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Geographical distribution, genetic diversity, and reproductive traits of mixed polyploid populations in *Parasenecio kamtschaticus* (Senecioneae; Asteraceae)

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Abstract

In order to clarify the genetic differentiation and reproductive traits of mixed polyploid populations in *Parasenecio kamtschaticus* complex, geographical distribution, genetic diversity, and reproductive performance were compared among three intraspecific types composed of two cytotypes and two varieties in Japan. Diploid *P. kamtschaticus* var. *kamtschaticus* (2*x*-*kamtschaticus*) was distributed at the center of the distribution range, tetraploid *P. kamtschaticus* var. *kamtschaticus* (4*x*-*kamtschaticus*) existed widely throughout the entire range, and tetraploid *P. kamtschaticus* var. *bulbifera* (4*x*-*bulbifera*), producing bulbils, was restricted to higher elevations. Genetic structure was analyzed using allozyme markers. The genetic diversity of 4*x*-*kamtschaticus* was higher than that of 2*x*-*kamtschaticus*, with that of 4*x*-*bulbifera* being intermediate. Populations of 4*x*-*bulbifera* and 2*x*-*kamtschaticus* were genetically discriminable from each other in principle coordinate analysis, and the genetic structure of 4*x*-*kamtschaticus* populations largely overlapped with those of the other types. Flower and achene production levels were highest in the 4*x*-*kamtschaticus* populations and lowest in the 4*x*-*bulbifera* populations. Germination activity of achenes was highest in the 2*x*-*kamtschaticus* populations and lowest in the 4*x*-*bulbifera* populations. Fruit-set success of 4*x*-*bulbifera* decreased with elevation because of a shorter growing season, indicating the importance of vegetative reproduction by bulbils at higher elevations. Unexpectedly, the inbreeding coefficients of the 4*x*-*bulbifera* populations were the lowest among the three types. Occasional achene production by outcrossing might maintain the high genetic diversity of the 4*x*-*bulbifera* populations. The evolution of polyploidy and subsequent bulbil production might enable *P. kamtschaticus* to disperse a wider range of environmental conditions.

Keywords: Allozymes, Bulbil, Diploid, Elevation, Genetic diversity, Tetraploid

Introduction

Polyploidy is a central feature of plant diversification. All angiosperms have ultimately been derived from multiple ancestral polyploidy events (Jiao et al. 2011), and nearly 35% of vascular plants are recent polyploid species (Wood et al. 2009). There are two types of polyploidization in nature: allopolyploidy and autopolyploidy. Allopolyploidy is formed by the hybridization of two related species, whereas autopolyploidy is largely the consequence of crosses between conspecific individuals, both of which are followed by whole-genome duplication. As an evolutionary advantage of polyploidy, high genetic diversity within a population is considerable (Ramsey and Schemske 2002; Soltis et al. 2003; Comai 2005). Allopolyploid populations contain the genome set of two different species, and autopolyploids can have more than two different alleles at any given locus owing to the polysomic inheritance. The high genetic diversity of polyploid populations may contribute to a higher ability to adapt to different environments than diploids (Adams and Wendel 2005; Hegarty and Hiscock 2008; Rosche et al. 2016). Furthermore, polyploidization affects the genetic structure of populations. Autopolyploid populations need many more generations to attain genetic equilibrium under random mating than diploid populations, and the effective population size of autopolyploids is estimated to be two times larger than that of diploid populations (Moody et al. 1993; Ronfirt et al. 1998). As a consequence, the effects of bottlenecks and genetic drift in polyploid populations are much smaller, and genetic differences among populations are estimated to be smaller than those of diploid populations.

Mating between diploids and polyploids often results in the formation of infertile offspring. In the early stage of polyploidization, therefore, the limitation of effective outcrossing due to frequency-dependent mating is crucial (Husband and Schemske 1997; Burton and Husband 2000). Repeated polyploidization within a population, improvement of selfing ability, and a decrease in inbreeding depression may mitigate the disadvantage of frequency-dependent mating and accelerate the establishment of polyploid populations (Husband and Sabara 2003; Brochmann et al. 2004). Therefore, shift to selfing, changes in flowering phenology and pollinators, and/or differentiation of growing habitats have been thought to be important processes in the establishment of tetraploid populations from the ancestral diploid populations (Stebbins 1950; Soltis et al. 2003). Furthermore, asexual seed production or vegetative reproduction may be advantageous for the establishment of polyploid populations (Otto and Whitton 2000). However, the prevalence of asexual reproduction may decrease genetic diversity within populations, resulting in the acceleration of genetic differentiation among populations (Young et al. 2002; Wang et al. 2004). Our knowledge about the relationship between polyploidization and frequency of non-sexual reproduction is still limited (Ellstrand and Roose 1987; Schinkel et al. 2016), and there is a need for the accumulation of empirical studies on this issue.

Parasenecio kamtschaticus (synonym *Parasenecio auriculata*; Asteraceae) is a perennial herb growing in the understory of cool-temperate forests in northern Japan. This species features two varieties, namely, *P. kamtschaticus* var. *kamtschaticus* Kadota and *P. kamtschaticus* var. *bulbifera* (Koidz.) Kadota, in Hokkaido, northern Japan. Although *P. kamtschaticus* var. *kamtschaticus* commonly grows in lowland to montane forests, *P. kamtschaticus* var. *bulbifera* populations are mainly distributed in subalpine to alpine sites in central Hokkaido. Interestingly, *P. kamtschaticus* var. *bulbifera* has two modes of reproduction: sexual reproduction via seed production and vegetative reproduction via bulbil formation. A recent study reported that *P. kamtschaticus* var. *kamtschaticus* in Hokkaido is composed of diploid and tetraploid populations, and all populations of *P. kamtschaticus* var.

bulbifera are tetraploids (Nakagawa 2006). Tetraploid populations of *P. kamtschaticus* var. *kamtschaticus* are widely distributed throughout the whole distribution range, but diploid populations are limited to the middle of the distribution range. Therefore, *P. kamtschaticus* complex provides an interesting opportunity to study polyploidal evolution and mating systems in terms of adaptation to different habitats and range shift.

In order to understand the evolutionary background of the existence of varieties and cytotypes in *P. kamtschaticus* complex, comparisons of genetic structure and reproductive traits are crucial. Furthermore, advantage of vegetative reproduction should be clarified in terms of the effectiveness of sexual reproduction. Generally, increases in asexual (via apomictic seed production) or vegetative reproduction (via bulbil or stolon) at higher elevations and higher latitudes have been identified (Young et al. 2002; Brochmann et al. 2004; Pluess and Stöcklin 2005; Steiner et al. 2012). Low pollinator activity and/or short growing period often restrict sexual seed production, and recruitment by vegetative reproduction may be more secure than that by seeds under harsh environmental conditions. Thus, we expect that seed production of *P. kamtschaticus* var. *bulbifera* will be limited at higher elevations.

In the present study, we aim to clarify the genetic and reproductive differences among three intraspecific types, combining two cytotypes (diploid and tetraploid) and two ecomorph types (*P. kamtschaticus* var. *kamtschaticus* and *P. kamtschaticus* var. *bulbifera*). Genetic analysis was conducted using allozyme markers because the choice of available genetic markers was limited when we started this study in 2003. Although the power of genetic analysis of allozyme markers is lower than that of direct molecular methods, enzyme electrophoresis is still useful for genetic studies on polyploid plant species (e.g., Chung et al. 2015; Duchoslav and Stanková 2015) because of its reliability for determining disomic/tetrasomic inheritance pattern in polyploid complex. The purposes of this study are as follows: (1) conformation of geographical distribution patterns reported by Nakagawa (2006) and clarification of polyploidy type (allotetraploid or autotetraploid), (2) comparisons of genetic diversity and structure, and (3) comparisons of reproductive characteristics among the three types of *P. kamtschaticus*. Furthermore, we measured fruit-set success of the tetraploid var. *bulbifera* under natural conditions along the elevational gradients to test the prediction that bulbil production is advantageous at higher elevations if fruit-set success decreases at higher elevations.

Materials and methods

Plant material

Parasenecio kamtschaticus (Maxim.) Kadota is a perennial herbaceous species distributed in East Asia. There are two varieties in Japan: *P. kamtschaticus* var. *kamtschaticus*, which is distributed from northern Honshu to Hokkaido, and *P. kamtschaticus* var. *bulbifera*, which is endemic to the mountainous regions of Hokkaido. *Parasenecio kamtschaticus* var. *bulbifera* is characterized by the formation of bulbils at leaf axils and reproduces vegetatively in addition to seed production (Online Resource 1). There are two cytotypes in *P. kamtschaticus* var. *kamtschaticus*, namely, diploid ($2n = 2x = 60$) and tetraploid ($2n = 4x = 120$) populations (hereafter 2*x*-*kamtschaticus* and 4*x*-*kamtschaticus*, respectively), and *P. kamtschaticus* var. *bulbifera* populations are tetraploids (hereafter 4*x*-*bulbifera*; Nakagawa 2006). Flowering of *P. kamtschaticus* complex usually occurs in mid- to late summer (mainly in August), and achenes mature from September to early October.

Population sampling

We collected leaf samples from 30 populations in 2003 (seven populations), 2004 (16 populations), and 2007 (seven populations) in Hokkaido and northern Tohoku, Japan (Table 1 and Fig. 1). At three mountainous sites (Mt. Yoichi, Mt. Soranuma, and Mt. Shokanbetsu), where *P. kamtschaticus* populations were distributed across wide elevational ranges, sampling was conducted in both low (montane zone below 1,000 m elevation) and high elevational populations (subalpine zone above 1,000 m elevation). In each population, we sampled one leaf from each of the 30–80 arbitrarily selected individuals. The interval of sampling was >2 m to avoid sampling from the same genets. Collected leaves were taken back to the laboratory in a cooler box and then stored at −28°C prior to the electrophoresis experiment.

Enzyme electrophoresis

Approximately 100 mg of leaf tissue was homogenized in 2.0 mL of extract buffer [0.1 M Tris-HCl (pH 7.5), 20% (v/v) glycerol, 0.75% Tween 80, 10 mM dithiothreitol, 0.1% (v/v) β-mercaptoethanol, and 75 mg/mL polyvinylpolypyrrolidone]. The extracts were loaded on polyacrylamide vertical slab gels after refining by centrifugation at 1500 rpm for 20 min. Electrophoresis was conducted at 4°C and 12 mA/cm^2 for 150 min. The gels were stained for the following enzyme systems: aspartate aminotransferase (AAT; 2.6.1.1), esterase (EST; 3.1.1), phosphoglucomutase (PGM; 2.7.5.1), leucine aminopeptidase (LAP; 3.4.11.1), and shikimic acid dehydrogenase (SKD; 1.1.1.25). Loci were numbered starting with the most anodal (for details, see Online Resource 2 and 3). In the same way, alleles were identified using letters alphabetically.

Genetic data analysis

To describe the levels of genetic diversity, the following statistics were calculated: individual sample size (n), observed number of alleles per locus (A), effective number of alleles (A_e; Nielsen et al. 2003), allelic richness (AR), genetic diversity corrected for sample size (H_e), observed heterozygosity (H_o), and inbreeding coefficient (F_{is}). Computations of these statistics were performed using SpageDi 1.5 (Hardy and Vekemans 2002).

The structure of genetic diversity across all populations was investigated on the basis of pairwise genetic distances [$F_{ST}/(1 - F_{ST})$ values] between pairs of populations, where F_{ST} means fixation index, by principal coordinate analysis (PCoA) using the *dudi.pco* function in the R package "ADEGENET" (Jombart 2008).

Reproductive traits

Basic reproductive characteristics, namely, capitulum number per plant, fruit set under natural conditions, individual achene size, and germinability of achenes, were compared among three types (2*x*-*kamtschaticus*, 4*x*-*kamtschaticus*, and 4*x*-*bulbifera*) across six populations in 2004: Tomakomai and Nopporo for 2*x*-*kamtschaticus*, Utoro and Wakkanai for 4*x*-*kamtschaticus*, and Mt. Hirayama and Mt. Shokanbetsu (high) for 4*x*-*bulbifera* (see Table 1). In each population, we arbitrarily selected 31–63 plants in the fruiting season (September to October), counted the original number of capitula per plant, and harvested capitula with achenes. In the laboratory, we counted the number of mature achenes per capitulum to calculate proportion of fruit set under natural conditions. After removal of the pappus, achenes from the same population were pooled and stored in paper bags at room temperature for 1 month. Achene mass was measured for five replications of 30 achenes in each population, and mean achene weight was calculated. Germinability was assessed after keeping achenes under wet and cool (4°C) conditions for 1 month. In the preliminary

experiment, the germination activity peaked at 15°C (18 h)/5°C (6 h) with 12-h light/dark conditions. Thus, the germination experiment was performed under these conditions for five replications of 30 achenes in each population for 30 days. Germinability was expressed as the final proportion of germinated achenes.

In the 4*x*-*bulbifera* populations of Mt. Hirayama and Mt. Shokanbetsu (high), we analyzed the relationship between elevation and fruit set along the elevational gradient in 2004. We sampled all capitula on the main floral stem of individuals in the fruiting season along the hiking trail from low to high elevation, in which all individuals were ranked in order of elevation, and fruit set was measured in the laboratory. In total, 63 individuals were sampled at elevations from 1,000 to 1,700 m on Mt. Hirayama, and 42 individuals were sampled at elevations from 970 to 1,490 m on Mt. Shokanbetsu.

Statistical analysis

All statistical analyses were performed using an open source system, R version 3.4.4 (R Development Core Team, 2018; https://www.r-project.org). The elevational distribution of sampling populations was compared among three types by generalized linear model (GLM) postulating Gaussian error distribution. Each reproductive trait was compared between intraspecific types by generalized linear mixed models (GLMMs) using the library of the "lme4" package, in which intraspecific type was an explanatory variable and population was treated as a random factor. Poisson error distribution with log-link function was used for GLMM of capitulum number, Gaussian error distribution was used for GLMM of achene weight, and binomial error distribution with logit-link function was used for GLMMs of fruit set and germinability. The relationship between elevation and fruit set of *P. kamtschaticus* var. *bulbifera* was analyzed in each population (Mt. Hirayama and Mt. Shokanbetsu) by GLM, postulating a binomial error distribution with logit-link function.

Results

Distribution patterns of three intraspecific types

Among five enzyme systems analyzed, genotyping of the banding patterns of dimeric *AAT* was difficult due to unclear and irregular bands. Thus, we deleted *AAT* from the analysis. On the basis of the banding patterns of allozyme electrophoresis using remaining four enzyme systems, seven polymorphic loci (SKD1, SDK2, PGM1, PGM2, PGM3, EST, and LAP) were detected (Online Resource 2 and 3). Of 23 populations of *P. kamtschaticus* var. *kamtschaticus*, 10 populations were recognized as diploids, and 13 were recognized as tetraploids. All seven *P. kamtschaticus* var. *bulbifera* populations were recognized as tetraploids (Table 1 and Fig. 1). Unbalanced heterozygosity was detected in each tetraploid population, indicating that the polyploidy system of *P. kamtschaticus* was autotetraploidy.

The locations of the 30 populations sampled in this study and 44 populations reported by Nakagawa (2006) were plotted on a map of northern Japan (Fig. 1). Diploid populations of *P. kamtschaticus* var. *kamtschaticus* (2*x*-*kamtschaticus*) were distributed in southwestern to eastern Hokkaido, that is, the middle part of the distribution range, tetraploid populations of *P. kamtschaticus* var. *kamtschaticus* (4*x*-*kamtschaticus*) were widely distributed in the whole range from Tohoku to northern Hokkaido, and tetraploid populations of *P. kamtschaticus* var. *bulbifera* (4*x*-*bulbifera*) were distributed only in the mountainous regions of central Hokkaido (Fig. 2). Elevational distribution ranges were similar between the 2*x*- and 4*x*-*kamtschaticus* populations, but the 4*x*-*kamtschaticus* populations in the Tohoku region were located at higher elevations than those in Hokkaido. By contrast, the elevational distribution of the 4*x*-*bulbifera*

populations was significantly higher than that of the 2*x*- and 4*x*-*kamtschaticus* populations ($p < 0.001$).

Genetic diversity and structure

Genetic variations in individual populations based on the seven polymorphic loci are summarized in Table 2, in which 3 of 30 populations (site nos. 10, 20, and 22 in Table 1) were excluded because of the lack of genetic data for some loci. All indices of the genetic diversity (i.e., *A*, *A*e, *AR*, *H*e, and *H*o) in the diploid populations (2*x*-*kamtschaticus*) were smaller than those of the tetraploid populations (4*x*-*kamtschaticus* and 4*x*-*bulbifera*). Inbreeding coefficient F_{is} was highest in 4*x*-*kamtschaticus* and lowest in 4*x*-*bulbifera*.

In the principal coordinate analysis based on the genetic distance between 30 populations, the contribution of the first PCoA axis was 58.0%, and that of the second PCoA axis was 17.3% (Fig. 3). The diploid populations (2*x*-*kamtschaticus*) and the 4*x*-*bulbifera* populations were clearly separated as different clusters, and the 4*x*-*kamtschaticus* populations were widely dispersed in the plot and overlapped with the other types. However, the distributions of the 4x-*kamtschaticus* populations were classified into three clusters: southern (site nos. 19–23), eastern (site nos. 11–14), and northern (site nos. 15–18) parts of the entire distribution range (Fig. 1).

Reproductive performance

The reproductive traits of 2*x*-*kamtschaticus*, 4*x*-*kamtschaticus*, and 4*x*-*bulbifera* measured in six populations are summarized in Table 3. The number of capitula per plant, that is, flower production, was highest in the 4*x*-*kamtschaticus* populations and lowest in the 4*x*-*bulbifera* populations. Fruit set under natural pollination ranged from 0.07 (4*x*-*bulbifera* in Mt. Hirayama) to 0.36 (4*x*-*kamtschaticus* in Wakkanai), and fruit set of the 4*x*-*bulbifera* populations was significantly lower than those of the 2*x*- and 4*x*-*kamtschaticus* populations. Individual achene weight was highest in the 4*x*-*kamtschaticus* populations and lowest in the 4*x*-*bulbifera* populations, although the significance level was marginal ($p < 0.10$). Germinability was highest in the 2*x*-*kamtschaticus* populations (> 40%) and lowest in the 4*x*-*bulbifera* populations (< 10%). These results indicated that the sexual reproductive performance was lowest in 4*x*-*bulbifera*.

The fruit set of 4*x*-*bulbifera* in two populations (Mt. Hirayama and Mt. Shokanbetsu) significantly decreased with elevation ($p < 0.001$ by GLM; Fig. 4), and plants growing at elevations above 1,500 m did not set any achene. This was because the aboveground parts of plants died back before achene maturation at higher elevations, probably because of freezing temperatures repeatedly occurring in late September.

Discussion

Our study confirmed the geographical distribution of *P. kamtschaticus* complex as reported by Nakagawa (2006): autotetraploid populations (4*x*-*kamtschaticus*) are widely present throughout the entire distribution range, diploid populations (2*x*-*kamtschaticus*) are present only at its center, and bulbiferous populations (4*x*-*bulbifera*) are limited to higher elevations. Furthermore, we detected significant differences in genetic structure and reproductive performance among intraspecific types.

Comparisons of diploid and tetraploid populations

Several studies reported the expansion or range shift of polyploid populations relative to

the ancestral diploid populations (Murray and Young 2001; Borgen and Hultgrård 2003; Yu et al. 2010; Kirchheimer et al. 2016). This may reflect the advantages of polyploidy for colonizing diverse habitats owing to the high allelic diversity, heterozygosity, and/or heterosis effects (Soltis and Soltis 2000; Adams and Wendel 2005; Comai 2005; Birchler et al. 2010). In this study, we detected wider geographical distributions for the tetraploid populations than those for the diploid ones. Interestingly, 4*x*-*kamtschaticus* showed a wider range shift not only northward but also southward (Tohoku area). However, the distribution of 4*x*-*kamtschaticus* in the Tohoku area was limited to higher elevations, indicating that 4x-*kamtschaticus* is more tolerant of cool weather conditions than 2*x*-*kamtschaticus*, that is, the conditions prevailing at higher elevations in southern areas and higher latitudes in northern ones. It is reported that tetraploids often occupy cooler and harsher environments than their diploid ancestors (Borgen and Hultgrård 2003; Brochmann et al. 2004; Kirchheimer et al. 2016).

As reported in previous studies (Hardy and Vekemans 2001; Borgen and Hultgåed 2003; Brockmann et al. 2004; Cosendai et al. 2013), tetraploid populations maintained high genetic diversity (number of alleles, allelic richness, and level of heterozygosity) in comparison with diploid populations. High genetic diversity in polyploids may mitigate the inbreeding depression and bottleneck effects as mentioned above, and chromosome doubling sometimes leads to a breakdown of self-incompatibility (Stebbins 1950; Soltis and Soltis 2000; Barringer 2007). It is known that 2*x*-*kamtschaticus* is an obligate outcrosser, for which pollinator visits are crucial for achene production (Kudo et al. 2008). In our study, 4*x*-*kamtschaticus* tended to show a higher inbreeding coefficient than 2*x*-*kamtschaticus*, although there was a large variation among populations. This implies the possibility of achene production by selfing in the tetraploid populations.

Furthermore, gene duplication may increase phenotypic variability, which may accelerate adaptation to various habitats (Soltis et al. 2015). 4*x*-*kamtschaticus* tended to produce a larger number of flowers and larger achenes than 2*x*-*kamtschaticus*. Fruit set of the 4*x*-*kamtschaticus* populations under natural conditions was similar to or even higher than that of the 2*x*-*kamtschaticus* populations. Therefore, the level of achene production was higher in the 4*x*-*kamtschaticus* populations. High reproductive activity in 4*x*-*kamtschaticus* may be advantageous for the maintenance of populations by sexual reproduction in cooler and harsher environments. On the other hand, germinability in vitro was lower in 4*x*-*kamtschaticus* than in 2*x*-*kamtschaticus*. To evaluate the ecological significance of the reproductive performance of 4*x*-*kamtschaticus*, further studies on population dynamics in the field are necessary.

Comparisons of sexual and bulbiferous populations

The distribution pattern of bulbiferous populations (4*x*-*bulbifera*) indicates the advantages of vegetative reproduction at higher elevations as expected. The occurrence of asexual and vegetative reproduction in harsh environments is often coupled with polyploidy, but its relationship has not been clearly explained (Schinkel et al. 2016). In *Rutidosis leiolepis* having cytological complexity, for instance, the importance of vegetative reproduction increased with elevation, but the shift of the reproductive mode was independent of the level of polyploidy (Young et al. 2002). In our study, 4*x*-*bulbifera* populations were restricted to the subalpine and alpine zones (940–1,700 m elevation), and 4*x*-*kamtschaticus* populations were common in the lowland to montane zones (5–800 m elevation) within Hokkaido. Although there are a few populations of 2*x*-*kamtschaticus* at higher elevations, such as at Mt. Yoichi (1,200 m elevation) and Mt. Soranuma (1,050 m elevation), bulbil formation was not observed. These results imply a physiological linkage between polyploidy and vegetative reproduction (Schinkel et al. 2016).

The fruit set of 4*x*-*bulbifera* clearly decreased with elevation. This is because the aboveground parts often withered before achene maturation because of frost damage. Flowering of *P. kamtschaticus* varieties usually occurs during August to mid-September, and achenes mature after mid-September. The nighttime temperature after mid-September often decreases below zero in subalpine and alpine zones. Thus, the short growing season at higher elevations strongly restricts achene production. Individual achene size and germinability showed the lowest levels in 4*x*-*bulbifera* among intraspecific types. Bulbils of 4*x*-*bulbifera* usually started to develop soon after leaf expansion and were dispersed before the fruiting season (see Online Resource 1). Furthermore, the rapid growth of offspring originating from bulbils in comparison with that of seedlings may be beneficial under conditions with short growing period and a harsh environment (Stöcklin et al. 2009). Therefore, the importance of vegetative reproduction relative to sexual reproduction increases with elevation. When there is a trade-off between sexual and vegetative reproduction, decreasing resource investment in sexual reproduction is accelerated with increasing vegetative reproduction (Pluess and Stöcklin 2005). Flower production of 4*x*-*bulbifera* was lowest among the intraspecific types, indicating a smaller investment in sexual reproduction. By contrast, bulbil formation occurs even in non-flowering small-sized plants. These characteristics indicate the importance of bulbil production in 4*x*-*bulbifera*.

The acceleration of vegetative reproduction often results in lower genetic diversity within populations (Young et al. 2002; Steiner et al. 2012). Interestingly, higher genetic diversity was retained in the 4x-*bulbifera* populations despite their lower level of sexual reproduction. This may partly reflect the advantages of gene duplication in tetraploids as mentioned previously (Hardy and Vekemans 2001; Ramsey and Schemske 2002; Comai 2005). Furthermore, occasional sexual reproduction may play a role in maintaining the genetic diversity even in populations in which vegetative or asexual reproduction is predominant (Diggle et al. 1998; Gabrielsen and Brochmann 1998; Cosendai et al. 2013). A demographic study on an alpine clonal plant (*Geum reptans*) revealed that sexual seed production in favorable years can have a significant effect on population dynamics (Weppler et al. 2006). Therefore, maintenance of sexual reproduction may also be important for populations at higher elevations (Cosendai et al. 2013).

Implications of polyploidal evolution

The PCoA of the subset of *P. kamtschaticus* revealed that the distributions of the 2*x*- and 4*x*-*kamtschaticus* populations strongly overlapped, with a larger dispersion in the 4*x*-*kamtschaticus* populations, and the distributions of the 2*x*-*kamtschaticus* and 4*x*-*bulbifera* populations were clearly segregated in the coordinate scale (Fig. 3). These patterns of genetic similarity appear to reflect the geographical distributions of intraspecific types across latitudes and elevations (Fig. 2). Repeated formation of tetraploids from diploid populations has been reported in several polyploid species (Hardy and Vekemans 2001; Borgen and Hultgård 2003; Koch and Bernhard 2004; Rosche et al. 2016). Especially for migration to higher elevations, local adaptation of the reproductive mode, that is, a shift to vegetative reproduction, may be required (Stöcklin et al. 2009). Therefore, two evolutionary processes, namely, gene duplication by polyploidy and subsequent ability to form bulbils, might contribute to the migration to subalpine and alpine environments by this species.

In conclusion, the formation of tetraploids and shift to vegetative reproduction enabled *P. kamtschaticus* to expand its distribution range toward cooler habitats with a shorter growing period. Our results suggest the advantages of polyploidy for adapting to new environmental

conditions. However, the specific life-history strategy and its contribution to fitness have not been evaluated in this study. To clarify the ecological and physiological significance of polyploidy, field-based experimental studies on population dynamics, growth rate, and tolerance to cold climate are necessary (e.g., Hegarty and Hiscock 2008).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Author contributions GK planned this study and wrote the first draft of the paper, GK and ASH conducted the field survey, analyzed the data, discussed the results, and prepared the final version of this paper.

Information on Electronic Supplementary Material

Online Resource 1. **a** *Parasenecio kamtschaticus* var. *kamtschaticus* and **b** *P. kamtschaticus* var. *bulbifera* at the flowering stage.

Online Resource 2. Banding patterns of seven loci observed in individuals of *Parasenecio kamtschaticus* cytotypes. The identified genotype is shown in each band for diploid (lower) and tetraploid (upper). SKD: shikimic acid dehydrogenase, PGM: phosphoglucomutase, EST: esterase, and LAP: leucine aminopeptidase.

Online Resource 3. Photographs of zymogram patterns of four enzyme systems. **a** SKD: shikimic acid dehydrogenase, **b** PGM: phosphoglucomutase, **c** EST: esterase, and **d** LAP: leucine aminopeptidase.

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Figures

Figure 1

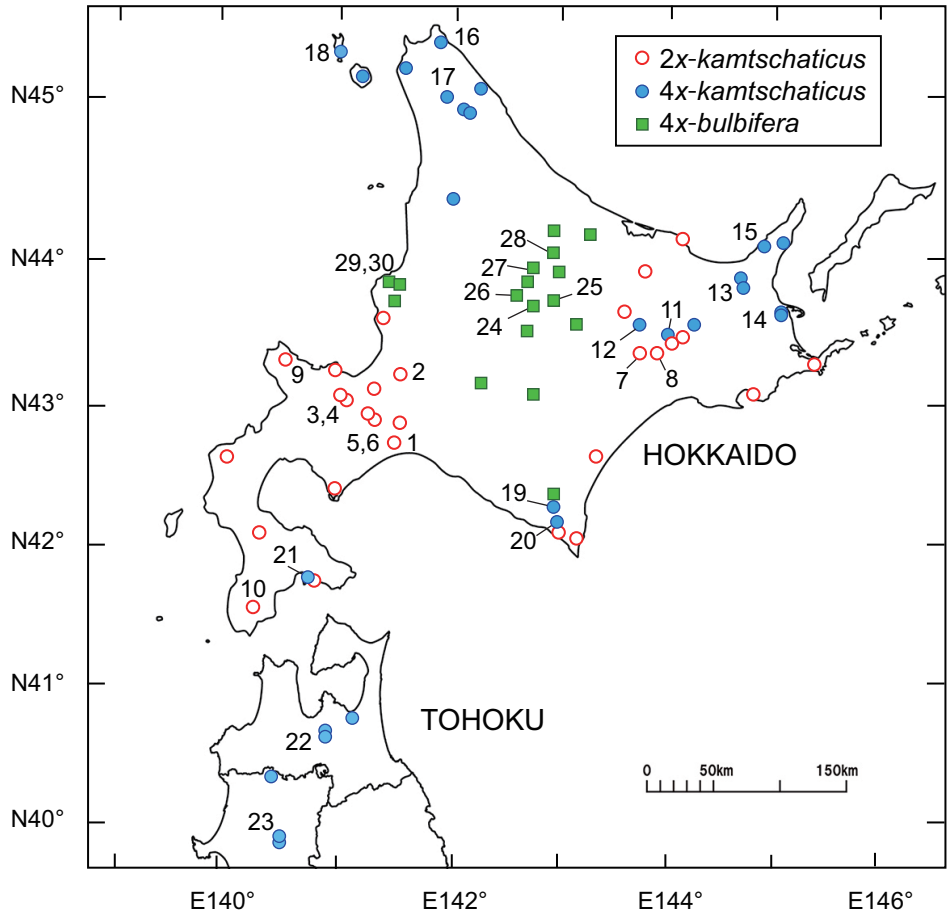

Fig. 1 Location of *Parasenecio kamtschaticus* populations of three intraspecific types (diploid var. *kamtschaticus*, tetraploid var. *kamtschaticus*, and tetraploid var. *bulbifera*) in Hokkaido and Tohoku. The populations studied in this paper are numbered (1–30). See Table 1 for the details of the individual populations. Plots without numbers indicate populations studied by Nakagawa (2006).

Figure 2

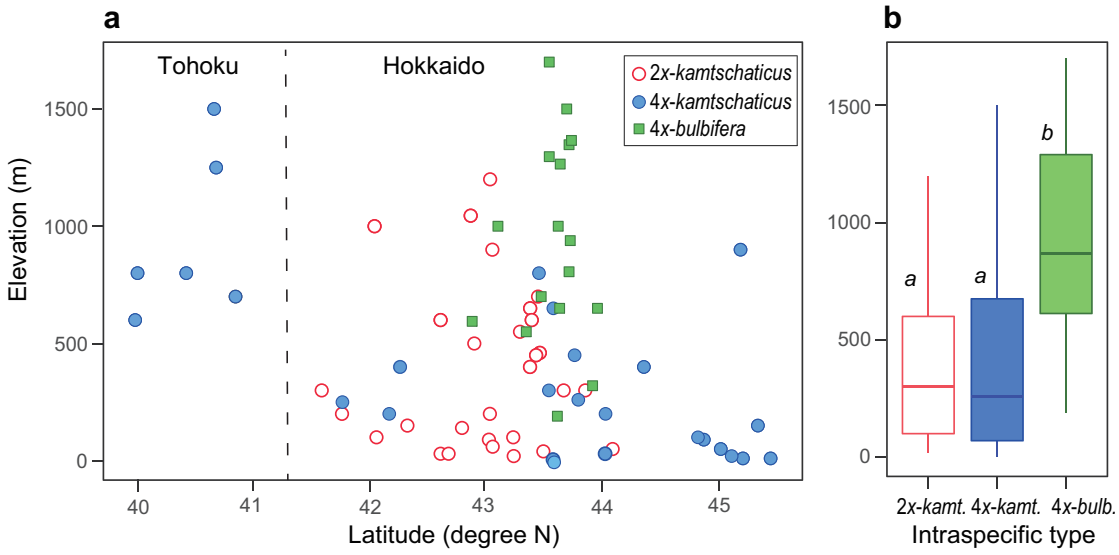

Fig. 2 **a** Latitudinal and elevational distributions of *Parasenecio kamtschaticus* populations of three intraspecific types. **b** Elevational distribution ranges of each type. Box plot includes median, first quartile, third quartile, and 1.5 times the interquartile range (whiskers). Different letters (*a*, *b*) indicate a significant difference ($p < 0.001$, GLM).

Figure 3

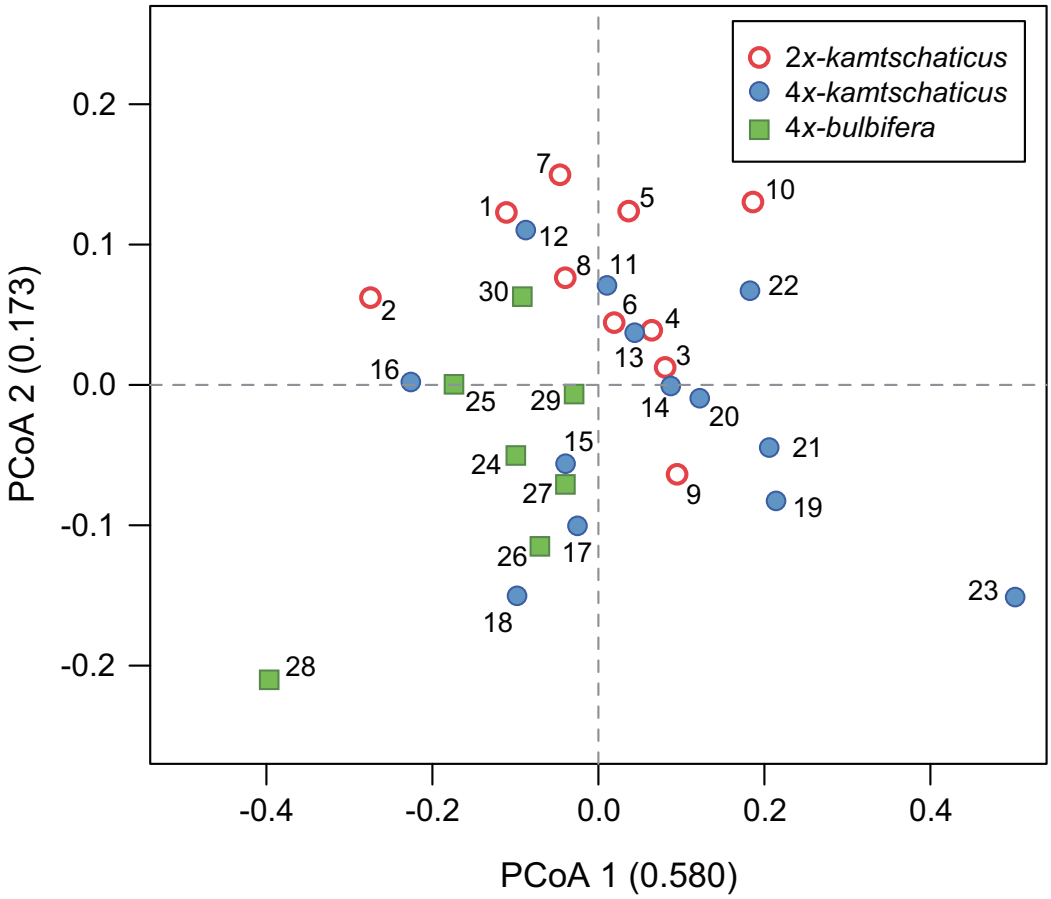

Fig. 3 Results of principal coordinate analysis (PCoA) of 30 populations, including three intraspecific types based on pairwise genetic distances [$F_{ST}/(1 - F_{ST})$ values] between pairs of populations. The number of each population corresponds to the location in Fig. 1 (see Table 1 for details). Explanatory contributions of the first (PCoA 1) and second axes (PCoA 2) are indicated in parentheses.

Figure 4

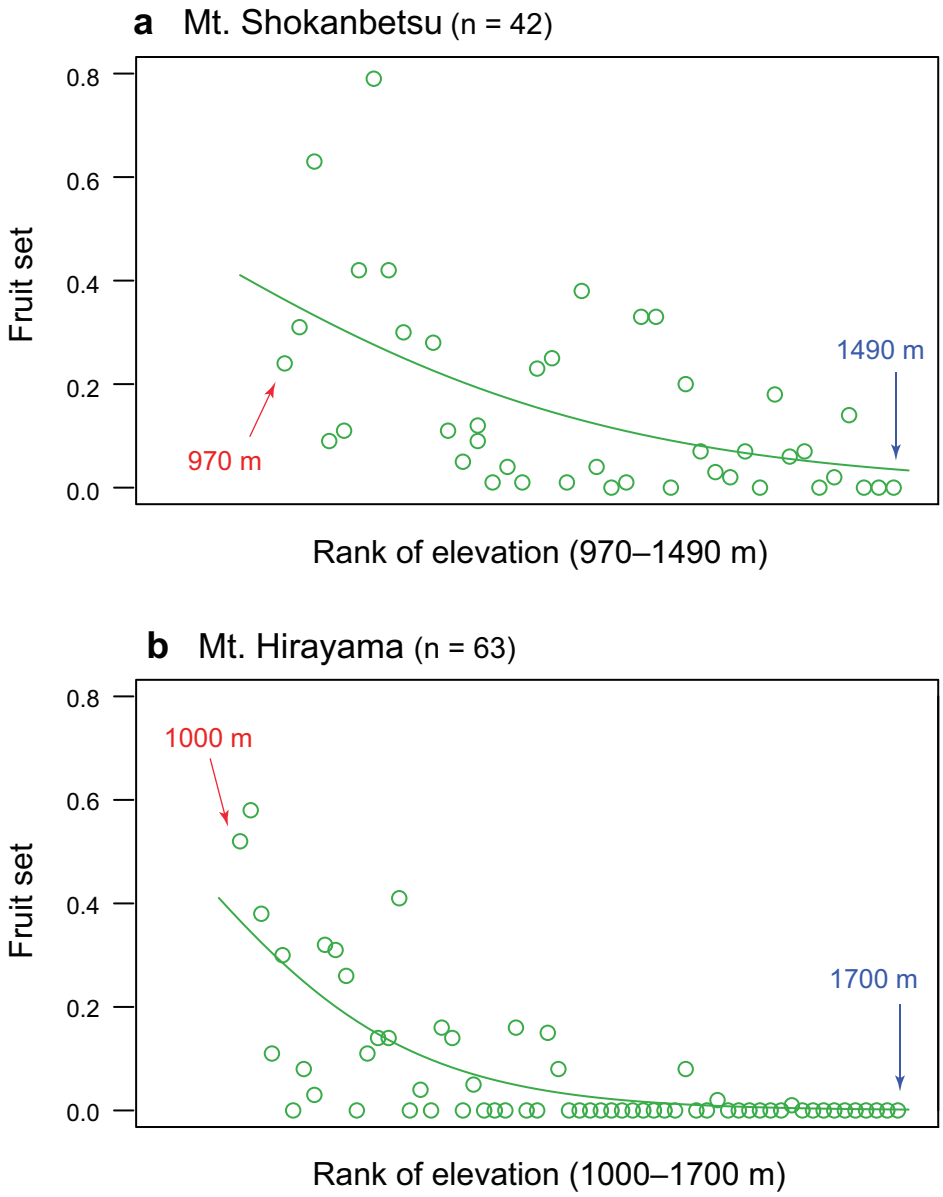

Fig. 4 Relationship between elevation and fruit set of *Parasenecio kamtschaticus* var. *bulbifera* under natural conditions on **a** Mt. Shokanbetsu and **b** Mt. Hirayama. Sampling points are arranged in order of elevation within the distribution range on each mountain.

Table 1 Location of sampling sites of *Parasenecio kamtschatica* complex, i.e., *P. kamtschaticus* var. *kamtschaticus* (diploidy and tetraploidy) and *P. kamtschaticus* var. *bulbifera* (tetraploidy), in the main distribution area. *n*: the number of sampling plants.

Site no.	Site name	Taxon	Ploidy	Location			*n*	Year
				Latitude (°N)	Longitude (°E)	Elevation (m)		
1	Tomakomai	*kamtschaticus*	2x	42.6744	141.5994	30	40	2003
2	Nopporo	*kamtschaticus*	2x	43.0547	141.5153	60	40	2003
3	Mt.Yoichi (h)	*kamtschaticus*	2x	43.0328	141.0208	1200	35	2004
4	Mt.Yoichi (l)	*kamtschaticus*	2x	43.0519	141.0075	900	38	2004
5	Mt.Soranuma (h)	*kamtschaticus*	2x	42.8650	141.2533	1050	30	2003
6	Mt.Soranuma (l)	*kamtschaticus*	2x	42.8961	141.2964	500	40	2003
7	Onnetoh	*kamtschaticus*	2x	43.3914	143.9781	600	38	2004
8	Akan	*kamtschaticus*	2x	43.3767	144.1042	400	36	2004
9	Shyakotan	*kamtschaticus*	2x	43.2903	140.5298	550	40	2007
10	Daisengen	*kamtschaticus*	2x	41.5805	140.1923	300	39	2007
11	Mt.Oakan	*kamtschaticus*	4x	43.4539	144.1644	800	80	2004
12	Tsubetsu	*kamtschaticus*	4x	43.5392	143.9861	300	30	2004
13	Shari Pass	*kamtschaticus*	4x	43.7928	144.7953	260	40	2004
14	Bekkai	*kamtschaticus*	4x	43.5833	145.2158	5	40	2004
15	Utoro	*kamtschaticus*	4x	44.0242	144.9281	30	40	2004
16	Wakkanai	*kamtschaticus*	4x	45.4511	142.0081	10	30	2004
17	Teshio	*kamtschaticus*	4x	45.0214	142.0497	50	30	2004
18	Rebun	*kamtschaticus*	4x	45.3414	141.0228	150	40	2004
19	Mt.Rakko	*kamtschaticus*	4x	42.2568	143.0859	400	39	2007
20	Mt.Apoi	*kamtschaticus*	4x	42.1628	143.1179	200	27	2007
21	Mt.Hakodate	*kamtschaticus*	4x	41.7592	140.7039	250	25	2007
22	Mt.Hakkoda	*kamtschaticus*	4x	40.6705	140.8699	1250	31	2007
23	Mt.Moriyoshi	*kamtschaticus*	4x	39.9926	140.5183	800	40	2007
24	Hisago Numa	*bulbifera*	4x	43.5483	142.8703	1700	30	2004
25	Numanohara	*bulbifera*	4x	43.5425	142.9581	1300	30	2004
26	Tenninkyo	*bulbifera*	4x	43.6197	142.7878	1000	30	2004
27	Mt.Tohma	*bulbifera*	4x	43.6911	142.8178	1500	40	2004
28	Mt.Hirayama	*bulbifera*	4x	43.7597	143.0072	1350	60	2003
29	Mt.Shokanbetsu (h)	*bulbifera*	4x	43.7158	141.5228	1350	40	2003
30	Mt.Shokanbetsu (l)	*bulbifera*	4x	43.7436	141.5250	940	35	2003

Table 2 Statistics of genetic variation within diploid and tetraploid populations of *Parasenecio kamtschaticus complex* in northern Japan based on seven loci. Of 30 populations observed, data of three populations are excluded due to the lack of genetic data for some loci.

Population	*n*	*A*	*Ae*	*Rs*	*He*	*Ho*	F_{is}
a *P. kamtschaticus* var. *kamtschaticus* (diploid)							
Tomakomai	40	2.57	1.30	1.90	0.210	0.141	0.331***
Nopporo	40	2.29	1.67	2.12	0.305	0.264	0.133*
Mt.Yoichi (h)	35	2.57	1.38	2.06	0.226	0.154	0.322***
Mt.Yoichi (l)	38	3.14	1.46	2.16	0.244	0.166	0.322***
Mt.Soranuma (h)	30	2.29	1.32	1.75	0.154	0.126	0.188*
Mt.Soranuma (l)	40	2.71	1.49	2.07	0.242	0.158	0.349***
Onnetoh	38	2.71	1.39	1.99	0.197	0.205	-0.045
Akan	36	2.57	1.49	2.09	0.243	0.242	0.004
Shakotan	40	2.57	1.40	2.13	0.248	0.151	0.396***
Mean		2.60	1.43	2.03	0.230	0.179	0.222
b *P. kamtschaticus* var. *kamtschaticus* (tetraploid)							
Mt.Oakan	80	3.00	1.48	2.30	0.269	0.187	0.309***
Tsubetsu	40	2.86	1.59	2.23	0.334	0.248	0.263***
Shari Pass	40	2.29	1.56	2.08	0.294	0.264	0.103***
Bekkai	40	2.86	1.55	2.16	0.292	0.240	0.179***
Utoro	30	2.86	1.91	2.39	0.412	0.349	0.155***
Wakkanai	30	2.71	1.49	2.10	0.282	0.228	0.197***
Teshio	30	2.86	1.73	2.30	0.338	0.254	0.253***
Rebun	40	2.86	1.97	2.29	0.407	0.366	0.104***
Mt.Rakko	39	2.43	1.52	2.06	0.270	0.126	0.537***
Mt.Hakodate	25	2.00	1.55	1.85	0.269	0.176	0.360***
Mt.Moriyoshi	40	2.00	1.21	1.50	0.117	0.082	0.304***
Mean		2.61	1.60	2.11	0.298	0.229	0.252
c *P. kamtschaticus* var. *bulbifera* (tetraploid)							
Hisago numa	30	2.71	1.60	2.11	0.330	0.279	0.158***
Numanohara	40	2.43	1.50	1.97	0.291	0.275	0.056*
Tenninkyo	40	2.86	1.59	2.07	0.318	0.290	0.091**
Mt.Tohma	40	2.71	1.63	2.13	0.332	0.262	0.213***
Mt.Hirayama	60	2.43	1.52	2.05	0.260	0.196	0.250***
Mt.Shokanbetsu (h)	40	3.00	1.44	2.19	0.269	0.231	0.145***
Mt.Shokanbetsu (l)	35	3.00	1.37	2.10	0.253	0.205	0.196***
Mean		2.73	1.52	2.09	0.293	0.248	0.158
Overall mean		2.64	1.52	2.08	0.274	0.217	0.218

n: sample size, *A*: average number of alleles per locus, *Ae*: effective number of alleles, *Rs*: allelic richness, *He*: expected heterozygosity, or gene diversity, *Ho*: observed heterozygosity, and F_{is}: inbreeding coefficient. *** $p < 0.001$, * $p < 0.05$

Table 3 Comparisons of reproductive traits of *Parasenecio kamtschaticus* complex between taxonomic groups, i.e., var. *kamtschaticus* 2x, var. *kamtschaticus* 4x, and var. *bulbifera*. Capitulum number per plant, proportion of fruit set under natural conditions, individual achene weight, and germination rate of achenes were compared using GLMMs in which site was incorporated in a random factor. Mean ± se.

Taxon	Site	*n*	Capitulum no.	Fruit set	Achene size (mg)	Germination rate
kamtschaticus (*k*2x)	Tomakomai	40	38.6 ± 3.3	0.19 ± 0.02	1.46 ± 0.01	0.40 ± 0.003
	Nopporo	40	50.2 ± 4.7	0.31 ± 0.03	1.84 ± 0.01	0.48 + 0.005
kamtschaticus (*k*4x)	Utoro	41	103.4 ± 7.4	0.33 ± 0.03	2.25 ± 0.02	0.25 ± 0.003
	Wakkanai	31	59.3 ± 6.3	0.36 ± 0.04	1.87 ± 0.02	0.29 ± 0.005
bulbifera (*b*)	Mt.Hirayama	63	24.6 ± 2.4	0.07 ± 0.02	1.33 ± 0.01	0.05 ± 0.003
	Mt.Shokanbetsu (h)	42	26.3 ± 3.0	0.15 ± 0.03	1.44 ± 0.01	0.10 ± 0.002
GLMM results			*k*4x > *k*2x > *b* **	*k*2x, *k*4x > *b* ***	*k*4x > *k*2x, *b* +	*k*2x > *k*4x > *b* ***

+ $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$

Geographical distribution, genetic diversity, and reproductive traits of mixed polyploid populations in *Parasenecio kamtschaticus* (Senecioneae; Asteraceae).
Plant Systematics and Evolution

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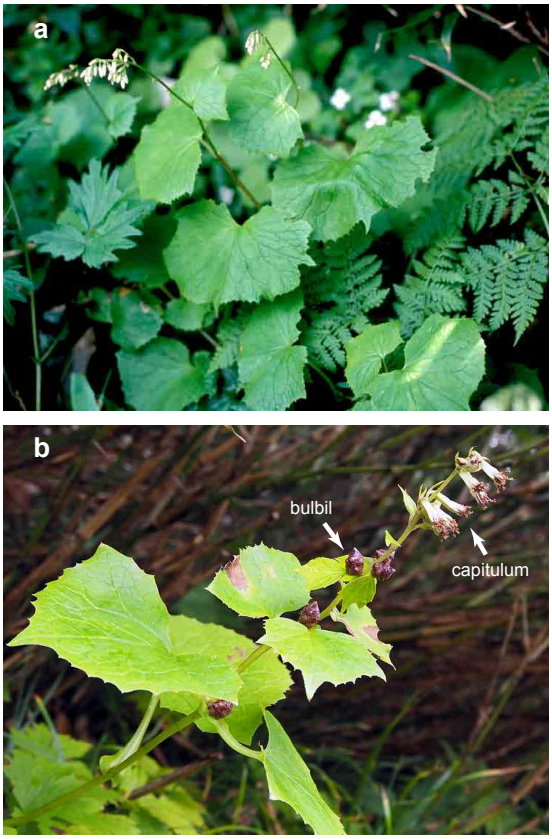

Fig S1. *Parasenecio kamtschaticus* var. *kamtschaticus* (a) and *P. kamtschaticus* var. *bulbifera* (b) at the flowering stage.

Geographical distribution, genetic diversity, and reproductive traits of mixed polyploid populations in *Parasenecio kamtschaticus* (Senecioneae; Asteraceae). Plant Systematics and Evolution

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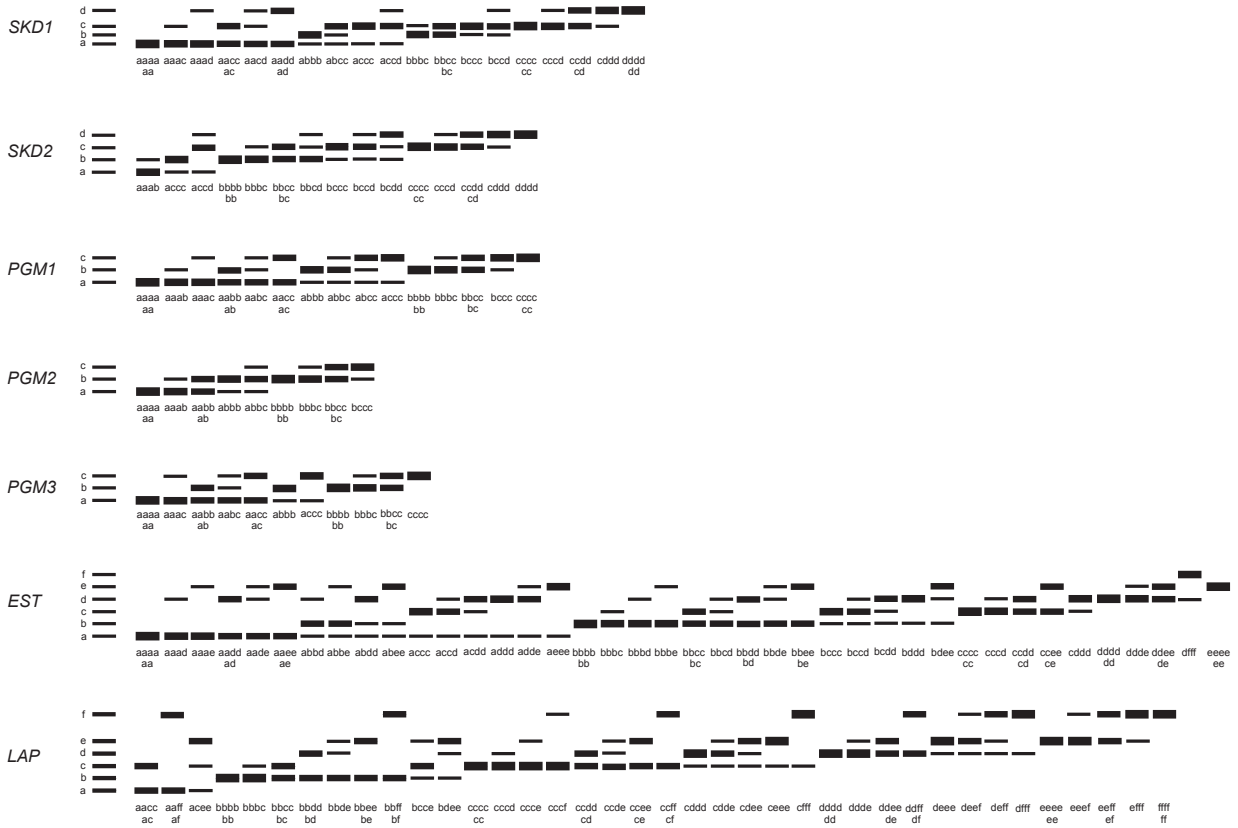

Fig. S2. Banding patterns of seven loci observed in individuals of *Parasenecio kamtschaticus* cytotypes. The identified genotype is shown in each band for diploid (lower) and tetraploid (upper). SKD: shikimic acid dehydrogenase, PGM: phosphoglucomutase, EST: esterase, and LAP: leucine aminopeptidase.

Geographical distribution, genetic diversity, and reproductive traits of mixed polyploid populations in *Parasenecio kamtschaticus* (Senecioneae; Asteraceae).
Plant Systematics and Evolution

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