsu	118	Nishikawa (1980)
– ^b	60	Koyama (1995)
<i>P. auriculata</i> var. <i>kamtschatica</i>		
Russia		
Southern primosky	ca.70	Sokolovskaya (1960)
Ossora, Koryak	ca. 60	Sokolovskaya (1968)
Japan/Hokkaido		
Mt. Apoi	60	Arano (1964)
Mt. Sokuryo	60	Arano (1964)
Chikyu peninsula	60	Nishikawa (1984)
Mt. Otobe-dake (900 m a.s.l.)	60	Koyama (1966)
Shibetcha-cho (250 m a.s.l.)	60	Koyama (1966)
Nupuro-Mapporo (200 m a.s.l.)	120	Koyama (1966)
Wakoto peninsula	120	Nishikawa (1984)
Sounkyo (700 m a.s.l.)	120	Koyama (1966)
Japan/Honshu		
Towada	60	Koyama (1961)
Lake towada (450 m a.s.l.)	120	Koyama (1966)
Mt. Hakkoda (1,500 m a.s.l.)	120	Koyama (1966)
Mt. Goyozan (950–1,300 m a.s.l.)	120	Koyama and Hotta 2190 ^c

^aAccording to Koyama (1995)

^bInformation on locality was not provided

^cSited from a herbarium specimen in KYO

the evolutionary outcome of tetraploidization in *P. auriculata*. Although Koyama (1966) pointed out that the tetraploid of var. *kamtschatica* has larger phyllaries and florets than the diploid, differentiation of gross morphology between the diploid and tetraploid was not yet to be analyzed in detail. In addition, previous cytological reports (Table 1) have not clarified the geographical trend in the distribution of polyploids in this species, though they have indicated the coexistence of diploids and tetraploids in Japan.

In considering the geographical range of *Parasenecio auriculata* in Japan, climate is an important factor in the determination of plant distribution. This region is located in a climatic cline from cool-temperate to sub-arctic zones, as one end of the gradual shift initiated from the sub-tropical zone along the Japanese archipelago. Correspondingly, the forest vegetation in this region is represented by the transition from broad-leaved deciduous forests to sub-alpine coniferous forests along the longitudinal or altitudinal axis (Kira et al. 1967). A value for Kira's (1948) warmth index of 45(°C month) is considered to roughly accord with the conditions at the border between two forest zones (Nogami and Ohba 1991), while the development of "pan-mixed forest" is also recognized in the southern part of the Kuromatunai depression as an intermediate zone (Tatewaki 1955). Such a vegetational shift is attributable to the differentiation of the geographical range among plant species according to their climatic tolerance (Masuda 1972; Sakai and

Kurahashi 1975; Uemura et al. 1986). In this regard, Uemura et al. (1986) indicated that *P. auriculata* var. *kamtschatica* was distributed under conditions of "warm and small rain" in Hokkaido. However, their study site did not represent the whole geographical range of *P. auriculata* in Japan, and is not enough to discriminate the climatic relationship to the polyploid distribution in this species.

In this study, for a better understanding of the polyploid evolution in *Parasenecio auriculata*, the geographical distribution of the diploid and tetraploid was examined in Japan. The influence of climate on the distribution of the polyploids is also discussed based on the results obtained. In addition, a morphological analysis was performed to confirm the correlation between ploidal level and morphological differentiation in this species.

Materials and methods

Plant materials

Plant materials from *Parasenecio auriculata* for chromosome observation were collected at 44 localities in Japan, with 11 localities of var. *bulbifera* and 33 localities of var. *kamtschatica* (Table 2). Four of the 44 localities were analogous to the collection sites of previous reports on chromosome number (see Table 1), though the precise location of

Table 2. Geographical location, sample size and results of chromosome observation of *Parasenecio auriculata* at 44 localities analyzed in this study

No.	Locality	Longitude (N)	Latitude (E)	Altitude (m)	Sample size ^a	Chromosome number					
						60	61	62	90	120	121
<i>P. auriculata</i> var. <i>bulbifera</i>											
1	Maruseppu	43°55'30"	143°20'25"	320	9 (4)	–	–	–	–	8	1
2	Mt. Teshio	43°57'30"	142°53'35"	650	3 (2)	–	–	–	–	3	–
3	Sounkyo ^b	43°43'25"	142°57'05"	800	3 (–)	–	–	–	–	3	–
4	Mt. Asahi	43°38'45"	142°47'50"	1,260	5 (1)	–	–	–	–	5	–
5	Mt. Tokachi	43°27'50"	142°39'20"	700	1 (–)	–	–	–	–	1	–
6	Nukabira	43°22'00"	143°11'40"	550	3 (1)	–	–	–	–	3	–
7	Mt. Yubari	43°06'00"	142°13'00"	1,000	4 (1)	–	–	–	–	4	–
8	Mt. Memuro	42°53'50"	142°47'10"	600	3 (–)	–	–	–	–	3	–
9	Mt. Rakko	42°15'20"	143°05'20"	400	3 (–)	–	–	–	–	3	–
10	Mt. Shokanbetsu	43°38'20"	141°38'00"	650	9 (4)	–	–	–	–	9	–
11	Mt. Ogon	43°36'55"	141°27'35"	190	1 (–)	–	–	–	–	1	–
Total (var. <i>bulbifera</i>)					42 (13)	–	–	–	–	41	1
<i>P. auriculata</i> var. <i>kamtschatica</i>											
12	Rausu	44°01'40"	145°09'35"	200	5 (2)	–	–	–	–	5	–
13	Nekita pass	43°45'40"	144°51'00"	450	10 (2)	–	–	–	–	10	–
14	Lake mashu	43°34'40"	144°30'25"	650	7 (3)	–	–	–	–	7	–
15	Bekkai	43°34'30"	145°13'35"	5	6 (2)	–	–	–	–	6	–
16	Mt. Rishiri	45°11'30"	141°14'30"	900	2 (1)	–	–	–	–	2	–
17	Kabuto-numa	45°12'45"	141°42'20"	10	6 (3)	–	–	–	–	6	–
18	Lake Kutcharo	45°06'55"	142°20'50"	20	6 (2)	–	–	–	–	6	–
19	Pin'neshiri	44°52'30"	142°13'20"	90	3 (–)	–	–	–	–	3	–
20	Shotonbetsu	44°49'30"	142°18'25"	100	8 (2)	–	–	–	–	8	–
21	Kamagafuchi	44°21'30"	142°08'25"	400	5 (2)	–	–	–	–	5	–
22	Ochiishi	43°14'10"	145°30'00"	20	13 (3)	13	–	–	–	–	–
23	Akkeshi	43°01'20"	144°50'50"	90	6 (2)	6	–	–	–	–	–
24	Teshikaga	43°27'40"	144°19'40"	460	6 (5)	6	–	–	–	–	–
25	Sokodai	43°26'45"	144°12'30"	700	4 (4)	3	1	–	–	–	–
26	Mt. Oakan	43°25'45"	144°08'30"	450	12 (2)	9	2	1	–	–	–
27	On'neto	43°22'40"	143°58'30"	650	15 (2)	12	1	1	1	–	–
28	Toyosato	43°51'10"	143°45'05"	300	7 (2)	6	1	–	–	–	–
29	Tsubetsu	43°40'00"	143°53'25"	300	5 (2)	4	1	–	–	–	–
30	Notoro Peninsula	44°05'20"	144°15'20"	50	6 (2)	6	–	–	–	–	–
31	Lake Yudo	42°36'20"	143°33'15"	30	7 (3)	7	–	–	–	–	–
32	Shoya	42°03'10"	143°17'55"	100	7 (2)	7	–	–	–	–	–
33	Gokibiru	43°29'30"	141°23'50"	40	4 (1)	4	–	–	–	–	–
34	Mt. Akaiwa	43°13'55"	140°58'15"	100	9 (7)	9	–	–	–	–	–
35	Mt. Moiwa	43°01'50"	141°19'15"	200	11 (4)	11	–	–	–	–	–
36	Nishimori	42°47'25"	141°29'40"	140	11 (1)	11	–	–	–	–	–
37	Mt. Sokuryo ^c	42°19'10"	140°57'35"	150	9 (3)	9	–	–	–	–	–
38	Mt. Kariba	42°36'20"	139°59'30"	600	8 (4)	8	–	–	–	–	–
39	Mt. Otobe ^c	42°02'10"	140°16'40"	1,000	5 (–)	5	–	–	–	–	–
40	Mt. Hakodate	41°45'20"	140°42'30"	200	4 (1)	3	–	–	–	1	–
41	Mt. Eboshi	40°50'15"	141°02'45"	700	4 (1)	–	–	–	–	4	–
42	Mt. Hakkoda ^c	40°39'10"	140°55'52"	1,500	2 (–)	–	–	–	–	2	–
43	Mt. Tashiro	40°24'45"	140°23'50"	800	7 (1)	–	–	–	–	7	–
44	Mt. Moriyoshi	39°58'20"	140°37'55"	600	7 (1)	–	–	–	–	7	–
Total (var. <i>kamtschatica</i>)					227 (70)	139	6	2	1	79	0
Total (Overall)					269 (83)	139	6	2	1	117	1

^aNumbers in *parentheses* indicate sample size for the morphological analysis. "–" indicates no sample was available at the site

^bKoyama (1966) reported the chromosome number of var. *kamtschatica* at the site

^cLocalities analogous to those of previous studies (see Table 1)

the site is unclear. In Sounkyo, Koyama (1966) reported the chromosome number of var. *kamtschatica* ($2n = 120$; Table 1), but the present study dealt with samples of var. *bulbifera*. For each locality, 1–15 individuals (average, 6.11) were collected, which were cultivated at the botanical

garden or at the laboratory of the Graduate School of Science, Kyoto University. Voucher specimens deposited in the herbarium of Kyoto University (KYO) were obtained from materials collected in the field and additionally from cultivated plants. Unfortunately, specimens were not available

for all individuals examined, because some cultivated plants were senesced before flowering.

Chromosome observations

Somatic chromosomes were examined with the standard aceto-orcein squash method using root tips. Root tips were pretreated with a 0.005 M-hydroxyquinoline solution for 24 h at 4°C and fixed with a 45% acetic acid solution for 15–30 min. Then, they were macerated in a 1:2 mixture (v/v) of 45% acetic acid and 1 N HCl for 30 s at 60°C, and stained and squashed with a 1% of acetic orcein solution. The exact number of chromosomes was determined by observation under an Olympus BX-51 microscope.

Climatic conditions

Climatic data such as monthly mean temperature, annual mean temperature, monthly precipitation, annual precipitation and monthly snow depth were obtained from the 44 localities from the Japan Metrological Agency (2002). These variables were estimated for each 1-km² mesh covering the whole of Japan, based on 30 years (1971–2000) of census data collected by automated meteorological data acquisition system (AMeDAS) using multiple regression analysis. Utilizing this data, climatic conditions at the localities were summarized by six indexes; Kira's (1948) warmth index [WI (°C-month)], mean temperature in the coldest month [MTCM (°C)], annual mean temperature [AMT (°C)], precipitation in summer [May to September; PS (mm)], precipitation in winter [October to May; PW (mm)], and maximum snow depth [MSD (cm)]. To analyze the relevance to polyploidal distribution, localities were grouped according to the main cytotype for each variety or to distinctive geographical ranges within the varieties (see "Results"), and differences among the groups were tested with the Kruskal–Wallis test using Statview Version 5.0 (SAS Institute, USA) followed by non-parametric multiple

comparisons according to the procedure described by Zar (1999).

Morphological variation

Morphological variation in *Parasenecio auriculata* was analyzed with voucher specimens for which chromosome numbers were known. In the analysis, specimens of cultivated plants were not included because their variation in size seems to be dependent on the conditions of cultivation such as availability of sunlight or pot size. As a result, one to four specimens were available for each locality (a total of 83 specimens), although for some localities no specimens were available (Table 2). For each specimen, 16 quantitative, primary characters were measured, and six derived characters (i.e., ratio between characters) were calculated (Fig. 1; Table 3). Because of the reniform or ovate-reniform leaves of this species, two leaf lengths (Nos. 5 and 6 in Table 3) were defined, and the foliar characters (Nos. 4–9 in Table 3) were measured on the largest leaf of the specimen. The floral characters (i.e., those associated with heads or florets; Nos. 12–22 in Table 3) were represented by the means for measurements of three to five (mostly five) heads, phyllaries or florets randomly taken from the inflorescence.

For individual characters, the difference in means among cytotypes or varieties was tested by one-way analysis of variance (ANOVA). Prior to the statistical test, the variables were logarhystic- or arcsine-transformed to meet the assumption of a normal distribution (Sokal and Rohlf 1995). Computations of one-way ANOVA and Schffé's multiple comparisons were performed with Statview Version 5.0 (SAS Institute). A principal components analysis (PCA) was also performed with a specimen as an operational unit. From 22 characters, either of the two characters used to calculate the derived characters were excluded from the data set, resulting in a set of 16 characters (Table 3). Before the analysis, each of the values were standardized by ranging. The PCA was carried out by Excel Statistics Version 1.1 (Social Survey Research Information Co. Ltd.).

Fig. 1. Definition of characters for morphological analysis. **a** largest leaves. **b** heads, phyllaries and florets. Numbers are equivalent for those in Table 3

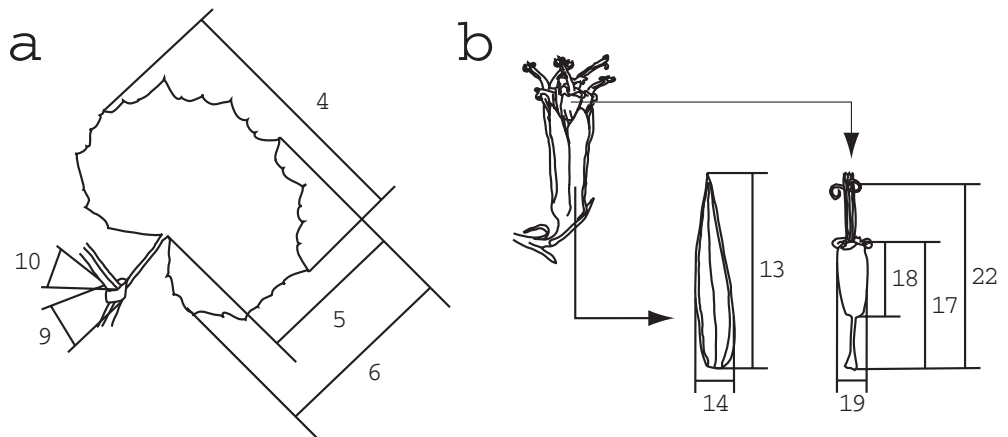


Table 3. List of characters used for the morphological analysis

No.	Character	PCA ^a
1	Shoot height (cm)	x
2	Number of leaves	x
3	Number of heads	x
4	Leaf width (cm) ^b	x
5	Leaf length (from a petiole tip) (cm) ^b	
6	Maximum leaf length (cm) ^b	
7	Ratio between leaf length and width (5/4) ^c	x
8	Ratio between maximum leaf length and leaf width (6/4) ^c	x
9	Auriculate diameter (mm) ^b	x
10	Stem diameter at a petiole base (mm) ^b	
11	Ratio between stem and auriculate diameter (10/9) ^c	x
12	Number of phyllaries per head	x
13	Phyllary length (mm)	x
14	Phyllary width (mm)	
15	Ratio between phyllary width and length (14/13) ^c	x
16	Number of florets per head	x
17	Floret length (mm)	x
18	Floral tube length (mm)	
19	Floral tube width (mm)	
20	Ratio between floral tube length and floret length (18/17) ^c	x
21	Ratio between floral tube width and floret length (19/17) ^c	x
22	Style length (mm)	x

^aCharacters used for a principal components analysis (PCA), indicated by “x”

^bMeasured on the largest leaf

^cDerived characters

Results

Cytotype distribution

In total, six cytotypes were observed among 269 individuals of *Parasenecio auriculata* examined in this study (Figs. 2, 3; Table 2). For var. *bulbifera*, all individuals had a chromosome number of $2n = 120$ (Fig. 2a) except for one individual in Maruseppu with $2n = 121$ (Fig. 2b), and the cytotypes with $2n = 118$ reported by Nishikawa (1980) and $2n = 60$ by Koyama (1995) were not found in this study. For var. *kamtschatica*, the diploid cytotype with $2n = 60$ (Fig. 2c) is most frequently observed (60.2% of 227 samples), while small numbers of diploidal aneuploids with $2n = 61$ (Fig. 2d) and 62 (Fig. 2e) were also found. In addition, one individual in Onneto had a chromosome number of $2n = 90$ (Fig. 2f), a triploid cytotype. Finally, tetraploids with $2n = 120$ (Fig. 2g) comprised 34.8% of the samples, representing the second major cytotype in this variety.

At most of the 44 localities, samples were mostly single polyploidal. Although sample sizes in several localities were limited to one or a few (Table 2), the site could be categorized as a diploid or tetraploid locality. The coexistence of different polyploidal levels was observed at two localities. In Onneto, one sample was a triploid with $2n = 90$, while the other 14 samples were diploid ($2n = 60$) or diploidal aneuploid ($2n = 61$ and 62). In addition, four samples from Mt. Hakodate included three diploids and one tetraploid. At the

localities included in the previous reports, no difference was detected between those reports and the samples examined in this study (see Table 1).

The geographical distribution of cytotypes based on the data available from previous studies (Table 1) and this study (Table 2) were shown in Fig. 4b. As mentioned above, var. *bulbifera* is predominantly tetraploid, and endemic to central Hokkaido. In var. *kamtschatica*, diploids were distributed relatively continuously in Southern Hokkaido, while Koyama (1961) reported $2n = 60$ in Towada, northern Honshu (Table 1). In addition, diploidal aneuploids with $2n = 61$ and 62 were found only in eastern Hokkaido. On the other hand, tetraploids of this variety were distributed disjunctly in northern and eastern Hokkaido and also the northern part of Honshu, except for the one individual at Mt. Hakodate (Southern Hokkaido). However, there was a less evident pattern in the altitudinal distribution of cytotypes (Fig. 4c). While the localities of var. *bulbifera* (central Hokkaido) and tetraploid of var. *kamtschatica* in northern Honshu tended to be distributed at higher altitudes than those of diploid and tetraploid of var. *kamtschatica* in Hokkaido, altitudinal ranges of the cytotypes or the varieties were overlapping one another, with the wider range for the tetraploid of var. *kamtschatica* from 5 m at Bekkai to 1,500 m at Mt. Hakkoda.

Climatic conditions

Six climatic indexes for 44 localities are summarized in Table 4. Kruskal–Wallis tests revealed a significant difference among localities for var. *bulbifera* (tetraploid), and the diploid and tetraploid of var. *kamtschatica*, in all indexes except for PS. Note that Mt. Hakodate (No. 40 in Table 2), where the diploid and tetraploid were symmetrically distributed, was not included in the tests. Three parameters related to thermal conditions (WI, MTCM and AMT) indicated that var. *bulbifera* is distributed under colder climates than var. *kamtschatica*, within which the tetraploid was distributed under colder climates than the diploid. On the other hand, PW and MSD indicated the tetraploid of var. *kamtschatica* was distributed in the most snowy environment, followed by var. *bulbifera*, and the diploid of var. *kamtschatica*.

Likewise, Kruskal–Wallis tests were also performed to test the difference of climatic conditions among three geographically distinct regions for tetraploid of var. *kamtschatica*; northern Honshu, northern and eastern Hokkaido (see Fig. 4). As a result, no significant difference was found at the three thermal parameters; WI ($H = 3.17$; $P > 0.05$), MTCM ($H = 3.74$; $P > 0.05$) and AMT ($H = 1.92$; $P > 0.05$). All of the mean values of these parameters for three distinct regions ranged between those for var. *bulbifera* and diploid of var. *kamtschatica*, except for the lower WI of eastern Hokkaido [WI = 40.7 (± 5.1); mean (\pm SE)]. In contrast, three parameters on precipitation showed statistical significance among the three regions; PS ($H = 9.90$; $P = 0.007$), PW ($H = 8.86$; $P = 0.012$) and MSD ($H = 6.76$; $P = 0.034$). Northern Honshu was characterized by the highest

Fig. 2. Chromosomes at mitotic metaphase of *Parasenecio auriculata*. **a** var. *bulbifera*, $2n = 120$. **b** var. *bulbifera*, $2n = 121$. **c** var. *kamtschatica*, $2n = 60$. **d** var. *kamtschatica*, $2n = 61$. **e** var. *kamtschatica*, $2n = 62$. **f** var. *kamtschatica*, $2n = 90$. **g** var. *kamtschatica*, $2n = 120$. Scale bars are 10 μm

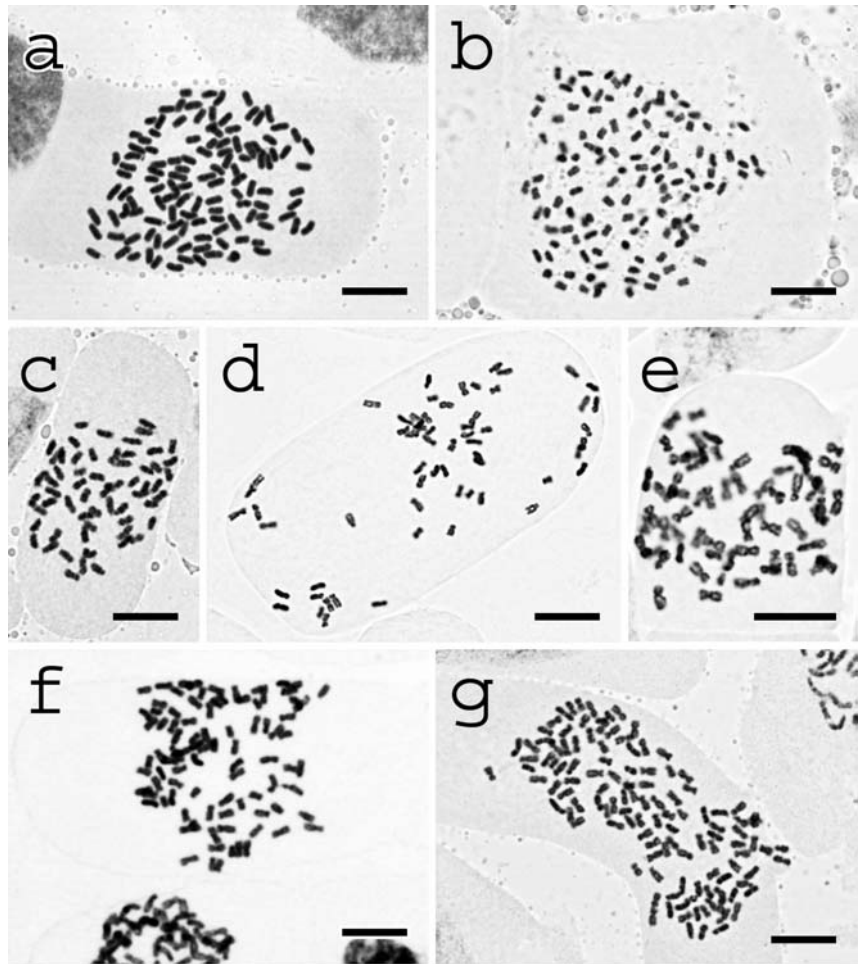


Table 4. Mean (\pm SE) and range (max/min) of six climatic indexes for the localities of *Parasenecio auriculata* var. *bulbifera* (tetraploid), and diploid, tetraploid and sympatric (both diploid and tetraploid) localities of *P. auriculata* var. *kamtschatica*, categorized based on the main cytotype at each locality (see Table 2)

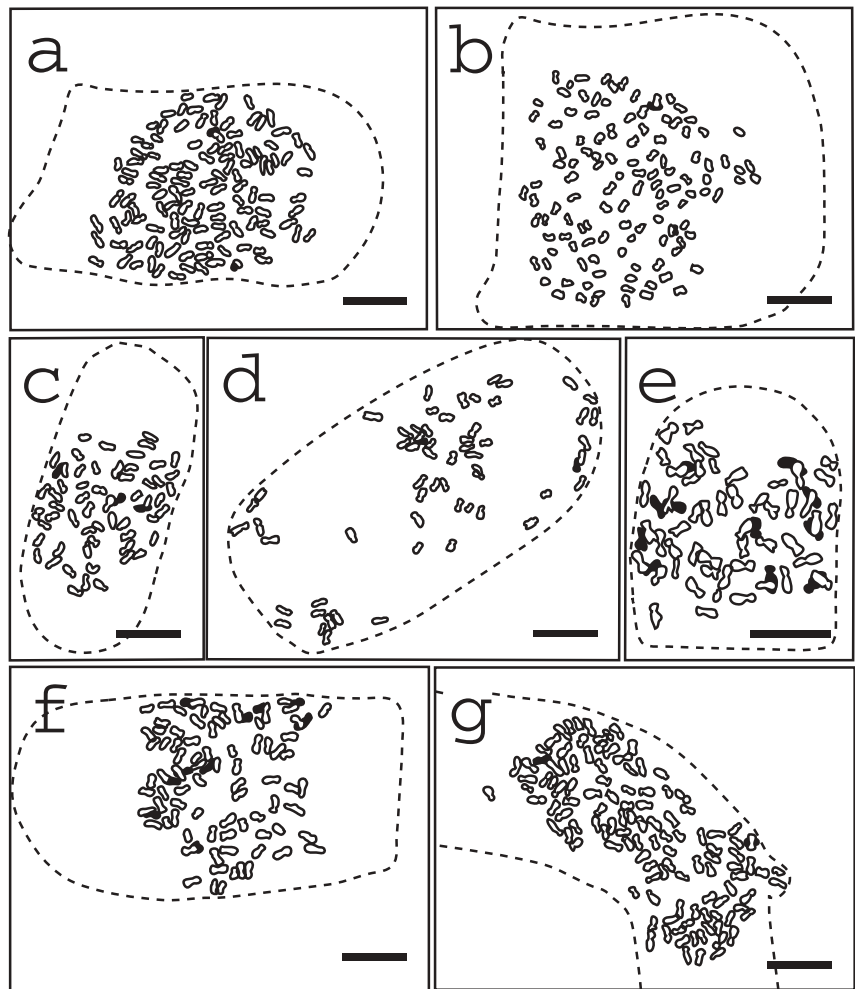
Climatic index	<i>P. auriculata</i> var. <i>bulbifera</i> (11)		<i>P. auriculata</i> var. <i>kamtschatica</i>				<i>H</i>	
			Diploid (18)		Tetraploid (14)			Sympatric (1)
	Mean \pm SE	Max/Min	Mean \pm SE	Max/Min	Mean \pm SE	Max/Min		
WI	41.1 \pm 2.4 ^a	59.3/30.5	49.7 \pm 2.0 ^b	63.1/34.8	45.4 \pm 2.2 ^{ab}	55.1/30.5	60.9	6.57*
MTCM	-10.5 \pm 0.6 ^a	-5.3/-12.7	-7.2 \pm 0.7 ^b	-2.4/-12.3	-8.1 \pm 0.6 ^{ab}	-4.2/-10.9	-3.1	9.53**
AMT	3.2 \pm 0.5 ^a	6.9/1.1	5.4 \pm 0.4 ^b	8.3/2.0	4.5 \pm 0.4 ^{ab}	6.8/2.1	7.9	8.75*
PS	698 \pm 51.2	1121.5/442.2	618.0 \pm 35.3	965.7/397.3	671.9 \pm 57.8	1193.3/436.1	610.9	1.80
PW	357 \pm 32.9 ^{ab}	564.6/177.5	270.2 \pm 24.2 ^a	455.1/146.7	399.2 \pm 34.6 ^b	631.7/196.0	201.1	8.18*
MSD	120 \pm 10.4 ^{ab}	208/80	87.4 \pm 13.6 ^a	277/23	157.2 \pm 19.3 ^b	289/43	24	12.98**

Numbers in parentheses indicate number of locality in each category. Results of the Kruskal–Wallis test (*H* values) were also provided, and means (\pm SE) with same superscripts are not significantly different ($P > 0.05$) by non-parametrical multiple comparison tests (Zar 1999). Note that one sympatric locality was not included in the tests. WI Kira (1948)'s warmth index ($^{\circ}\text{C}$ month), MTCM mean temperature at coldest month ($^{\circ}\text{C}$), AMT annual mean temperature ($^{\circ}\text{C}$), PS precipitation in summer (mm), PW precipitation in winter (mm), MSD maximum snow depth (cm) * $P < 0.05$; ** $P < 0.01$

precipitation in summer and winter [PS = 937.7 (\pm 192.6), PW = 559.4 (\pm 64.2), MSD = 219.5 (\pm 74.2); mean (\pm SE)]. Multiple comparisons showed significantly lower summer rainfall in northern Hokkaido [PS = 508.3 (\pm 53.3), PW = 370.2 (\pm 54.4), MSD = 161.3 (\pm 50.3); mean (\pm SE)] and

lower snowfall in eastern Hokkaido [PS = 651.6 (\pm 111.6), PW = 282.7 (\pm 98.0), MSD = 88.8 (\pm 38.8); mean (\pm SE)]. This relationship on precipitation was obviously inconsistent with the results of comparisons between the cytotypes and the varieties (see Table 4).

Fig. 3. a–g Respective explanatory drawings of Fig. 1a–g. The *dashed lines* represent cellular margins, and overlapped chromosomes are shows by *filled outlines*



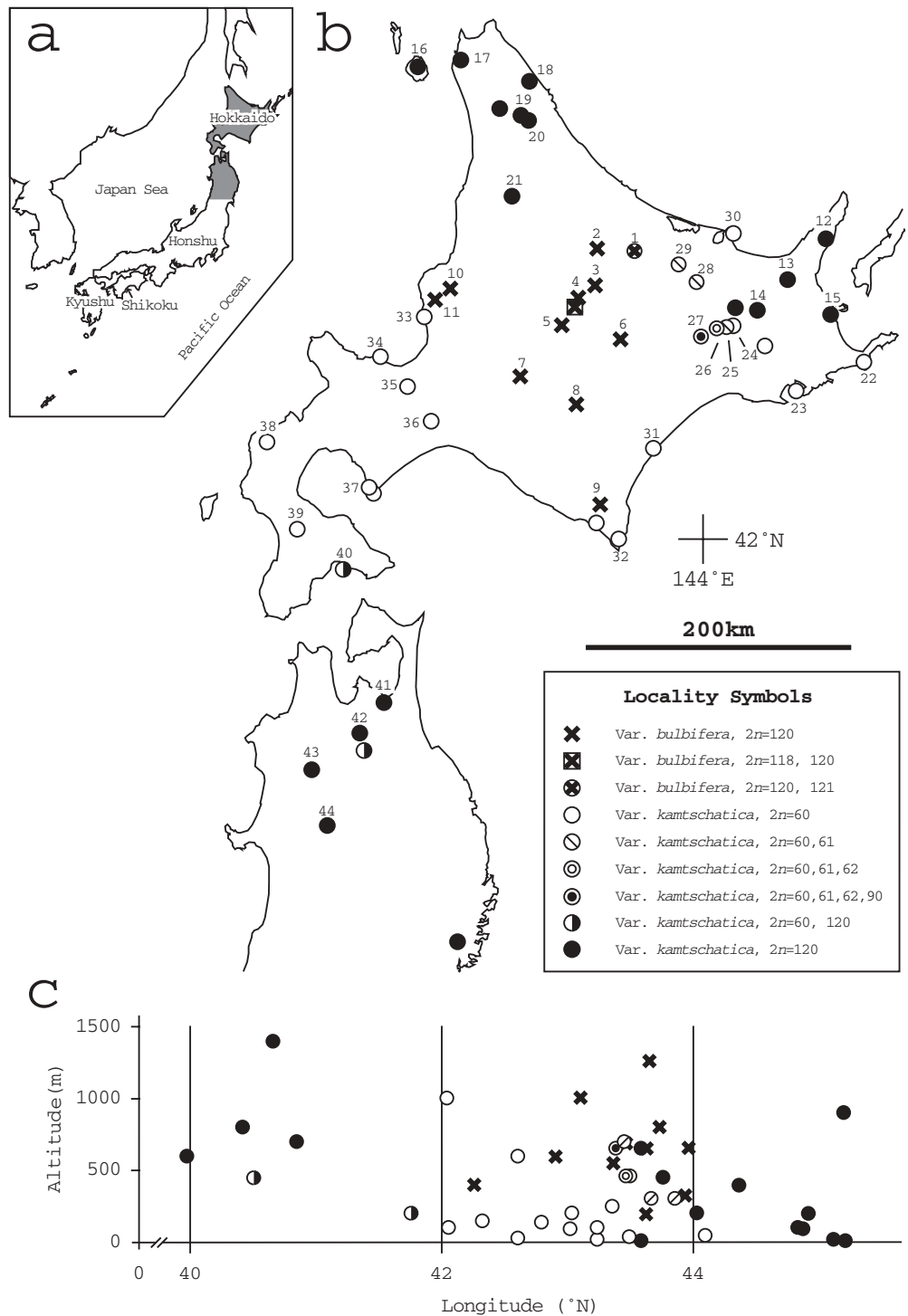
Morphological variation

Table 5 summarizes the morphological variation among 22 characters analyzed in 83 specimens of *Parasenecio auriculata*, classified into three groups, var. *bulbifera* (tetraploid) and the diploid and tetraploid of var. *kamtschatica*. To simplify the analysis, three specimens of var. *kamtschatica* with $2n = 61$ were included in the diploid group. For all 16 primary characters, the variation was continuous among these groups, but significant differences were also detected by one-way ANOVA except for numbers of leaves, heads and phyllaries per head (Nos. 2, 3, and 12 in Table 5). For most of the characters, the tetraploid of var. *kamtschatica* exhibited larger values than the diploid, while var. *bulbifera* tended to display an intermediate range of values. Two exceptions were the number of heads, which was largest in the diploid of var. *kamtschatica*, and the floral tube length, which was longest in var. *bulbifera*. Likewise, all derived characters varied continuously and were indistinguishable among the three groups of specimens, despite that the ANOVA revealed a significant difference in three of six characters (Nos. 11, 15 and 21).

In the principal component analysis, the first two components (PC1 and PC2) accounted for 32.1 and 13.8% of

the total variance. The character loading on these components were given in Table 5. The PC1 is positively associated with the characters related to plant size such as shoot height, leaf width, auriculate diameter, phyllary length and style length (Nos. 1, 4, 9, 13 and 22 in Table 5). The PC2 has a positive loading for ratio between phyllary width and length and the ratio between floral tube width and floret length (Nos. 15 and 21), and a negative loading for number of heads, phyllary length and floret length (Nos. 3, 13 and 17). A plot of the scores of PC1 and PC2 is shown in Fig. 5. In the plot, the ranges of specimens of var. *bulbifera*, and the diploid and tetraploid of var. *kamtschatica* largely overlap one another. However, specimens tended to be located along the PC1 axis in the order, diploid of var. *kamtschatica*, var. *bulbifera* and tetraploid of var. *kamtschatica*, supporting the plant size differentiation indicated by the univariate analysis. In addition, var. *bulbifera* tended to concentrate on the negative side of the PC2 axis. Three specimens of var. *kamtschatica* with $2n = 61$ (represented by stars) were positioned within the range of the diploid ($2n = 60$). On the other hand, the ranges of specimens of three disjunct regions for tetraploid of var. *kamtschatica* (assigned different symbols in Fig. 5) overlaps one another, reflecting the continuous morphological variation among the three

Fig. 4. **a** Map of the study area (represented by shaded areas), **b** and **c** geographical and altitudinal distribution of the diploid and tetraploid of *Parasenecio auriculata* in Japan. In **b**, numbers attached to symbols are equivalent to those in Table 2, otherwise sited only from previous studies (see Table 1)



regions. However, specimens from northern Hokkaido (filled circles) tend to show higher PC1 scores, indicating a tendency for the larger plant size at the region.

Discussion

The occurrence of two polyploidal levels in *Parasenecio auriculata*, a diploid with $2n = 60$ and a tetraploid with

$2n = 120$ (Table 1), was confirmed in this study (Table 2). The cytotypes with $2n = 60$ and 120 accounted for 51.6 and 44.6% of the samples overall, obviously representing two major cytotypes in this species. As for varietal basis, var. *bulbifera* is predominantly comprised of tetraploids, while var. *kamtschatica* includes both diploids and tetraploids. This indicates the discordance of polyploidal constitution between the two varieties. On the other hand, small numbers of aneuploids with $2n = 61, 62$ or 121 were also observed. However, it is difficult to speculate on the

Table 5. Mean (\pm SE) and range (max/min) of 22 morphological characters of *Parasenecio auriculata* var. *bulbifera* (tetraploid), diploids (including three diploidal aneuploids) and tetraploids of *P. auriculata* var. *kamtschatica*

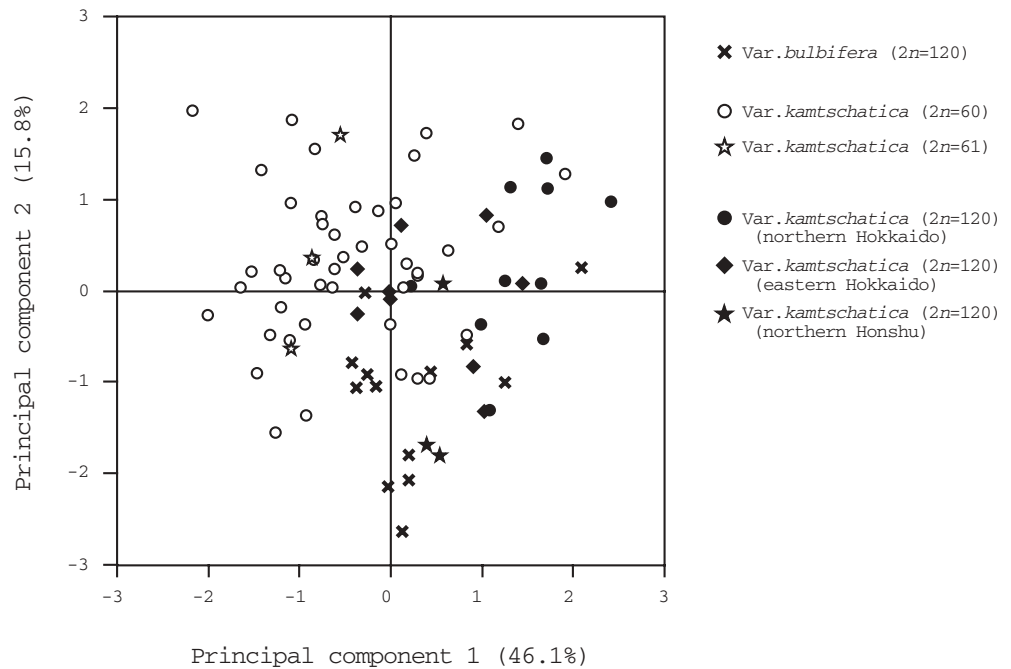
Character No.	<i>P. auriculata</i> var. <i>bulbifera</i> (13)		<i>P. auriculata</i> var. <i>kamtschatica</i>				F	PCA	
			Diploid (48)		Tetraploid (22)			PC1	PC2
	Mean \pm SE	Max/Min	Mean \pm SE	Max/Min	Mean \pm SE	Max/Min			
1	85.7 \pm 7.3 ^{ab}	141/53.5	72.1 \pm 3.6 ^a	139.0/33.5	110 \pm 6.4 ^b	176.0/78.5	15.1***	0.863	0.268
2	4.9 \pm 0.3	7/3	4.8 \pm 0.2	8/3	5.3 \pm 0.2	7/4	2.03	0.668	0.336
3	35.2 \pm 6.4	104/14	57.8 \pm 5.5	176/9	53.3 \pm 7.2	137/9	2.09	0.431	0.633
4	18.8 \pm 1.5 ^a	28.1/13.3	19.2 \pm 0.6 ^a	29.3/9.5	24.1 \pm 1.3 ^b	41.1/15.3	8.92***	0.737	0.311
5	10.1 \pm 0.8 ^{ab}	15.5/6.4	9.7 \pm 0.4 ^a	16.5/4.1	12.6 \pm 0.7 ^b	18.9/8.2	8.64***	–	–
6	12.4 \pm 1.1 ^{ab}	21.3/8.8	12.5 \pm 0.5 ^a	22.0/5.5	15.0 \pm 0.7 ^b	22.7/10.3	5.39**	–	–
7	0.55 \pm 0.02	0.70/0.43	0.50 \pm 0.01	0.68/0.39	0.53 \pm 0.02	0.67/0.43	3.63*	0.293	–0.36
8	0.67 \pm 0.03	0.85/0.56	0.65 \pm 0.01	0.80/0.53	0.63 \pm 0.01	0.73/0.50	0.42	0.106	–0.33
9	19.10 \pm 1.94 ^{ab}	34.6/10.4	14.6 \pm 1.41 ^a	50.51/3.37	22.8 \pm 1.69 ^b	42.92/14.66	10.23***	0.820	0.142
10	3.33 \pm 0.36 ^a	6.27/1.8	3.19 \pm 0.14 ^a	5.80/1.37	4.72 \pm 0.27 ^b	7.53/3.41	14.50***	–	–
11	0.18 \pm 0.01 ^a	0.22/0.14	0.28 \pm 0.02 ^b	0.60/0.11	0.22 \pm 0.01 ^b	0.30/0.13	6.77**	–0.6	0.152
12	5.0 \pm 0.0	5.0/5.0	5.0 \pm 0.0	5.0/4.3	5.0 \pm 0.0	5.0/4.8	0.66	0.158	0.056
13	8.94 \pm 0.26 ^a	10.7/7.35	7.84 \pm 0.15 ^b	10.68/5.78	9.14 \pm 0.22 ^a	11.46/7.17	16.87***	0.702	–0.45
14	1.30 \pm 0.04 ^{ab}	1.52/1.05	1.27 \pm 0.03 ^a	1.73/0.90	1.58 \pm 0.05 ^b	1.92/1.19	18.00***	–	–
15	0.15 \pm 0.00 ^a	0.17/0.13	0.17 \pm 0.00 ^{ab}	0.25/0.11	0.17 \pm 0.01 ^b	0.23/0.12	3.36*	–0.07	0.513
16	5.1 \pm 0.1 ^a	5.6/4.6	5.3 \pm 0.1 ^a	6.2/4.3	5.6 \pm 0.1 ^b	6.2/5.0	6.56**	0.462	0.202
17	6.60 \pm 0.12 ^a	7.31/5.87	5.83 \pm 0.08 ^b	7.14/4.74	6.74 \pm 0.14 ^a	8.01/5.77	24.89***	0.691	–0.43
18	3.96 \pm 0.10 ^a	4.62/3.55	3.30 \pm 0.06 ^b	4.35/2.24	3.86 \pm 0.07 ^a	4.72/3.39	24.11***	–	–
19	1.29 \pm 0.05 ^a	1.11/1.70	1.26 \pm 0.02 ^b	0.95/1.62	1.53 \pm 0.04 ^a	1.20/1.78	21.57***	–	–
20	0.60 \pm 0.01	0.66/0.54	0.57 \pm 0.01	0.70/0.46	0.58 \pm 0.01	0.66/0.50	2.50	–0.06	–0.32
21	0.20 \pm 0.01 ^a	0.24/0.16	0.22 \pm 0.00 ^b	0.28/0.15	0.23 \pm 0.01 ^b	0.29/0.17	4.90**	–0.05	0.421
22	9.19 \pm 0.17 ^a	10.2/8.51	7.83 \pm 0.09 ^b	9.09/6.43	9.24 \pm 0.18 ^a	10.79/7.77	45.43***	0.744	–0.39

Character number is the same as in Table 3. Numbers in *parentheses* are the sample size for each group. Results of one-way ANOVA for each character with log- or arcsine-transformed variables are provided with *F*-values, and means (\pm SE) with same *superscripts* are not significantly different ($P > 0.05$) by Sheffé's multiple comparisons. Loading of the characters to the first and second components (PC1 and PC2) in a principal component analysis (PCA) is also indicated

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

“–” indicates the character was not included in the analysis

Fig. 5. Plot of scores of first and second principal components of 83 specimens of *Parasenecio auriculata*, based on a set of 16 morphological characters (see Tables 2, 3)



evolutionary significance of these aneuploids because their inheritance or reproductive ability is largely unknown. In addition, one triploid individual with $2n = 90$ was also found at Onneto (Table 2). This cytotype could potentially be derived from the intercytotype mating between the diploid ($2n = 60$) and tetraploid ($2n = 120$), despite that the tetraploid was not found in the samples either this locality or adjacent sites (such as Mt. Oakan, Teshikaga and Sokodai). Since the cytotype is also likely to be derived from the occasional formation of an unreduced gametophyte, the origin of the triploid remains unclear.

The morphological analysis revealed significant differentiation of quantitative characters among the cytotypes of *Parasenecio auriculata* (Table 5). While the ranges of the characters overlapped one another, the tetraploid of var. *kamtschatica* has the largest plant size, followed by var. *bulbifera* (tetraploid), and the diploid of var. *kamtschatica*. This relationship was confirmed by the PCA (Fig. 5). Whereas moderate differentiation of plant size variation was also revealed among three geographically distinct regions for tetraploid of var. *kamtschatica* (cf. Fig. 5), this exemplified size enlargement at higher polyploid levels in *P. auriculata*, which is consistent with the tendency found for other diploid-tetraploid pairs in other taxa (e.g., Mosquin 1967; Gauthier et al. 1998; Naiki and Nagamasu 2004). However, it also clearly illustrated that cytotypes could not be distinguished based only on quantitative characters, although var. *bulbifera* is clearly distinguishable from var. *kamtschatica* by bulbils at leaf axils. Grant (1981) pointed out that morphological resemblance between a polyploid and its putative progenitor is one of the most important characteristics of autopolyploidization, while cytological or genetic evidences are also important to clarify the origin of polyploids. In this regard, the continuous morphological variation among cytotypes in var. *kamtschatica* could be interpreted as a support for a autotetraploidal origin of the $2n = 120$ cytotype from the $2n = 60$ cytotype. Correspondingly, var. *bulbifera*, which showed a continuous variation on quantitative characters with var. *kamtschatica*, may be an autotetraploidal variety in *P. auriculata*, despite its phylogenetic relationship with var. *kamtschatica* or other varieties in this species still remains uncertain.

In *Parasenecio auriculata* in Japan, the cytotype distribution showed a clear geographical pattern (Fig. 4b). While the diploid of var. *kamtschatica* was continuously distributed in southern Hokkaido, the tetraploid distributed disjunctly in northern and eastern Hokkaido, and also at the northern Honshu. In addition, var. *bulbifera*, predominantly tetraploid, was endemic to central Hokkaido. Comparisons of the climatic indexes among the localities revealed differences in habitat among the cytotypes and the varieties (Table 4). Statistical significance was detected for the parameter associated with warmth and snowfall. Moreover, among three disjunct regions for the tetraploid of var. *kamtschatica*, significant differences were detected for rainfall and snowfall conditions. Such climatic relationships reflects the differentiation of climatic preferences within and among the cytotypes or the varieties, and is likely explain geographical distribution of cytotypes in Japanese

P. auriculata. However, precipitation conditions seem to be less implicative for explanation of the cytotype distribution, because the relationship among the cytotypes and the varieties was not concordant with those among the disjunct ranges for the tetraploid of var. *kamtschatica*. In contrast, the relationship for thermal condition was sufficiently consistent; var. *bulbifera* inhibited at the coldest condition, followed by the tetraploid of *kamtschatica* and the diploid. This exemplifies a confident tendency of the tetraploid to be distributed under colder climate than the diploid within this species. In this regard, warmthness, rather than precipitation, may be a critical factor to account for the cyto-geographical structure of Japanese *P. auriculata*.

Interestingly, the mean for Kira's (1948) WI among localities for the tetraploid of var. *kamtschatica* is 45.4, while for var. *bulbifera* and the diploid of var. *kamtschatica* it is 41.1 and 49.7, respectively. This means that the geographical distribution of cytotypes in Japanese *P. auriculata* is formed around WI = 45, which is considered the boundary between broad-leaved deciduous and sub-alpine coniferous forests (Nogami and Ohba 1991). Indeed, comparison with a vegetation map (Miyawaki 1977) revealed that the geographical range of both the tetraploid of var. *kamtschatica* and var. *bulbifera* largely overlaps that of sub-alpine coniferous forests in Hokkaido, whereas the range of the diploid of var. *kamtschatica* overlaps that of broad-leaved deciduous forests (Fig. 6). However, the relationship is less evident in

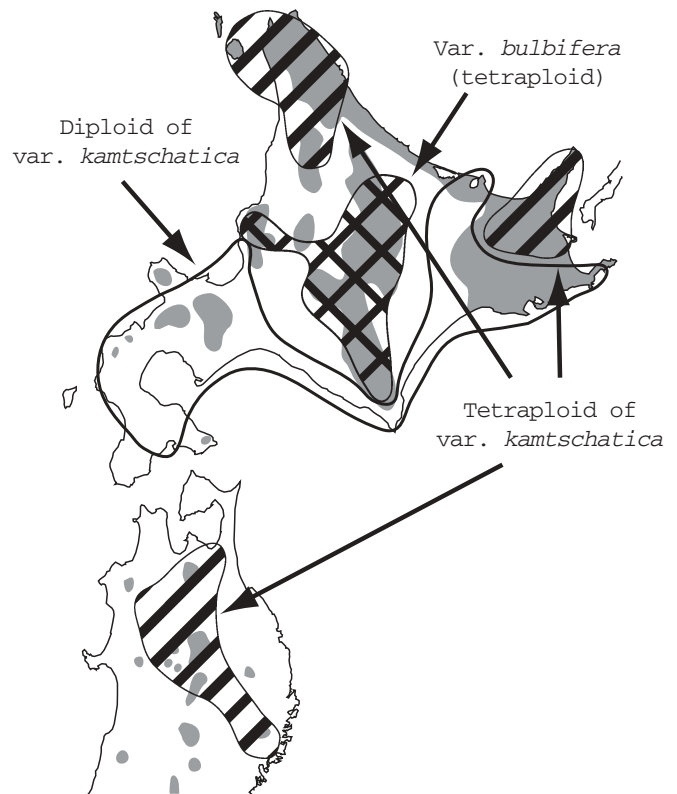


Fig. 6. A schematic illustration of the distribution of *Parasenecio auriculata* var. *bulbifera* (tetraploid) and the diploid and tetraploid of *P. auriculata* var. *kamtschatica* in Japan. Shaded areas represent ranges of subalpine coniferous forests (partly including the alpine zone) based on Miyawaki (1977)

Honshu, and the delimitation by vegetation does not necessarily accord with local forest types at the localities (Nakagawa personal observation). Nonetheless, the cyto geographical structure of *Parasenecio auriculata* in Hokkaido roughly corresponds to the shift in vegetation associated with the climatic cline along the Japanese archipelago, and the climatic gradient might play an important role in determining the geographical distribution of cytotypes in this species.

Consequently, cyto geographical structure and its climatic relevance in Japanese *Parasenecio auriculata* imply that the tetraploidization is likely associated with an acquisition of the tolerance to colder climate. On the other hand, the chromosome number of $2n = 60$ or associated numbers were also reported at Sakhalin, southern Primorsky and Koyak in Russia (Table 1). This means that the geographical distribution of the diploid extends further into the northern region than Hokkaido. In this regard, it is questionable whether such a widespread distribution is accompanied by greater climatic adaptability of the diploid than is to be expected from its distribution in Japan. In addition, the tetraploid has been reported only in Japan, and the distribution within the species range is largely unknown. Thus, more cytological information from a wider geographical range is still required, although results obtained in this study provides a foundation to elucidate the ecological significance of polyploidization in *P. auriculata*.

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