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RESEARCH ARTICLE

Short Title: Hirao et al.—Fellfield–snowbed ecotypes of an alpine herb

Ecotypic divergences of the alpine herb *Potentilla matsumurae* adapted to fellfield–snowbed habitats across a series of mountain sky islands

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PREMISE OF THE STUDY: Divergent selection due to environmental heterogeneity can lead to local adaptation. However, the ecological and evolutionary processes of local adaptation that occurs across multiple regions are often unknown. Our previous studies reported on the ecotypic divergence within a local area of variation of *Potentilla matsumurae*, an alpine herb adapted to the fellfield–snowbed environment. Here we investigated large-scale geographic patterns of ecotypic differentiation in this species to infer local adaptation and selective forces across multiple regions.

METHODS: We compiled information on the overall distributions of fellfield and snowbed habitats on the mountains in Japan across the distribution of the species. Next, we conducted common garden experiments to test the adaptive divergence of the fellfield–snowbed plants derived from multiple regions. Finally, we evaluated phylogeographic structures based on cpDNA and allozyme variations and inferred the evolutionary history of ecotype differentiation.

KEY RESULTS: The mosaic distribution of the fellfield–snowbed ecotypes across isolated mountaintops constitutes indirect evidence for habitat-specific natural selection. The significant difference in survivorship between the ecotypes observed in a controlled snow environment provides more substantial evidence of local selection. Phylogeographic structures support the hypothesis that ecotypic divergence events from fellfield to snowbed populations occurred independently in at least two distinct regions.

CONCLUSIONS: Ecotypic divergence of *P. matsumurae* has occurred across a series of mountain sky islands. Local selection in snowy environments is a driving force that maintains the divergent ecotypes across multiple mountain regions and can contribute to the diversification of plants in heavy-snow regions.

KEY WORDS: common garden experiment; ecotype; genetic structure; Japan; local adaptation; microhabitat; parapatric; phylogeography; Rosaceae; survival rate.

INTRODUCTION

Divergent selection due to environmental heterogeneity drives the establishment of local adaptation (Schluter, 2000; Nosil, 2012). Varying patterns of local adaptation are expected to emerge when selective pressure is sufficiently strong relative to other evolutionary forces, particularly gene flow (Kawecki and Ebert, 2004). During the last nearly hundred years, numerous reciprocal transplant and common garden experiments and studies of populations along environmental clines have demonstrated that local adaptation of plant species is widespread (Linhart and Grant, 1996; Leimu and Fischer, 2008; Lascoux et al., 2016); however, its extent and scale greatly varies among species and populations. In populations, adaptive genetic variation can be continuously distributed along environmental gradients or discretely in specific environments. Furthermore, in the latter case, divergent selection along with restricted gene flow can lead to the formation of discernable “ecotypes”. Here we define an ecotype as a group of individuals belonging to the same species that share a set of traits that are adaptive to a specific habitat (see Lowry, 2012).

Since the seminal work of Turesson (1922), evidence for genetically divergent ecotypes has accumulated across various spatial scales (e.g., Turesson, 1925, 1931; Clausen et al., 1940, 1947; Clausen, 1951; Jain and Bradshaw, 1966; Antonovics and Bradshaw, 1968; Galen et al., 1991; Lowry et al., 2008, 2014; Andrew et al., 2012; Sakaguchi et al., 2017). Most of the initially recognized ecotypes—including most of those reported in the classic studies of Clausen and his colleagues—were geographically isolated, where macroscale environmental disruption, acting as both a divergent selective force as well as a strong physical barrier to gene flow, can enforce allopatric differentiation of ecotypes across large areas. However, ecotypic divergence can also occur at a small spatial scale if local selective pressures linked to ecological isolation (such as differences in flowering time) are sufficiently strong to counteract gene flow (e.g., Jain and Bradshaw, 1966; Antonovics and Bradshaw, 1968; Andrew et al., 2012; Sakaguchi et al., 2018). Therefore, the clarification of the spatial scales of divergent selection is crucial to understand how adaptive differentiation occurs between conspecific populations. Furthermore, the driving forces leading the creation and maintenance of divergent ecotypes across discrete regions need to be recognized to understand the geographic pattern of local adaptation and its generality.

In the present study, we focus on the environmental heterogeneity of alpine ecosystems

and examine the habitat-specific selective forces leading to local adaptation across a series of isolated mountain regions. Alpine ecosystems in snowy regions are dominated by two contrasting habitats, fellfield and snowbed, arranged in a mosaic landscape (Fig. 1). Wind-blown fellfields constitute a harsh environment located on ridges and summits; snowbeds are moderate environments located on leeward slopes and microtopographic depressions. The fellfield habitat is climatically harsh and less fertile and promotes less competition and supports a longer growing season. Conversely, the snowbed habitat is climatically mild and more fertile but promotes more competition and supports a shorter growing season (Miller, 1982). Therefore, the environmental heterogeneity across fellfield and snowbed habitats can be a driving force for the creation and maintenance of local adaptation in alpine plants, even at a small spatial scale. On the other hand, viewed at a larger scale, highly elevated places, such as different mountain ranges surrounded by lower terrain, are geographically isolated; these mountain sky islands have served as barriers to historical gene flows over long periods of time. Therefore, a series of mountain sky islands provides a replicated natural experiment to investigate the patterns of local adaptation to heterogeneous environments at micro- to macroscale scales.

In alpine environments, snowmelt regime is an extrinsic factor that can strongly impact the small-scale, short-term gene flow that occurs through pollen dispersal among plant populations. Differences in snowmelt regimes can restrict gene flow among plant populations in diverse habitats, thereby leading to ecological isolation (reviewed in Kudo, 2016). Snowmelt time directly determines the timing of growth initiation and reproduction of plants of the same species (Holway and Ward, 1965; Kudo, 1991); therefore, phenological progress can be asynchronous among local populations along a snowmelt gradient that is determined by the heterogeneous alpine landscape. In fact, the snowmelt regime is an effective barrier to pollen-mediated gene flow and thus can cause spatial variation in the genetic structures of plant populations, even at the microscale (Stanton et al., 1997; Hirao and Kudo, 2004, 2008; Yamagishi et al., 2005). Restricted gene flow by phenological isolation can accelerate local adaptation to divergent environments inherently linked to the snowmelt regime. Thus, snowy alpine ecosystems offer a unique opportunity to identify selective forces driving local adaptation.

An excellent model system for examining selective forces causing divergence and the establishment of ecotypes at multiple spatial scales is provided by the alpine herb *Potentilla*

matsumurae, which grows in diverse environments from fellfield to snowbed habitats across the Japanese archipelago. Several life-history traits in this species, such as reproductive phenology, flower number per inflorescence, ovule number per flower, germination pattern, seedling establishment, and growth form, differ significantly between fellfield and snowbed populations within a local area (Shimono and Kudo, 2003; Shimono et al., 2009). The fellfield plants are dwarfed with small and thick leaves that are likely advantageous for survival in a dry, wind-blown habitat, whereas the snowbed plants are tall with large and thin leaves that likely allow the acquisition of more light in a moist but competitive habitat (see for examples; McGraw and Antonovics, 1983; McGraw, 1983, 1985a, 1985b, 1987a, 1987b). Moreover, flowering phenologies differ greatly between fellfield and snowbed plants, presumably as a result of prezygotic isolation between fellfield and snowbed populations (Shimono and Kudo, 2003; Shimono et al., 2009). Furthermore, the differences in morphological traits between fellfield and snowbed plants are maintained when the plants are grown under uniform conditions in a greenhouse (Shimono et al., 2009), thereby indicating genetic divergence of the fellfield and snowbed ecotypes.

A reciprocal sowing experiment between the fellfield and snowbed habitats in the Taisetsu Mountains revealed that seedlings of the snowbed ecotype could not survive at the fellfield site, but seedlings of the fellfield ecotype survived at the snowbed site throughout the 2-year study (Shimono and Kudo, 2003). Although both ecotypes had similar survivorship in the snowbed habitat, native snowbed seedlings were larger than fellfield seedlings. The complete immigrant inviability of the snowbed ecotype in the fellfield habitat indicates a clear home-site advantage of the fellfield ecotype, while a home-site survivorship advantage for the snowbed ecotype has not been clearly demonstrated, at least at the seedling stage. However, such home-site advantage is expected to occur over the long term. These observations led to the hypothesis that the snowbed ecotypes were derived from fellfield ecotypes and not vice versa.

Although the differentiation between the fellfield and snowbed ecotypes of *P. matsumurae* is supported only in the case of a single mountain region in northern Japan (Shimono and Kudo, 2003; Shimono et al., 2009), allozyme variation suggests that genetic differentiation between fellfield and snowbed populations has also occurred in the mountain region of Central Japan (Shimono et al., 2009). Therefore, fellfield and snowbed ecotypes

may exist at a broader scale across multiple mountain ranges in the Japanese archipelago. However, a broad-scale approach with a historical (phylogeographic) perspective is required to infer the evolutionary history of ecotype differentiation. Phylogeographic studies on Japanese alpine flora have demonstrated the existence of distinct lineages in Central Honshu and northern populations across multiple alpine plant species (e.g., Fujii et al., 1997; Fujii and Senni, 2006; Ikeda et al., 2006, 2009; Hirao et al., 2017), including *P. matsumurae* (Ikeda et al., 2006, 2008). On the basis of these findings, it has been proposed that the mountains of Central Honshu serve as refugia during historical climatic oscillations and populations in this area have been historically isolated from the populations of the more northern regions (reviewed by Fujii and Senni, 2006). However, the phylogeographical basis of ecotype differentiation in *P. matsumurae* remains unresolved because the previous studies did not consider microhabitat affinities.

In this study, we examined large-scale geographic patterns of ecotypic differentiation in *P. matsumurae* to assess local adaptation across a series of isolated regions and to infer the evolutionary history of ecotype differentiation. First, through literature and field surveys, we compiled the current distributions of fellfield and snowbed habitats of this species across the Japanese archipelago. Second, we performed two types of common garden experiments to test local adaptations to fellfield–snowbed environmental variations, using plants derived from 12 populations—seven fellfield populations and five snowbed populations—across nine mountain regions in Japan. A “mild” common garden experiment was first set up in a climatically mild environment to compare the performances of the genetically differentiated forms between fellfield and snowbed habitats from multiple regions. A “snowy” common garden experiment in a snowbed-like environment tested for a home-site advantage of the snowbed ecotypes in comparison with the fellfield ecotypes. In this experiment, we focused on the survivorship of both ecotypes only under snowy (i.e., snowbed-like) conditions but not under less snowy (i.e., fellfield-like) conditions. This is because our previous study (Shimono and Kudo, 2003) had already revealed that the snowbed ecotype is completely unable to survive in the fellfield habitat. However, the responses of the fellfield ecotypes after the seedling stage at the snowbed habitat are still unclear. Thus, we compared survivorship of established plants, that were grown in the mild common garden, in the snowy common garden. We then assessed the genetic structures of *P. matsumurae* populations to infer the divergent history of the fellfield and snowbed

ecotypes. Using chloroplast (cp) DNA sequences, we analyzed the phylogeographic structure of 23 *P. matsumurae* populations—15 fellfield and eight snowbed populations—across 16 mountainous regions throughout the distribution range. To complement the analyses of cpDNA sequences as a single organelle marker, we reevaluated the allozyme-based genetic structure of 15 populations across two distant regions in Japan, i.e., five fellfield and six snowbed populations from northern Japan and two fellfield and two snowbed populations from Central Japan.

We aimed to answer the following questions: (1) Are the fellfield and snowbed forms of *P. matsumurae* in mountain sky islands across multiple mountain ranges in the Japanese archipelago genetically divergent? (2) If so, do fellfield and snowbed ecotypes indicate habitat-specific survivorship under controlled conditions? (3) Did the divergent ecotypes found across isolated mountaintops in Japan originate from a single or multiple differentiation events? Finally, we considered the driving forces affecting the establishment and maintenance of the fellfield–snowbed ecotypes across multiple regions in the context of historical climate oscillations and current climate changes.

MATERIALS AND METHODS

Plant species

The alpine cinquefoil *Potentilla matsumurae* Th.Wolf is a perennial herb commonly distributed in alpine regions from Sakhalin and the Kuril Islands to Taiwan; however, it is mainly found in the Japanese archipelago (Ikeda, 2016). The self-compatible, hermaphroditic flowers are mainly pollinated by dipteran insects. The flowering season ranges from early June to early August, depending on the timing of snowmelt in local areas, and adjacent populations with different snowmelt schedules are temporally isolated by the timing of anthesis (Kudo, 2006). Seeds mature within approximately 1 month after flowering and are passively dispersed around the parent plants. *Potentilla matsumurae* is a tetraploid species with $4n = 28$ (Shimizu, 2002).

Distribution of habitat types

In our literature survey that determined the current distribution of fellfield and snowbed habitats of *P. matsumurae* across the high mountains of Japan, we retrieved data about

habitat types from 31 publications on Japanese alpine flora—including books, papers, and reports (e.g., Sato, 2007; see additional references in Appendix S1). In addition, we conducted field observations in 16 mountain regions, as mentioned below. We categorized habitat types within each mountain region (i.e., contiguous high-altitude terrain around mountain summits) as (1) fellfield only, (2) snowbed only, (3) both fellfield and snowbed, and (4) unknown. Next, to assess the potential distribution of the target species, we summarized the distributions of fellfield and snowbed vegetation in Japanese high mountain regions based on a 1:50,000 vegetation map obtained from the second to the fifth National Survey of the Natural Environment (Ministry of the Environment Japan: http://www.biodic.go.jp/english/kiso/top_list.html) and arranged by the Conservation GIS consortium Japan (<http://cgis.jp/>).

Leaf size variation under natural and controlled conditions

We measured leaf width, one of the most distinctive traits that distinguish between the ecotypes (Shimono et al., 2009), under natural field conditions and in the mild common garden experiment. In field surveys of 2007 and 2008, we randomly selected 26–109 individual plants from each of the 11 populations—six fellfield populations and five snowbed populations—in seven mountain regions (Table 1) and measured the width of the largest fully developed leaf. These natural fellfield and snowbed habitats provide contrasting physical environments (Kudo et al., 2010; for example, see in Appendix S2). In addition, we used leaf-width data obtained from natural fellfield and snowbed populations in the Taisetsu Mountains (from Shimono et al., 2009) as well as data from seven herbarium specimens (MAK3232) from a fellfield population in the Hakkoda Mountains. The total sample size was 687 plants from 14 populations—eight fellfield populations and six snowbed populations—in nine mountain regions. The mean number of sampled plants per population was 49.1.

In 2007 and 2008, we collected seeds from 12 source populations—seven fellfield populations and five snowbed populations in the nine mountain regions (Table 1)—from the field survey of 14 populations. No seed was collected from the TOT fellfield and the ONT snowbed populations used for the common garden experiments because mature seeds were not available at the time when the field trips to these populations were made. Fully mature seeds were sampled from 15–50 of the same plants (all growing at least 2 m apart)

measured for leaf width in each population and were kept at room temperature (approximately 20°C) until the experiment. In June 2009, seeds were germinated in Petri dishes as described by Shimono et al. (2009). One week after germination, approximately 10 cotyledon-stage seedlings (depending on the success in germination) from each maternal plant were randomly transplanted into trays measuring 29 × 38 × 1.3 cm containing potting soil (Golden Peat-Board, Sakata Seed Co., Yokohama, Japan) and placed on the ground of the first mild common garden, located at a low elevation with a mild climate in Matsumoto, Nagano Prefecture, Central Japan (36.25°N, 137.98°E; 610 m a.s.l.). At this site, the annual mean air temperature during 1981–2010 was 11.8°C, monthly mean temperature ranged from 24.7°C in August to –0.4°C in February, and the annual mean rainfall was 1031 mm, and average annual maximum snow depth was 27 cm, respectively (Matsumoto Weather Station, Japan Meteorological Agency). Two months after the first transplanting, seedlings with more than three true leaves were randomly selected, retransplanted into 12 × 10 cm pots containing a mixture of garden soil and peat moss at a density of one seedling per pot, and placed on the ground. Plants were grown under natural sunlight and watered as needed but received neither additional nutrients nor any protection against herbivory. In November 2009, we measured the width of the largest fully developed leaf in each of the established plants without herbivore damage. From each original source population, we obtained measurements from 6–63 plants (mean, 32.3 plants). After one growing season in the mild common garden, the survival rates of the potted plants were high and not different between the putative ecotypes (Appendix S3).

Survivorship in a snowy environment

A “snowy” common garden was established at Sugadaira Research Station, Mountain Science Center, University of Tsukuba (36°31′N, 138°21′E; 1300 m a.s.l., which is located in a snowy mountain region of Nagano Prefecture, Central Honshu, Japan (Appendix S4A). In most years, the Sugadaira region experiences cold temperatures and continuous snow cover from early December to early April. The continuous snow cover effectively maintains the ground temperature at a constant 0°C throughout the winter (Appendix S4B), thereby providing a snowbed-like environment. At the snowy common garden site, the annual mean air temperature was 6.5°C, monthly mean temperatures ranged from 19.4°C in August to

–5.6°C in February, and the annual mean rainfall and maximum snow depth were 1226 mm and 102 cm, respectively (data from 1971 to 2006, Sugadaira Research Station records). To assess the survivorship of established plants, but not seedlings, in the snowbed-like environment, 7–73 two-season-old plants (mean, 36.3 plants) that descended from 1–32 maternal plants from each of the 12 source populations were translocated from the mild common garden to the snowy common garden in autumn 2010 (Table 1). Survival data were collected in November 2012, following the two growing seasons at the snowy common garden.

Data analyses

We assessed the fixed effect of putative “ecotype” (fellfield or snowbed) on morphological and fitness response variables (i.e., leaf width and survivorship, respectively) using hierarchical Bayesian models designed explicitly to handle complex hierarchical structured data (see Appendix S5 for details). In full models, the sum-to-zero constrained random effects included “maternal family” (derived from maternal sources) nested within “population” nested within “mountain” region. In preliminary analyses, we used a model selection approach based on the deviance information criterion (Spiegelhalter et al., 2002) to select a constant set of fixed and random effects to compare across models (Appendices S6, S7). Hierarchical linear models for leaf width at the field sites and in the mild common garden were separately performed. Furthermore, the overall leaf-width data set from the natural sites and the mild common garden were jointly assessed by one comprehensive model that included the fixed effects of ecotype and location (natural site or common garden) and their interaction, simultaneously considering both fellfield and snowbed ecotypes, experimental location, and environmental responses of the ecotypes. In the comprehensive model for leaf width, the random effect of maternal family included the mother-and-child relationship between maternal plants in natural sites and their offspring in the mild common garden, as well as the sibling relationship among plants derived from same maternal plants in the mild common garden. A hierarchical logistic model for survivorship in the snowy common garden was performed with the same set of fixed and random effects as mentioned above. All the Bayesian models were implemented in the JAGS 4.2.0 program through the R packages RJAGS (Plummer, 2013).

Phylogeographic structure inferred from cpDNA

For the DNA analyses, leaf materials of *P. matsumurae* were collected from fellfield and snowbed populations in each of 16 mountain regions, broadly covering its distribution area in the Japanese archipelago (Fig. 2A). Approximately 10 individuals, separated by at least 2 m, were sampled per population. In total, 328 individuals from 23 populations—15 fellfield populations and eight snowbed populations—were sampled from the 16 regions (Table 1; Fig. 2A). Collected leaf materials were dried with silica gel and stored at room temperature. DNA was extracted from the dried leaf tissue by the cetyltrimethyl-ammonium bromide method (Stewart and Via, 1993) or by using a DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol.

We analyzed two noncoding cpDNA regions (the *rpl20-rps12* spacer and the *trnT-trnL* spacer), which were PCR-amplified and then sequenced using primers from Ikeda et al. (2006). PCR was carried out in a total volume of 10 μ L containing 2.5 U AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA), 1 \times PCR buffer, 0.5 μ M of each pair of primers, 0.2 mM of each dNTP, and ~20 ng of template DNA. The thermal cycling program included a denaturing step at 94°C for 9 min, followed by 30 cycles of 60 s at 94°C, annealing at the specific temperature of the primers (Ikeda et al., 2006) for 60 s, and extension for 60 s at 72°C, and a final extension for 7 min at 72°C. The PCR products were purified by using an ExoSAP-IT PCR Clean-up Kit with 0.1 \times dilution (GE Healthcare, Little Chalfont, UK). Cycle sequencing reactions of forward and reverse strands were performed with the same PCR primers and using the BigDye v.3.1 Cycle Sequencing Kit (Applied Biosystems) with 0.1 \times dilution. The sequencing products were analyzed on an ABI Prism 3130 automated sequencer (Applied Biosystems). We aligned and edited the sequences using muscle (Edgar, 2004) and MEGA7 (Kumar et al., 2016) programs. The newly generated haplotype sequences have been deposited in DDBJ/EMBL/GenBank, with accession numbers LC378550–LC378574. We did not include previously deposited sequences for *P. matsumurae* (AB241646–AB241659) in the following analyses because some insertions and deletions had been excluded from the deposited sequences (H. Ikeda, personal communication).

Phylogenetic relationships between cpDNA haplotypes (with *Potentilla nivea* as an outgroup) were initially examined by Bayesian inference analysis using the MrBayes 3.1.2 program (Ronquist et al., 2012). To select the optimum substitution models for the

phylogenetic inference, the Bayesian information criterion and the Kakusan4 program (Tanabe, 2011) were used. Two sets of four Markov chain Monte Carlo (MCMC) runs were executed over 10^7 generations. After convergence among runs was verified by examining the likelihood plots with the Tracer 1.6 program (Rambaut et al., 2014), and after confirming that all the effective sample size (ESS: Kass et al., 1998) values exceeded 200, the first 10% of the trees were discarded as burn-in. We also performed maximum likelihood analyses under a GTR + G model and 1000 bootstrap replicates using the RAxML 8.0.26 program (Stamatakis, 2014) implemented in the raxmlGUI software package version 1.3.1 (Silvestro and Michalak, 2012). In interpreting phylogenetic confidence, we considered nodes to be strongly supported when they received both a posterior probability of 0.97 and bootstrap proportion of 80% or higher. To further detect genealogical relationships among haplotypes, we constructed a median-joining haplotype network using the Network 5.0.0.1 program (Bandelt et al., 1999). Genetic diversity for each population, including both haplotype diversity (H_d) and nucleotide diversity (π), were inferred by using the Arlequin version 3.5 program (Excoffier and Lischer, 2010). Spatial analysis of molecular variance (SAMOVA, Dupanloup et al., 2002) was used to test for genetic differentiation among the 23 populations by dividing them into a number of genetically partitioned groups. This method allows groups of geographically proximate populations that are maximally differentiated from each other to be defined through a simulated annealing procedure without any prior assumption about group composition. Analyses were performed with the sampling points for each population for ($K = 2$ to 10), and the maximum fixation index F_{CT} (variance among groups relative to the total variance) value, and the minimum number of groups among the analyses were used to select the optimal number of groups (K).

Population genetic structure inferred from allozyme variation

We reevaluated allozyme variation in 330 individuals representing 15 populations—five fellfield populations and six snowbed populations from Taisetsu Mountains, Hokkaido (northern Japan) and two fellfield populations and two snowbed populations from Mt. Tateyama, Central Honshu (Central Japan)—using the dataset of 10 allozyme loci from Shimono et al. (2009). First, we assessed the neutrality of the 10 allozyme loci using the coalescent-based simulation of Beaumont and Nichols (1996), as implemented in FDIST2.

A null distribution of genetic differentiation (estimated as F_{ST}) was generated using 100,000 simulations, and putative outlier loci were found above or below the 99% confidence limit. We then tested for associations between allele frequencies at putative outlier loci under divergent selection and a habitat property (fellfield or snowbed) using a general linear mixed model with spatial autocorrelations, as implemented in the R package spaMM (Rousset and Ferdy, 2014). Second, we performed principal component analysis (PCA) to characterize allozyme genotypes with or without outlier loci, using the R package adegenet (Jombart, 2008). In addition, we tested two AMOVA (analysis of molecular variance) models, estimating the percent of molecular variance accounted for by each level of the nested sampling hierarchy in which the 15 populations were grouped according to a habitat property (fellfield or snowbed) or a regional property (northern or Central Japan). All AMOVA were run using the extended framework of Meirmans and Liu (2018), as implemented in the R poppr package (Kamvar et al., 2014). The significance of variance components in the AMOVA models was tested by 9999 random permutations as described in Excoffier et al. (1992).

RESULTS

Distributions of the habitat types

Cross-checking of the literature survey with the field observation provided confidence results of habitat type of *P. matsumurae* across the mountain regions. *Potentilla matsumurae* inhabited fellfields on high mountains across the Japanese archipelago, from Hokkaido through Tohoku to Central Honshu (Fig. 2A); and it was found on most of the Japanese high mountains where fellfield vegetation occurs (Appendix S8A). Conversely, it inhabited snowbeds in Hokkaido and Central Honshu but not in Tohoku (Fig. 2A), although snowbed vegetation is distributed in all three of these regions, mainly along the Japan Sea side (Appendix S8B). Mountain regions that contain only fellfield habitats are widely distributed in Japan from Hokkaido to Central Honshu, mainly on the Pacific Ocean side, whereas most mountain regions containing snowbed habitats have fellfield habitats as well, with the exception of Mt. Yoichi and Mt. Mekunnai in Hokkaido, where only snowbed habitats were found.

Genetic differentiation in leaf size

Potentilla matsumurae populations in natural snowbeds had significantly larger leaf widths than those in the natural fellfield populations across multiple mountain regions, although the means and variances differed greatly within and among populations (Fig. 3A; Table 2). Moreover, in the mild common garden experiment, morphological variation between the putative ecotypes were clearly maintained (Fig. 3B). In the hierarchical Bayesian models, the 95% credible intervals of the posterior distribution for the effect of ecotype (β_1) did not overlap with zero under either natural site or common garden conditions (Table 2). In addition, in the comprehensive model for the combined data set for the natural sites and the mild common garden, the effect of ecotype (β_1) was evident, as well as the effect of experimental location (natural field site or first mild common garden: β_2) was significant; in addition, the interaction between ecotype with location (coefficient β_3) was significant—the 95% credible intervals of the posterior distribution for the effect of interaction between ecotype and location (β_3) ranged in the negative values and did not overlap with zero, indicating that the snowbed ecotype has more morphological variation in response to environments than the fellfield ecotype (Fig. 3C; Table 2).

Survivorship in a snowy environment

The snowy common garden experiment revealed that most *P. matsumurae* plants derived from snowbed populations survived at the snowy site, while those derived from fellfields showed low survivorship, except for plants derived from the CHO fellfield populations (Fig. 4). In the hierarchical Bayesian model for survivorship, 95% credible intervals of the posterior distribution for the effect of habitat (β_1) were positive (they did not overlap zero), indicating that snowbed plants had a home-site advantage (Table 3). The posterior distributions for the random effects (family, population, and mountain) suggest substantial heterogeneity among families nested within populations, and among populations nested within mountains (Fig. 4; Table 3).

Phylogenetic relationships among cpDNA haplotypes

We identified a total of 24 cpDNA haplotypes for *P. matsumurae* based on the combined polymorphisms in the *rpl20–rps12* and *trnT–trnL* spacers (Appendix S9). The total

alignment length of the sequences is 1487 bp, based on an alignment of 328 individuals from 23 populations across 16 mountain regions. Haplotype frequencies within each population are presented in Fig. 2. Among the 24 haplotypes, 14 were restricted to specific populations. Haplotype diversity (H_d) and nucleotide diversity (π) in individual populations ranged from 0.000 to 0.727 and from 0.0000 to 0.0121, respectively.

The fellfield populations included 22 of the 24 haplotypes. Conversely, snowbed populations included only six haplotypes; of 113 samples from snowbed populations, 105 (>90%) each included four of these six haplotypes (A1, C1, L1, and G1), which were also found in fellfield populations; the other two of these six haplotypes (A7 and G2) were specific to snowbed populations. Both A7 and G2 represent sequences that can be obtained by a one-step mutation in haplotypes found in the fellfield populations. Per-population nucleotide diversity did not differ significantly between fellfield and snowbed populations ($\pi = 0.0014$ and 0.0027 , $SD = 0.0025$ and 0.0045 , respectively). The phylogenetic tree of the cpDNA haplotypes includes two major clades, corresponding to the northern and southern regions of the *P. matsumurae* distribution (Fig. 5). The northern clade includes haplotypes found in the Hokkaido and Tohoku populations, whereas almost all haplotypes included in the southern clade were found in the Central Honshu populations, with the exception of haplotype A7. The northern clade showed a deeper level of genetic structuring than in the southern clade; three main clusters were identified, two of them with very high support. On the median-joining network of cpDNA haplotypes, the phylogenetic structure of the southern clade (A1–A10 and C1) appears star-like, with branches originating from the most widespread haplotype (A1, from Central Honshu) (Fig. 2B). Conversely, the northern clade has a more complicated structure with more diverged haplotypes (Fig. 2B). The SAMOVA analysis identified two groups of populations ($K = 2$) as the optimal number of groups to best explain among-group spatial partitioning of the molecular variance: the northern group included all of the Hokkaido and Tohoku populations, and the southern group included all of the Central Honshu populations. The two-group genetic structure explains 66.60% of the overall molecular variance ($F_{CT} = 0.666$; $P < 0.001$); 24.33% of the variance is explained by among-population differences within each group ($F_{SC} = 0.729$; $P < 0.001$), and 90.93% of the variance is explained by differences among populations ($F_{ST} = 0.909$; $P < 0.001$).

Allozyme-based genetic structure

In the outlier test for the allozyme loci, three of the 10 loci analyzed were identified as potentially non-neutral loci, including *LAP-2* under divergent selection and *MDH* and *SKD* under balancing selection (Fig. 6). In addition, a generalized linear mixed model with spatial autocorrelations found that *LAP-2* was highly associated with a habitat property ($P < 0.001$). The PCA based on all the 10 loci (including the putative outliers) displayed marked differentiation between fellfield and snowbed plants, where fellfield plants in both northern and Central Japan, snowbed plants in northern Japan, and snowbed plants in Central Japan were clustered separately on the score plot of PC1 and PC2 axes (accounting for 13.8% and 9.2% of the variance, respectively) (Fig. 7A). In addition, the AMOVA based on the total loci demonstrated significant differences in genetic variation both between the habitats ($\Phi_{CT} = 0.211$; $P < 0.001$) and between the geographic regions ($\Phi_{CT} = 0.158$; $P < 0.01$) (Table 4). Conversely, the PCA based on the seven neutral loci (without the three outliers) found that the geographic difference between populations in northern and Central Japan along PC1 was more pronounced (15.2%); however, no clear-cut differentiation among fellfield and snowbed plants was detected (Fig. 7B). Similarly, the AMOVA based on the seven neutral loci showed a significant divergence between the geographic regions ($\Phi_{CT} = 0.242$; $P < 0.001$), but the difference between fellfield and snowbed habitats was not significant ($\Phi_{CT} = 0.071$; $P > 0.05$) (Table 4).

DISCUSSION

We found a consistent ecotypic divergence in *P. matsumurae* from the fellfield–snowbed environmental gradient across a series of the high mountains in the Japanese archipelago. The mild common garden experiment shows that the morphological difference associated with habitat types is genetically controlled across the entire distribution range (Fig. 3B, Table 2). The distributions of the divergent ecotypes among multiple isolated regions constitute indirect evidence for a habitat-specific natural selection. The snowy common garden experiment demonstrates that, across populations, snowbed plants have a survival advantage over fellfield plants in a snowy environment (Fig. 4; Table 3). Furthermore, the

fitness of transplants from the different populations substantially varies among the mountain regions (Fig. 4; Table 3). These results imply that divergent selection occurs independently in each mountain region.

Absence of the snowbed ecotype in the Tohoku region

Among the mountain regions studied, the snowbed ecotype was absent in the Tohoku region, even though snowbed vegetation is found there (Appendix S8B). This absence is likely due to the relatively lower elevations of the mountains in Tohoku; here, the tree line is typically close to ridgetops and summits, thus, only small areas of alpine environments exist (Appendix S10) (Kaizuka et al., 1986). Even in the lower mountains, the fellfield habitat exists around exposed summits, where strong winds during the winter prevent the establishment of forests. Conversely, small areas of snowbed habitat at a lower elevation may cause the environment to be biologically more competitive and result in the exclusion of the snowbed ecotype. Intriguingly, the survivorship of transplants from the fellfield population in Mt. Chokai (CHO)—the highest single-peak mountain in Tohoku—was equal to that of transplants from snowbed populations in other mountain regions (Fig. 3), thereby suggesting that plants of the CHO fellfield site have the ability to survive in snowbed environments. Indeed, some flowering plants were distributed at the head of a snowy valley, i.e., snowbed-like environment, on CHO (Appendix S11), which is an exceptional case in the Tohoku region. A relatively large area of the typical snowbed environment and/or topographically unclear spatial separation in CHO might account for the lack of plants belonging to the typical fellfield–snowbed ecotypes.

Evolutionary history of ecotype divergence

The fellfield and snowbed ecotypes occur even in close parapatry within mountain regions and moreover are distributed across isolated mountaintops. The mosaic distribution of the two ecotypes can occur via two evolutionary pathways: (1) multiple ecotypic differentiation in each disjunct geographical region, i.e., parallel local adaptation, and (2) single ecotypic differentiation followed by synchronized migrations of the two ecotypes in each habitat across geographical regions. The phylogeographic structure of *P. matsumurae* inferred from cpDNA variations suggests that ecotype divergence has occurred independently in at least two geographically separated regions, because genetic differentiation was high between the

northern (Hokkaido and Tohoku) and the southern (Central Honshu) regions (Figs. 2, 5). The phylogenetic tree comprises two distinct lineages, a northern and a southern lineage; in each lineage, the haplotypes within the fellfield and snowbed ecotypes are identical or nearly identical (Fig. 5). These distinct lineages substantially contributed to the high genetic differentiation between the northern and southern regions (SAMOVA: 66% of genetic variation). In addition, the phylogeographic tree shows that fellfield plants have more haplotype diversity than snowbed plants; almost all snowbed plants have haplotypes that are also found in fellfield plants (Figs. 2, 5), which supports the hypothesis that snowbed ecotype might be derived from the fellfield ecotype.

The population genetic structure based on multiple allozyme loci provides complementary evidence of the cpDNA phylogeography, which is based only on a single organelle maker. Neutral allozyme variation identified in this study resulted in significant differentiation between the northern and southern regions ($\Phi_{CT} = 0.242$; $P < 0.001$); however, no clear-cut differentiation was detected between fellfield and snowbed plants across the regions ($\Phi_{CT} = 0.071$; $P > 0.05$) (Fig. 7B; Table 4). In other words, putatively neutral loci retained a less apparent signal of ecotype divergence, but also showed divergence between the northern and southern regions as shown in the cpDNA phylogeography. However, genetic differentiation between the ecotypes is more pronounced if considering loci are indicative of adaptive divergence. The allozyme-based genetic structure inferred from all loci (including non-neutral loci) clearly demonstrates habitat-specific differentiation, where snowbed plants in northern Japan and snowbed plants in Central Japan are independently distinguished from fellfield plants (Fig. 7A; Table 4). These patterns are consistent with the hypothesis that the differentiation from fellfield to snowbed ecotype occurred independently in at least two distinct regions. Previously, based on allozyme variation in the same data set, we have reported that the genetic differentiation between fellfield and snowbed habitats in a single region is greater than that between populations of either habitat in different large geographic regions (Shimono *et al.*, 2009). Although allozyme variation is usually regarded as selection-neutral (Kimura, 1989), some allozyme studies have suggested that natural selection causes changes in gene frequencies that reflect the environmental gradients (Linhart and Grant, 1996). In this study, we found that of the 10 allozyme loci analyzed, *LAP2* constitutes a non-neutral locus under positive

selection (Fig. 6), which had specific alleles with habitat-related frequencies ($P < 0.001$). Therefore, the largest positive outlier loci *LAP2* might be under habitat-specific natural selection and then mainly contribute to the highly distinctive differentiation pattern between fellfield and snowbed populations (Fig. 7B). A similar result has been reported in *LAP* (and the other two allozyme loci) for ecotype variation in *Littoriana saxatilis* (Galindo et al., 2009), although the detailed mechanism on the adaptive divergence is unknown.

The genetic boundary between *P. matsumurae* in Central Honshu and the northern regions corresponds to that found by phylogeographic studies of several alpine plant species belonging to various taxa in Japan (e.g., Fujii et al., 1997; Ikeda et al., 2006, 2008, 2009; Hirao et al., 2017). This genetically disjunct structure in multiple alpine plant species strongly suggests historical isolation of alpine plant communities between Central Honshu and the northern regions during past climate oscillations (reviewed by Fujii and Senni, 2006). Therefore, the current distinct distributions of the snowbed ecotype in Hokkaido and Central Honshu should reflect at least a biphyletic origin owing to the historical isolation between these regions. Moreover, their distributions on multiple isolated mountain sky islands suggest a polyphyletic origin, which would result in cryptic phylogenetic structures in this species. Unfortunately, the answer to whether the ecotypic divergence is the result of two or more independent events cannot be determined from the results of the present study. Further investigation that takes into account genetic variation at a genome-wide scale will be necessary to reconstruct the high-resolution evolutionary history of the divergent ecotypes.

Ecological process of the ecotypic divergence

From a theoretical point of view, local adaptation depends on the relative strengths of natural selection and gene flow (reviewed by Felsenstein, 1976). Our common garden experiments suggest the substantial selective pressure of a snowbed-like environment against the fellfield ecotypes, while our previous experiment demonstrated the absolute selective pressure of a fellfield environment against seedlings of the snowbed ecotypes, which were unable to establish at a natural fellfield site (Shimono and Kudo, 2003). Gene flow between fellfield and snowbed populations of *P. matsumurae* is substantially limited, even in close parapatry, by the following ecological barriers (Shimono and Kudo, 2003; Shimono et al., 2009): (1) flowering phenology—this limits pollen-mediated gene flow,

thereby isolating fellfield populations from snowbed populations, and (2) the asymmetric barrier of immigrant inviability—i.e., immigrants of the snowbed ecotype are unable to survive in fellfields but not vice versa, could be a solid barrier to directional gene flow from snowbed to fellfield populations (but not vice versa). These ecological barriers, i.e., phenological separation and asymmetric immigrant inviability acting as prezygotic and postzygotic selection mechanisms, respectively, create a situation that allows ecotypes to be established and maintained. Moreover, if the asymmetric seed-mediated gene flow from fellfield to snowbed populations constructs a source–sink relationship between the populations, the derived ecotype can be more efficiently maintained. For instance, in the continent–island model (Haldane, 1927; Wright, 1931), local adaptation of a snowbed (as island) population would be maintained if selection (s) favoring local resident plants occurred at a higher rate than immigration (m) from fellfield (as continental) populations, i.e., $s/m > 1$. In fact, the results of our snowy common garden experiment reveal a relatively strong degree of selection operating in a snowbed-like environment (Fig. 4; Table 3). While pollen flow between fellfield and snowbed populations is limited due to phenological isolation, seed flow may be possible but probably at low frequency because the seeds of *P. matsumurae* are dispersed passively around the parent plants (Shimono et al., 2009). Conversely, in an alpine dwarf shrub, *Salix herbacea*, gene flow occurs substantially from fellfield to snowbed populations owing to the long-distance seed dispersal by wind, resulting in the restriction of ecological differentiation between the habitats (Cortes et al., 2014). Thus, distinct ecotypes of *P. matsumurae* may be maintained by the restricted gene flow between the habitats as well as strong selective pressure.

Historical stability of fellfield and snowbed habitats

On the Quaternary timescale, glacial–interglacial climatic oscillations were a major driving force of historical changes in the distribution of species in various taxa (e.g., Hewitt, 2000; Petit et al., 2003). A phylogeographic analysis using nuclear DNA of *P. matsumurae* (Ikeda et al., 2008) suggests that the current geographic distribution of this species reflects post-glacial fragmentation of populations. Fellfield environments might have provided a stable microrefugium for alpine plants during Quaternary climatic oscillations (i.e., nunatak survival; e.g., Schönswetter et al., 2005; Westergaard et al., 2011), suggesting that *P.*

matsumurae has persistently survived in fellfield habitat. Conversely, snowbed habitat and vegetation are regarded as particularly vulnerable to global warming (reviewed by Björk and Molau, 2007; e.g., Hedenås et al., 2011; Callaghan et al., 2013). Furthermore, snowbed environments are supposed to be unstable during past climate changes. Heavy-snowfall environments in the Japanese archipelago might be established in the post-glacial period (Nakagawa et al., 2002), which has been linked to the establishment of the snowbed environment mainly on the Japan Sea side (Appendix S7B). Little snowfall during the glacier period (Ono, 1984) likely resulted in the degradation of snowbed environments. Although it is unclear how the glacial–interglacial cycles influenced the snowbed plants, different historic situations between fellfield and snowbed habitats could have produced phylogeographic disparities among species that are currently co-distributed (Massatti and Knowles, 2014). Thus, ecological divergence in alpine plant species might reflect, in part, the histories of habitats, i.e., long-term persistence of fellfield versus instability of snowbed habitats in the alpine ecosystems.

Local adaptation to heavy-snow environment

The divergent pattern of *P. matsumurae* ecotypes is consistent with the ecotypic variation in an arctic–alpine dwarf shrub, *Dryas octopetala* (McGraw, 1983, 1985b, 1985a, 1987b, 1987a; McGraw and Antonovics, 1983; Bennington et al., 2012). For instance, leaf sizes of *D. octopetala* ecotypes are genetically controlled, with snowbed leaves typically 2–10 times longer than fellfield leaves. Interestingly, the snowbed ecotype (*D. octopetala* subsp. *alaskensis*) is supposed to be derived from the fellfield ecotype (*D. octopetala* subsp. *octopetala*) (Hultén, 1959). The evolution of snowbed ecotypes in these species provide an insight into the evolutionary history of some snowbed species, such as *Ranunculus pygmaeus* and *Sibbaldia procumbent*, that are restricted to snowbed habitat (Holway and Ward, 1963). Snowy environments are most common in high latitudes and altitudes, but exist also in lowland temperate regions. In the Japanese archipelago, the Sea of Japan side is an extremely heavy snow region, where the snowfall during the winter exceeds 3 m in the hilly areas. Adaptive divergence to snowy habitats has been reported in multiple plant species, e.g., *Aucuba japonica* var. *boreali*, *Euonymu oxyphyllus* var. *magnus*, *Daphniphyllum macropodum* subsp. *humile*, and *Camellia rusticana* (reviewed by Hotta, 1974; Kume and Tanaka, 1996; Kume and Ino, 2000; Iwasaki et al., 2012). Therefore,

natural selection in the snowy habitat can be a consistent driving force to the adaptation and diversification of plants from arctic to temperate zones.

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AUTHOR CONTRIBUTIONS

A.S.H. and G.K. planned and designed the research. Y.S., G.K., N.W., K.N., and A.S.H. conducted the fieldwork. A.S.H. performed experiments and analyzed the data. A.S.H. was the primary writer with contributions from all co-authors.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information at the end of the article.

Appendix 1. Additional references concerning the distribution of habitat types.

Appendix 2. Annual variation of ground temperature at fellfield and snowbed sites on Mt. Yarigatake, Japan.

Appendix 3. One-year survival rates in the mild common garden.

Appendix 4. The snowy common garden at Sugadaira, Japan.

Appendix 5. Hierarchical Bayesian models for leaf width and second-year survivorship.

Appendix 6. Model selection based on DIC for leaf size variation.

Appendix 7. Model selection based on DIC for second-year survivorship.

Appendix 8. Distribution of fellfield vegetation and snowbed vegetation on high mountains of the Japanese archipelago.

Appendix 9. DDBJ/EMBL/GenBank accession numbers of cpDNA haplotype sequences for *Potentilla matsumurae*.

Appendix 10. Diagram of alpine zones and the forest limit along latitudinal and altitudinal gradients in northern and Central Japan.

Appendix 11. Flowering plants of *Potentilla matsumurae* grown in the head of the snowy valley on Mt. Chokai, Tohoku region, Japan.

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TABLE 1. Population ID (with habitat postfix: F for fellfield and S for snowbed), geographic coordinates, elevation (m a.s.l.), and sample sizes of 23 *Potentilla matsumurae* populations from 16 mountain regions across the Japanese archipelago. n_1 , number of samples measured for leaf width at natural sites; n_2 , number of samples measured in the mild common garden; n_3 , number of samples measured to estimate survivorship in the snowy common garden; and n_4 , sample size for cpDNA analyses. Numbers in parentheses following n_2 and n_3 represent the number of maternal plants from which seedlings were obtained for transplanting. *¹ Data from Shimono et al. (2009); *² data from herbarium specimens.

Pop. ID	Mountain region (Abbreviation)	Habitat	District	Latitude (°N)	Longitude (°E)	Elevation				
						n (m a.s.l.)	n_1	n_2	n_3	n_4
POR.F	Mt. Poronupuri (POR)	Fellfield	Hokkaido	44.962	142.420	840	–	–	–	10
NIS.F	Mt. Nishibetsu (NIS)	Fellfield	Hokkaido	43.553	144.582	790	–	–	–	13
TAI.F	Taisetsu Mts. (TAI)	Fellfield	Hokkaido	43.559	142.861	1900	49* ¹	8 (8)	8 (5)	19
TAI.S	Taisetsu Mts. (TAI)	Snowbed	Hokkaido	43.556	142.864	1840	49* ¹	6 (4)	7 (4)	19
TOT.F	Mt. Tottabetsu (TOT)	Fellfield	Hokkaido	42.739	142.695	1950	46	–	–	20
TOT.S	Mt. Tottabetsu (TOT)	Snowbed	Hokkaido	42.733	142.696	1600	50	27 (10)	30 (10)	11
RAK.F	Mt. Rakko (RAK)	Fellfield	Hokkaido	42.273	143.111	1470	–	–	–	6
MUI.F	Mt. Muine (MUI)	Fellfield	Hokkaido	42.930	141.042	1400	–	–	–	6
YOU.F	Mt. Youtei (YOU)	Fellfield	Hokkaido	42.827	140.811	1890	–	–	–	9
YOU.S	Mt. Youtei (YOU)	Snowbed	Hokkaido	42.830	140.804	1800	–	–	–	10
HAK.F	Mt. Hakkoda (HAK)	Fellfield	Tohoku	40.659	140.877	1584	7* ²	17 (1)	17 (1)	9

Table 1 (Hirao et al.)

CHO.F	Mt. Chokai (CHO)	Fellfield	Tohoku	39.094	140.048	2150	43	46 (15)	46 (15)	21
IID.F	Mt. Iide (IID)	Fellfield	Tohoku	37.879	139.640	1960	–	–	–	16
TAT.F	Mt. Tateyama (TAT)	Fellfield	Central Honshu	36.571	137.612	2690	–	–	–	20
TAT.S	Mt. Tateyama (TAT)	Snowbed	Central Honshu	36.571	137.611	2650	–	–	–	20
YAR.F	Mt. Yarigatake (YAR)	Fellfield	Central Honshu	36.342	137.647	3100	49	34 (17)	36 (17)	11
YAR.S	Mt. Yarigatake (YAR)	Snowbed	Central Honshu	36.332	137.669	2120	50	62 (28)	73 (32)	11
NOR.S	Mt. Norikura (NOR)	Snowbed	Central Honshu	36.115	137.560	2630	109	41 (26)	45 (26)	8
YAT.F	Yatsugatake Mts. (YAT)	Fellfield	Central Honshu	35.972	138.370	2890	28	19 (9)	31 (12)	17
ONT.F	Mt. Ontake (ONT)	Fellfield	Central Honshu	35.917	137.486	2850	26	24 (10)	25 (12)	22
ONT.S	Mt. Ontake (ONT)	Snowbed	Central Honshu	35.910	137.489	2700	62	–	–	20
KIS.F	Mt. Kiso-komagatake (KIS)	Fellfield	Central Honshu	35.789	137.805	2950	69	53 (16)	62 (21)	17
KIS.S	Mt. Kiso-komagatake (KIS)	Snowbed	Central Honshu	35.780	137.813	2650	50	49 (20)	55 (25)	13

TABLE 2. Posterior distribution statistics of the hierarchical Bayesian models for leaf width in *Potentilla matsumurae*.

Effect	Parameter	Posterior		Lower	Upper
		Mean	SD	95% credible interval	95% credible interval
Data set from natural sites					
Intercept	β_0	33.35	1.57	30.79	35.86
Ecotype (snowbed)	β_1	17.67	2.35	13.87	21.50
Population	$\sigma_{\text{Population}}$	3.94	1.08	2.54	5.92
Data set from mild common garden					
Intercept	β_0	33.90	2.29	30.12	37.50
Ecotype (snowbed)	β_1	5.78	2.99	1.031	10.60
Family	σ_{Family}	4.64	0.86	3.23	6.03
Population	$\sigma_{\text{Population}}$	3.59	1.86	0.91	6.89
Mountain	σ_{Mountain}	3.08	2.19	0.29	7.08
Overall data set, from natural sites and mild common garden					
Intercept	β_0	6.53	4.41	-0.83	13.71
Ecotype (snowbed)	β_1	27.04	2.81	22.48	31.72
Location (mild common garden)	β_2	9.72	2.15	6.21	13.31
Interaction: Ecotype \times Location	β_3	-9.85	1.35	-12.10	-7.63
Family	σ_{Family}	3.24	0.60	2.23	4.17

Table 2 (Hirao et al.)

Population	$\sigma_{\text{Population}}$	3.66	1.10	2.22	5.68
Mountain	σ_{Mountain}	1.69	1.38	0.14	4.26

TABLE 3. Posterior distribution statistics of the hierarchical Bayesian model for second-year survivorship of plants grown in the second snowy common garden.

Effect	Parameter	Posterior		Lower 95% credible interval	Upper 95% credible interval
		Mean	SD		
Intercept	β_0	-0.917	0.657	-1.994	0.1200
Ecotype (snowbed)	β_1	2.360	0.897	0.8648	3.7196
Family	σ_{Family}	0.827	0.340	0.227	1.378
Population	$\sigma_{\text{Population}}$	0.925	0.547	0.117	1.876
Mountain	σ_{Mountain}	1.012	0.690	0.102	2.238

TABLE 4. Analysis of molecular variance (AMOVA) for *Potentilla matsumurae* populations using (A) total loci and (B) neutral loci (original data set of 10 allozyme loci in Shimono et al., 2009).

Analysis	Source of variance	df	SS	Variation (%)	Φ statistic	<i>P</i>
A) Total loci	Among regions: northern and Central Japan	1	9.07	15.8	$\Phi_{CT} = 0.158$	0.009
	Among populations within regions	13	24.63	28.5	$\Phi_{SC} = 0.339$	<0.001
	Within populations	315	49.47	55.7	$\Phi_{ST} = 0.443$	<0.001
	Among habitat groups: fellfield and snowbed	1	11.75	21.1	$\Phi_{CT} = 0.211$	<0.001
	Among populations within habitat groups	13	21.94	24.4	$\Phi_{SC} = 0.309$	<0.001
	Within populations	315	49.47	54.5	$\Phi_{ST} = 0.455$	<0.001
B) Neutral loci	Among regions: northern and Central Japan	1	7.79	24.2	$\Phi_{CT} = 0.242$	<0.001
	Among populations within regions	13	10.35	17.2	$\Phi_{SC} = 0.227$	<0.001
	Within populations	315	34.13	58.5	$\Phi_{ST} = 0.415$	<0.001
	Among habitat groups: fellfield and snowbed	1	3.19	7.1	$\Phi_{CT} = 0.071$	0.077
	Among population within habitat groups	13	14.96	28.4	$\Phi_{SC} = 0.306$	<0.001
	Among individuals within populations	315	34.13	64.4	$\Phi_{ST} = 0.356$	<0.001

FIGURE 1. Characteristic habitats of the studied *Potentilla matsumurae* populations. (A) Fellfield and snowbed habitats in alpine mosaic landscape. Wind-blown fellfields are located on ridgetops and summits with little snow; snowbeds are located on more protected leeward slopes and in microtopographic depressions where snow lingers. (B) Fellfield and (C) snowbed populations of *P. matsumurae*.

FIGURE 2. (A) Geographic distribution of *Potentilla matsumurae* and its cpDNA haplotypes in mountain areas in the Japanese archipelago. Red dots: only fellfield habitats; blue dots: both fellfield and snowbed habitats; black dots: only snowbed habitats; gray dots: habitat types unknown. Pie charts show the cpDNA haplotypes within each fellfield (F) or snowbed (S) population. The size of each pie chart is proportional to the number of sampled individuals per population listed in Table 1. (B) Median-joining network of cpDNA haplotypes. Each line represents one mutational step between two haplotypes, and small open circles represent missing intermediate haplotype states.

FIGURE 3. Morphological difference in leaf size between fellfield (red) and snowbed (blue) populations of *Potentilla matsumurae* measured (A) at natural sites, and (B) in the “mild” common garden, located in a mild environment. Each box plot includes median (horizontal line), first quartile (bottom of box), third quartile (top of box), 1.5 times the interquartile range (whiskers), and outliers (circles). The mountain regions are arranged along the *x*-axis from south to north. The abbreviations for the regions are explained in Table 1. (C) Differential response to leaf size between fellfield (red) and snowbed (blue) populations growing on natural sites and the mild common garden. Significant difference between the ecotypes and the interaction between ecotype \times location (i.e., growing conditions) are given in Table 2.

FIGURE 4. Survival rates of *Potentilla matsumurae* in the “snowy” common garden, a snowbed-like environment. (A) Per-population survival rate of transplants: red, fellfield; and blue, snowbed. The mountain regions are arranged along the *x*-axis from south to north. (B) Posterior distribution of the probability density of survivorship (survival rate) estimated by the hierarchical Bayesian model. Dotted and dashed lines denote population-level estimates for the survival rate of plants originating from fellfield and snowbed habitats, respectively. Population IDs are explained in Table 1.

FIGURE 5. Bayesian phylogenetic tree inferred for 24 chloroplast DNA haplotypes of *Potentilla matsumurae*. Numbers on each branch represent Bayesian posterior probability (values <0.90 are not shown) and bootstrap support for clades resolved in the ML analysis (values <75% are not shown). The red and blue circles at the branch tips are sized proportionally to the number of plants sampled from fellfield and snowbed populations, respectively.

FIGURE 6. Detection of outlier loci among 10 allozyme loci (data set from Shimono et al., 2009) using the FDIST2 program (Beaumont and Nichols, 1996). One locus above the 99% confidence intervals, *LAP-2*, has higher than expected F_{ST} values and is a candidate for being under divergent selection. Loci below the 99% confidence intervals, *MDH* and *SKD*, have lower than expected F_{ST} values and are candidates for being under balancing selection. The other seven loci are regarded as neutral ones. Filled circles are allozyme loci. Dashed and dotted lines are the 99% and 95% confidence intervals, respectively, under the infinite allele mutation model.

FIGURE 7. Principal component analysis (PCA) of individual genotypes from 15 *Potentilla matsumurae* populations—five fellfield and six snowbed populations from northern Japan and two fellfield and two snowbed populations from Central Japan—using (a) all loci and (b) neutral loci (excluding outliers, see Fig. 6) (the allozyme dataset from Shimono et al., 2009). Points represent genotypes of individuals; circles indicate individuals from same populations. Habitat properties are color-coded: red and blue represent plants in fellfield and snowbed habitats, respectively. Symbols indicate regional properties: closed circles and closed squares represent plants in northern and Central Japan, respectively. See Table 4 for significant trends in the genetic structures.

Fig.1

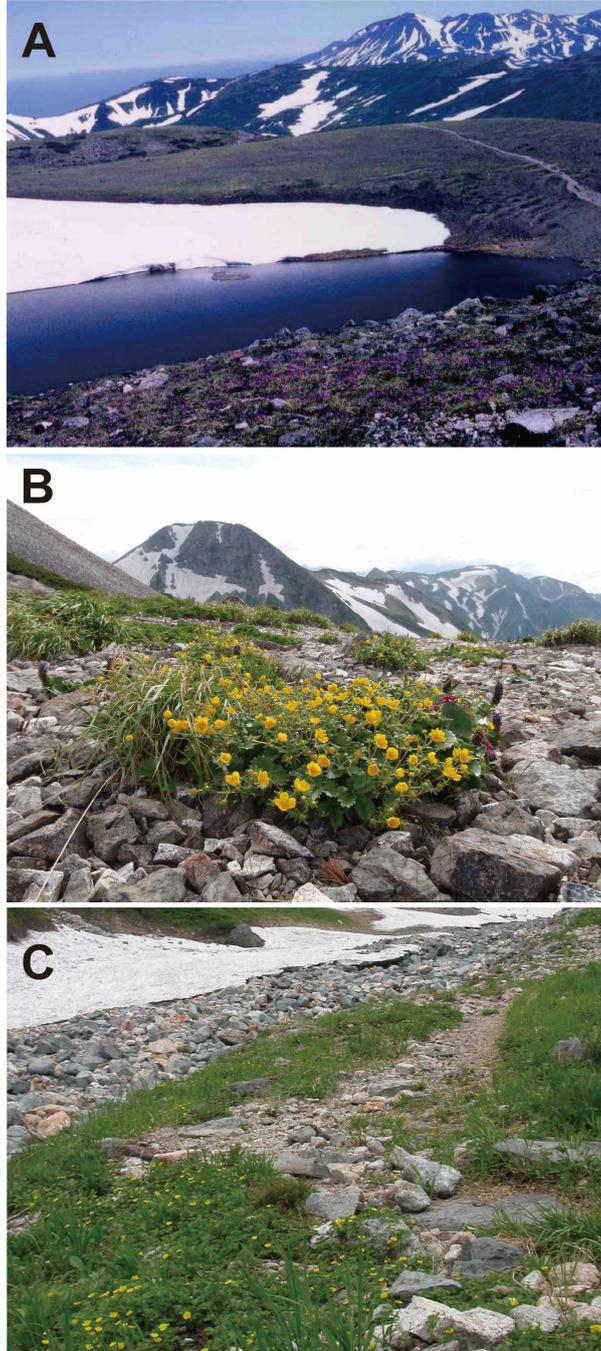


Fig.2

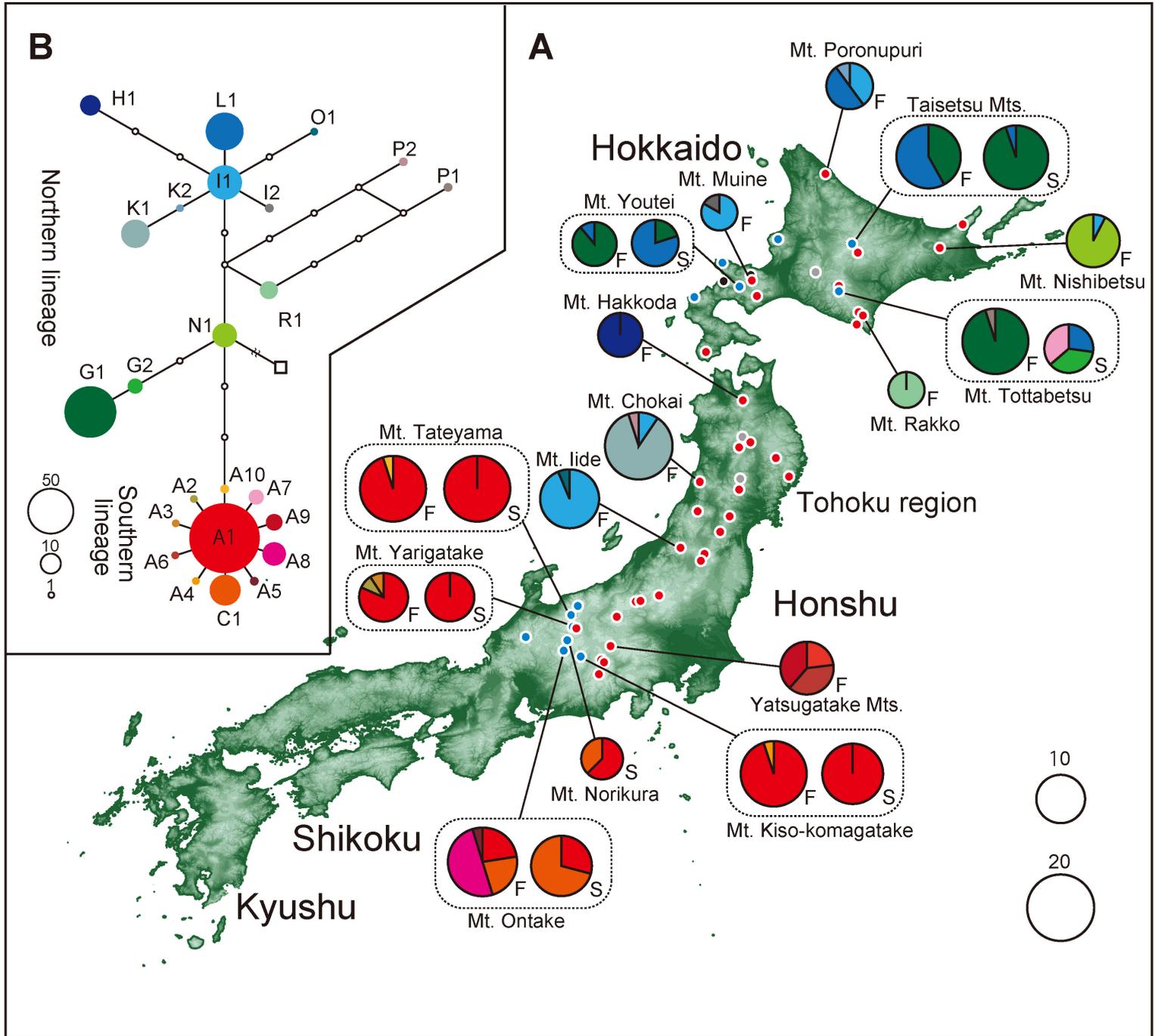


Fig.3

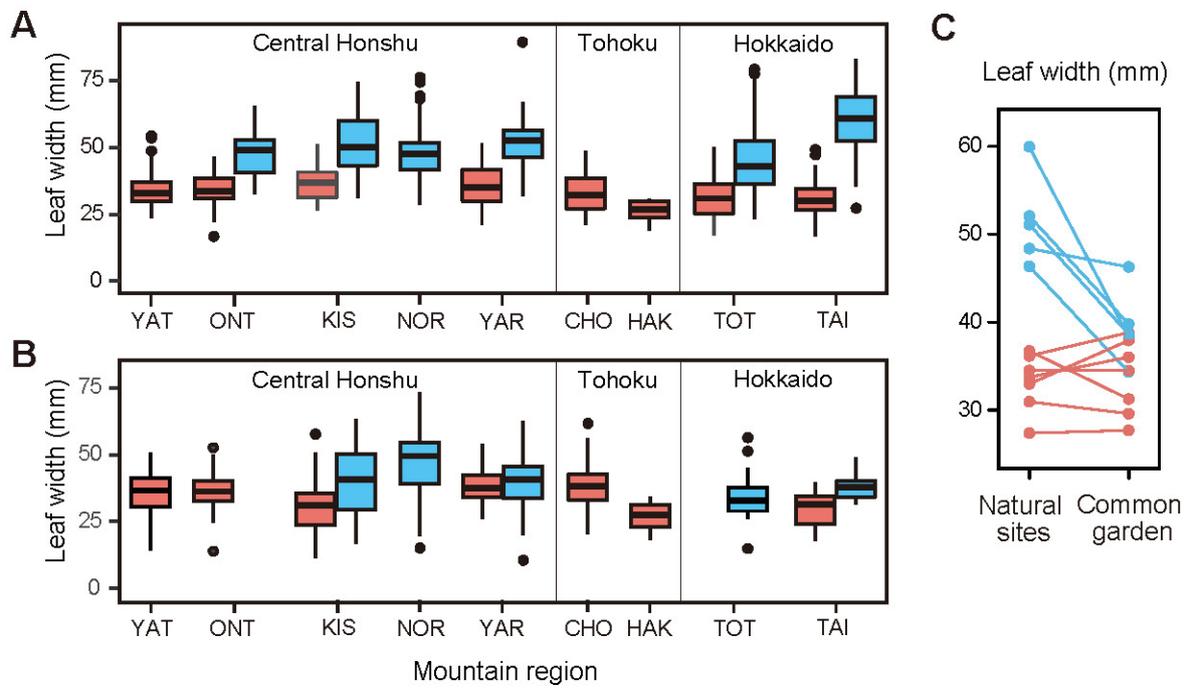


Fig.4

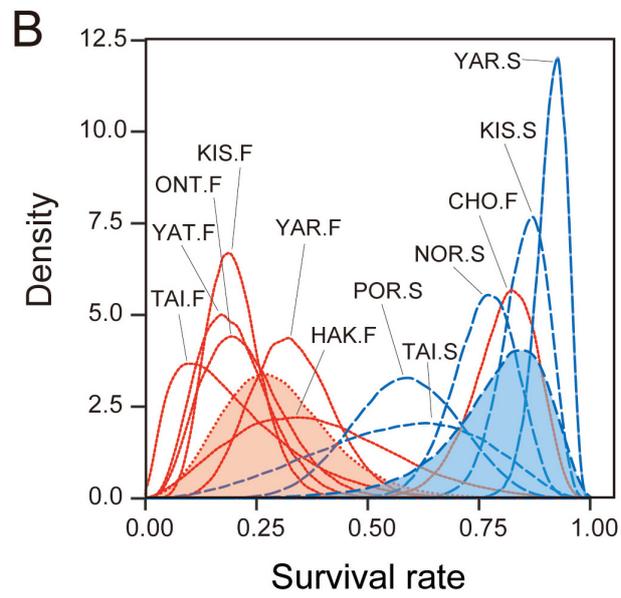
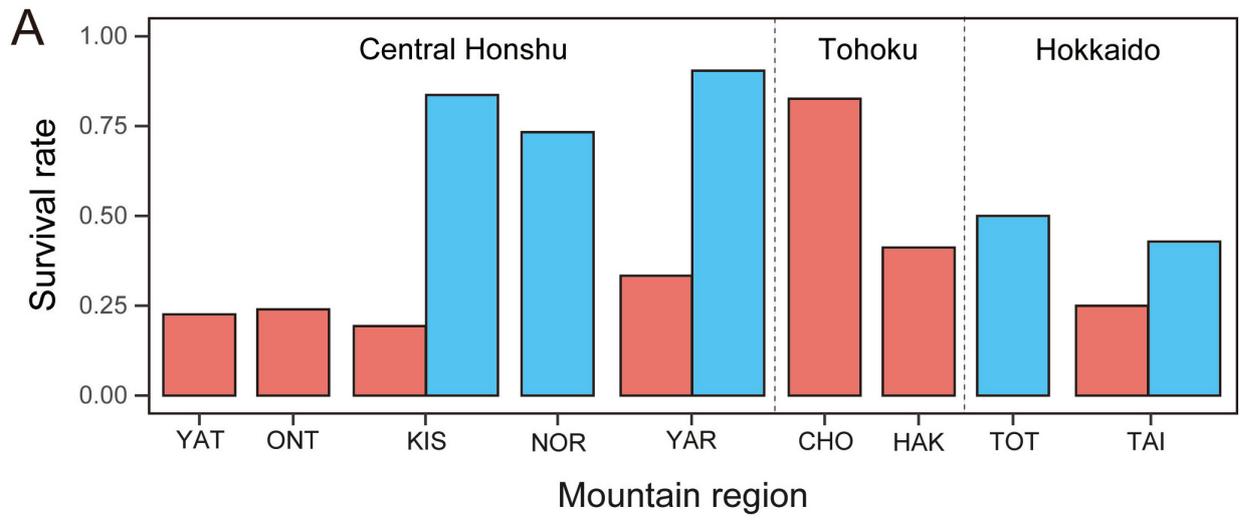


Fig.5

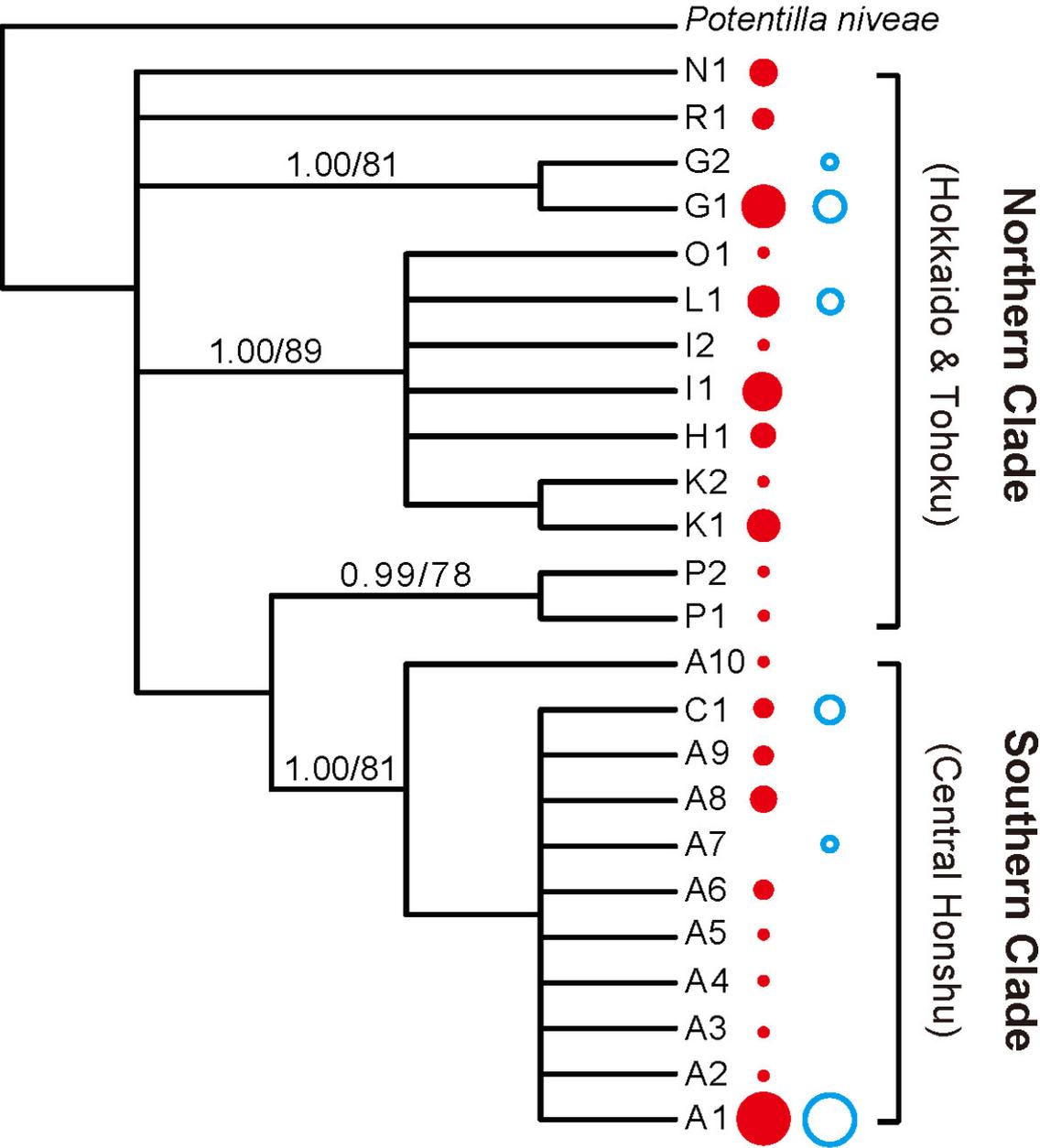


Fig.6

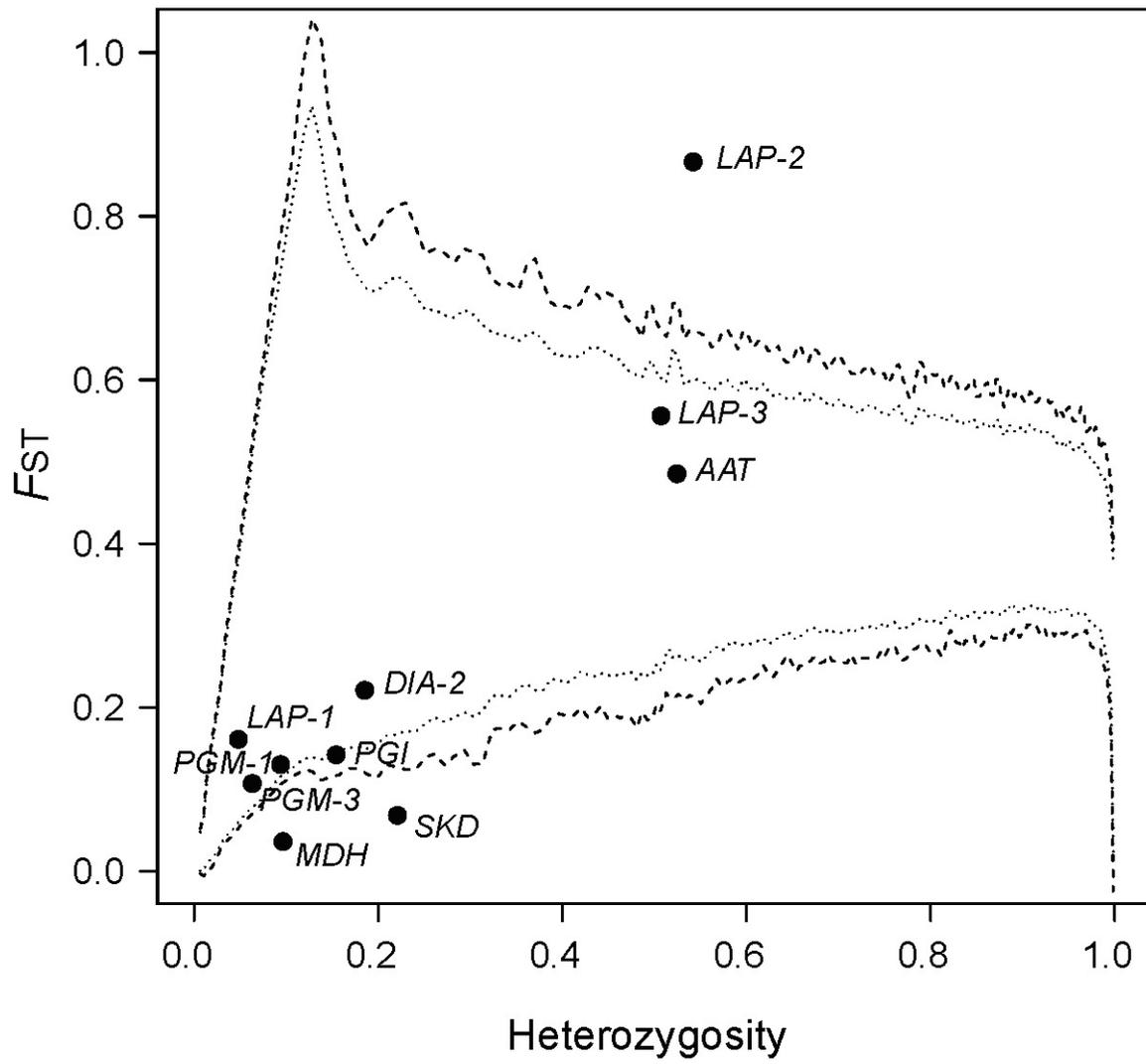
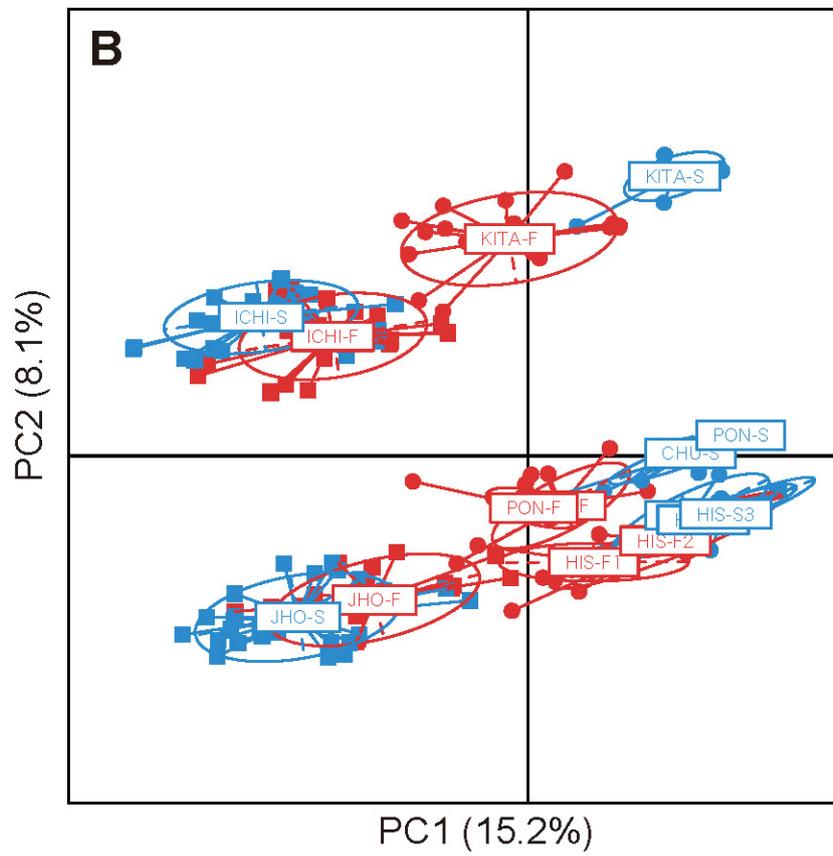
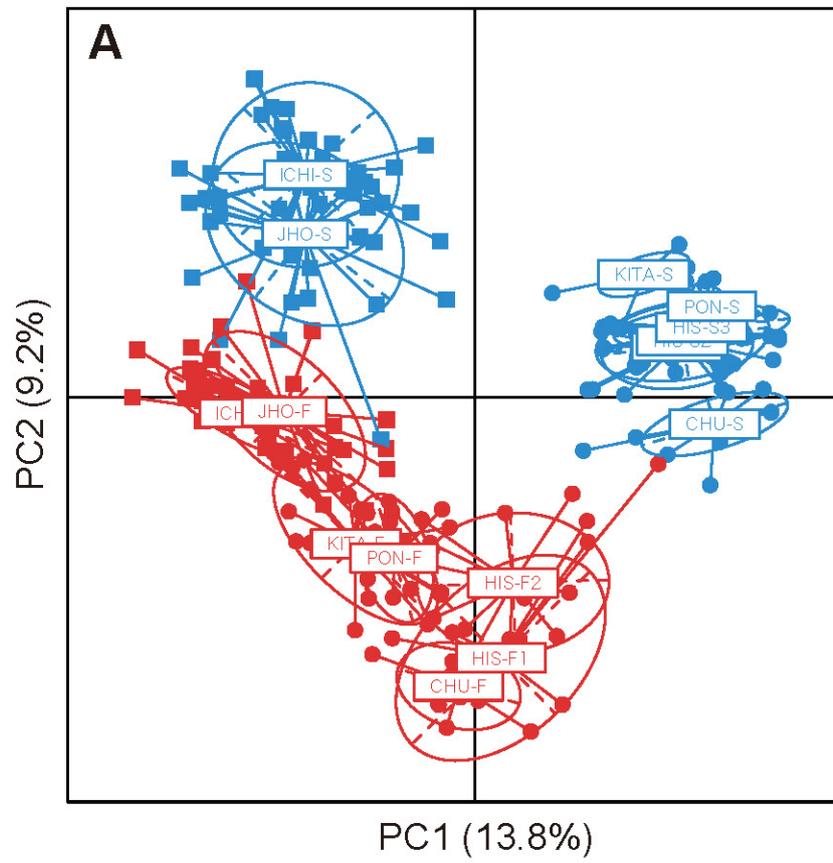


Fig.7

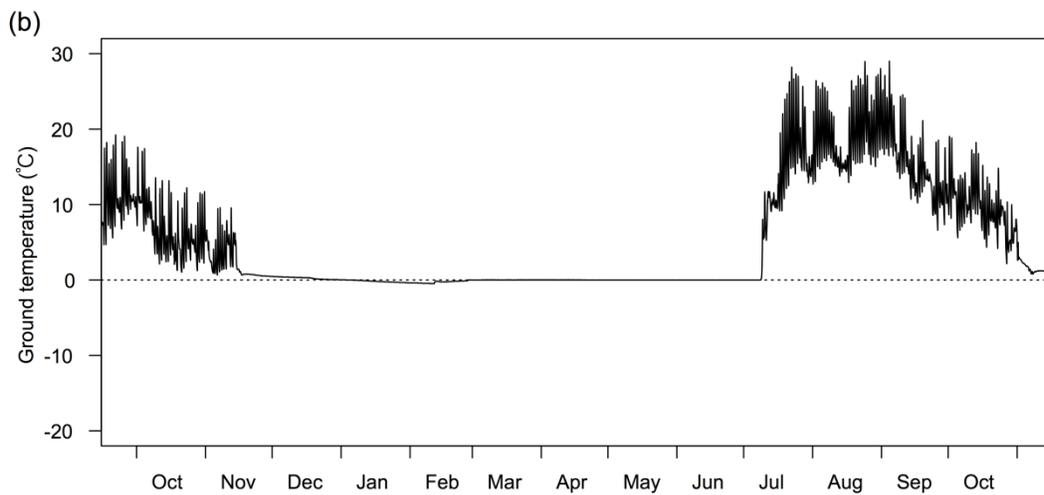
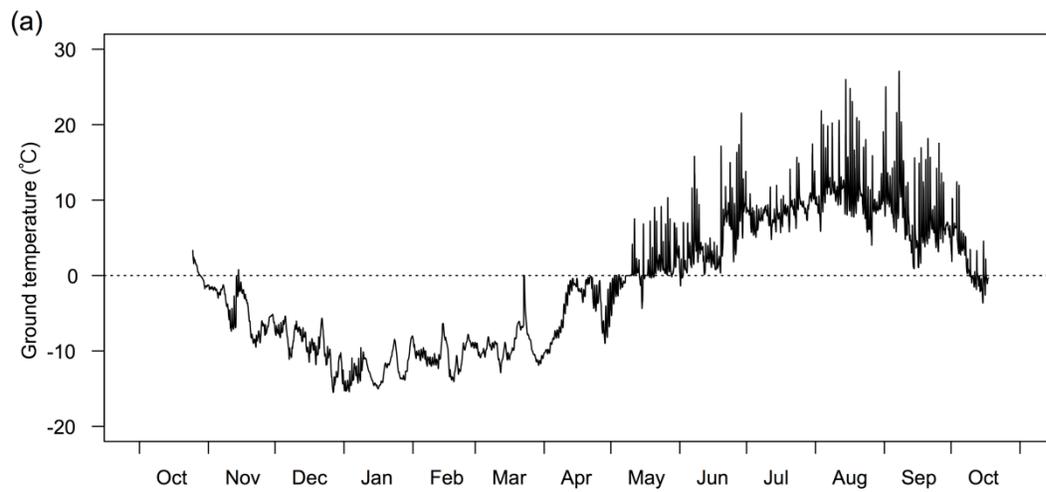


Appendix S1. Additional references concerning the distribution of habitat types of *Potentilla matsumurae*.

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Appendix S2. Annual variation of ground temperature, measured at 5 cm below the soil surface, at (a) fellfield and (b) snowbed sites on Mt. Yarigatake, central Honshu, Japan. Data were collected from October 2008 to October 2009 at the fellfield site and from November 2009 to November 2010 at the snowbed site.



Appendix S3. One-year survival rates of *Potentilla matsumurae* plants in the first mild common garden. Red and blue represent fellfield and snowbed populations, respectively. The mountain regions are arranged along the x-axis in order of latitude from south to north. The population IDs are explained in Table 1.

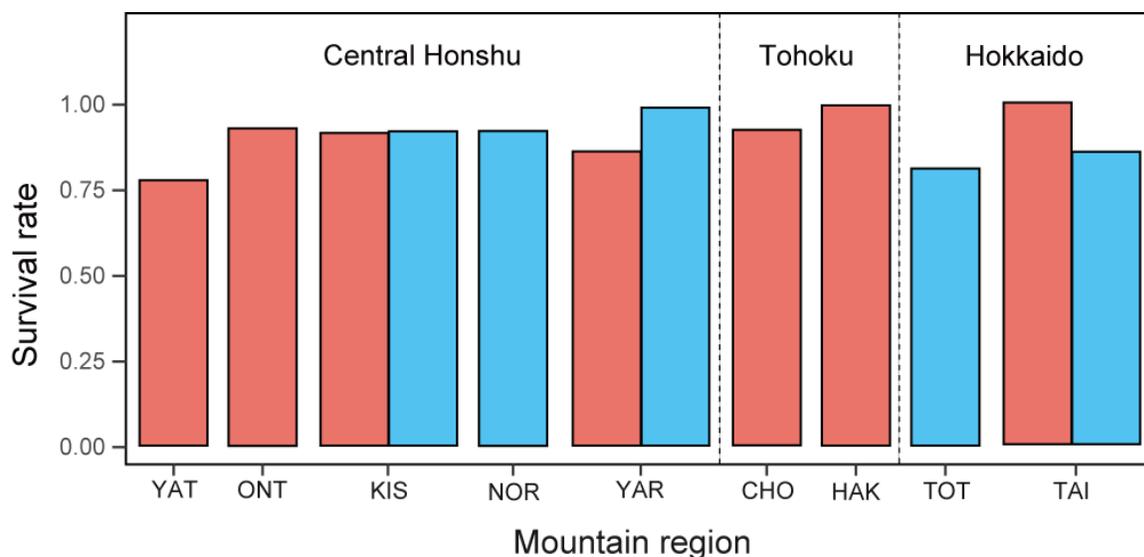


TABLE. Posterior distribution statistics of the hierarchical Bayesian model for 1-year survivorship of the potted plants grown in the first mild common garden.

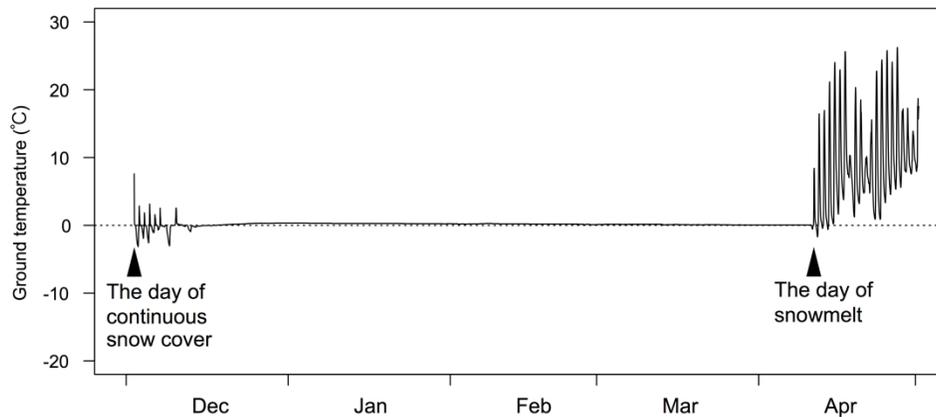
Effect	Parameter	Mean	SD	Lower 95% credible interval	Upper 95% credible interval
Intercept	β_0	2.928	0.811	1.641	4.745
Ecotype (snowbed)	β_1	0.376	1.007	-0.137	2.200
Family	σ_{Family}	1.407	0.545	0.328	2.564
Population	$\sigma_{\text{Population}}$	0.939	0.682	0.058	2.573
Mountain	σ_{Mountain}	0.704	0.619	0.028	2.280

Appendix S4. The snowy environment used for the second common garden at Sugadaira Research Station, Mountain Science Center, University of Tsukuba, Ueda city, Nagano Prefecture, central Honshu, Japan (36°31N, 138°21E; 1300 m a.s.l.). (a) Photograph of the snowy common garden location taken on 16 February 2018 (photo taken by Akira S. Hirao). (b) Ground temperature remained constant at 0°C during the winter owing to the effective snow cover. Data were collected from 1 December 2013 to 1 May 2014.

(a)



(b)



Appendix S5: Hierarchical Bayesian models for leaf width and survivorship of *Potentilla matsumurae*.

We analyzed the effects of habitat on leaf size and survivorship of *Potentilla matsumurae* using hierarchical Bayesian models. As preliminary analyses, we used a model selection approach based on the deviance information criterion (Spiegelhalter et al., 2002) to select a constant set of fixed and random effects to compare across models (Appendix S5, S6).

We assumed that leaf width of individual plant i at the field sites (L_{fi}) and in the first common garden (L_{ci}) depended on habitat type (fellfield or snowbed) and that it followed a normal distribution in each case:

$$L_{fi} = \beta_0 + \beta_1 H_i + rP_i$$

$$L_{ci} = \beta_0 + \beta_1 H_i + rF_i + rP_i + rM_i$$

where β_0 is the intercept, β_1 is the coefficient of the fixed effect of habitat (H_i : fellfield or snowbed), and rF_i , rP_i , and rM_i are the random effects of ‘maternal family’ (derived from material sources), ‘population’, and ‘mountain region’, respectively. In the latter model, random effects include ‘maternal family’ nested within ‘population’ nested within ‘mountain region’. Furthermore, we jointly assessed the overall leaf-width dataset L_i from the natural field sites and the first common garden to simultaneously concern genetic differentiation and phenotypic plasticity of the leaf trait as follows:

$$L_i = \beta_0 + \beta_1 H_i + \beta_2 E_i + \beta_3 H_i E_i + rF_i + rP_i + rM_i$$

where β_2 is the coefficient of the fixed effect of experimental condition (E_i : natural field site or first common garden), β_3 is the coefficient of the interaction term between the experimental condition and the habitat, and the other parameters are as defined above.

The prior distribution of β is non-informative and follows a normal distribution, $N(0, 10^4)$.

The inverse-variance parameters (rF_i , rP_i , and rM_i) are assumed to follow a gamma distribution, $N(0, \sigma_{\text{Family}})$, $N(0, \sigma_{\text{Population}})$, and $N(0, \sigma_{\text{Mountain}})$, respectively, where σ_{Family} , $\sigma_{\text{Population}}$, and σ_{Mountain} represent the standard deviations of individual characteristics under the linear predictor λ_i , assuming they follow a gamma distribution, $1/\sigma \sim \text{gamma}(10^{-4}, 10^{-4})$. The posterior samples were obtained by using the combination of three independent Markov chain Monte Carlo (MCMC) chains with 5×10^5 samplings at 10-step intervals after 5×10^4 burn-in MCMC steps. We confirmed the Markov chains convergence of all parameters using the Gelman-Rubin statistic R-hat with a value of below 1.1 (Gelman et al., 2013).

In the assessment of the survival experiment results, we assumed that the survival of transplants (S_i) followed a Bernoulli distribution in which the transplant survival probability, p_i , depended on habitat (fellfield or snowbed):

$$S_i \sim \text{Bernoulli}(p_i),$$

$$\text{logit } p_i = \beta_0 + \beta_1 H_i + rF_i + rP_i + rM_i$$

where the fixed factor (H_i) and the random factors (rF_i , rP_i , and rM_i) were identical to those used in the leaf trait analyses. We used the same vague prior distributions and settings of the MCMC analysis to implement the hierarchical Bayesian model for the survivorship as well as those for the leaf trait in the above analyses. All the models were implemented in the JAGS 4.2.0 program in the R package “RJAGS” (Plummer, 2013).

Appendix S6. Model selection based on the deviance information criterion (DIC) for leaf size variation under natural and controlled conditions

Model description	DIC
Dataset from natural sites	
HAB + pop + mou	4997.8
HAB + pop	4997.6
HAB + mou	5036.6
HAB	5064.0
Dataset from the first common garden	
HAB + fam + pop + mou	2864.6
HAB + fam + pop	2865.1
HAB + fam + mou	2867.3
HAB + fam	2877.0
HAB + pop	2893.3
HAB	2927.0
Overall data set from natural sites and the first common garden	
HAB × EXP + fam + pop + mou	7905.3
HAB × EXP + fam + pop	7905.5
HAB × EXP + pop + mou	7928.1
HAB × EXP + fam + mou	7942.4
HAB × EXP + pop	7928.0
HAB + EXP + fam + pop + mou	7951.6
HAB + EXP + fam + pop	7952.3
HAB + EXP + pop	7979.2

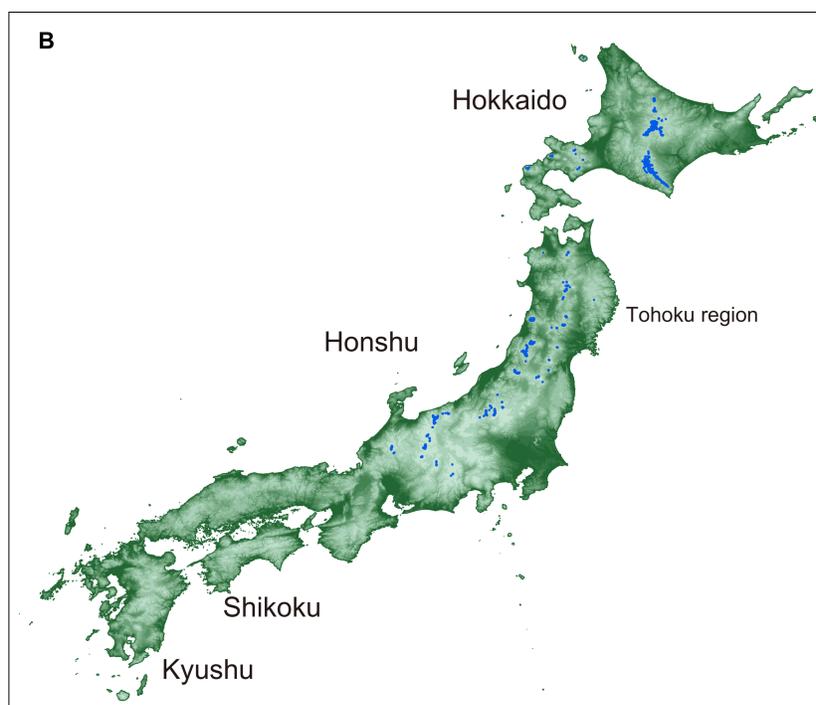
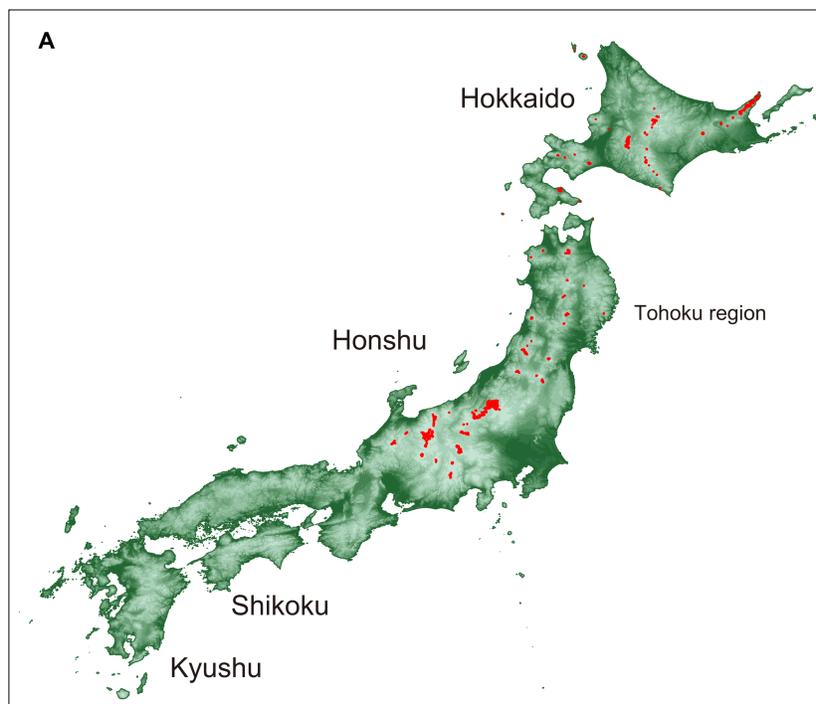
Models are defined by fixed effects (uppercase letters) and random effects (lowercase letters): HAB, habitat (fellfield or snowbed); EXP, experiment (natural sites vs. common garden); fam, maternal family; pop, population; and mou, mountain region. ‘×’ implies an effect that is both additive and interactive. In all cases, random effects include ‘fam’ nested within ‘pop’ nested within ‘mou’.

Appendix S7. Model selection based on DIC for second-year survivorship in a snowy environment

Model description	DIC
HAB + fam + pop + mou	457.1
HAB + fam + pop	457.5
HAB + fam + mou	457.2
HAB + pop + mou	463.1
HAB + pop	462.8

Models are defined by fixed effects (uppercase letters) and random effects (lowercase letters): HAB, habitat (fellfield or snowbed); EXP, experiment (natural sites vs. common garden); fam, maternal family; pop, population; and mou, mountain region. ‘×’ implies an effect that is both additive and interactive. In all cases, random effects include ‘fam’ nested within ‘pop’ nested within ‘mou’.

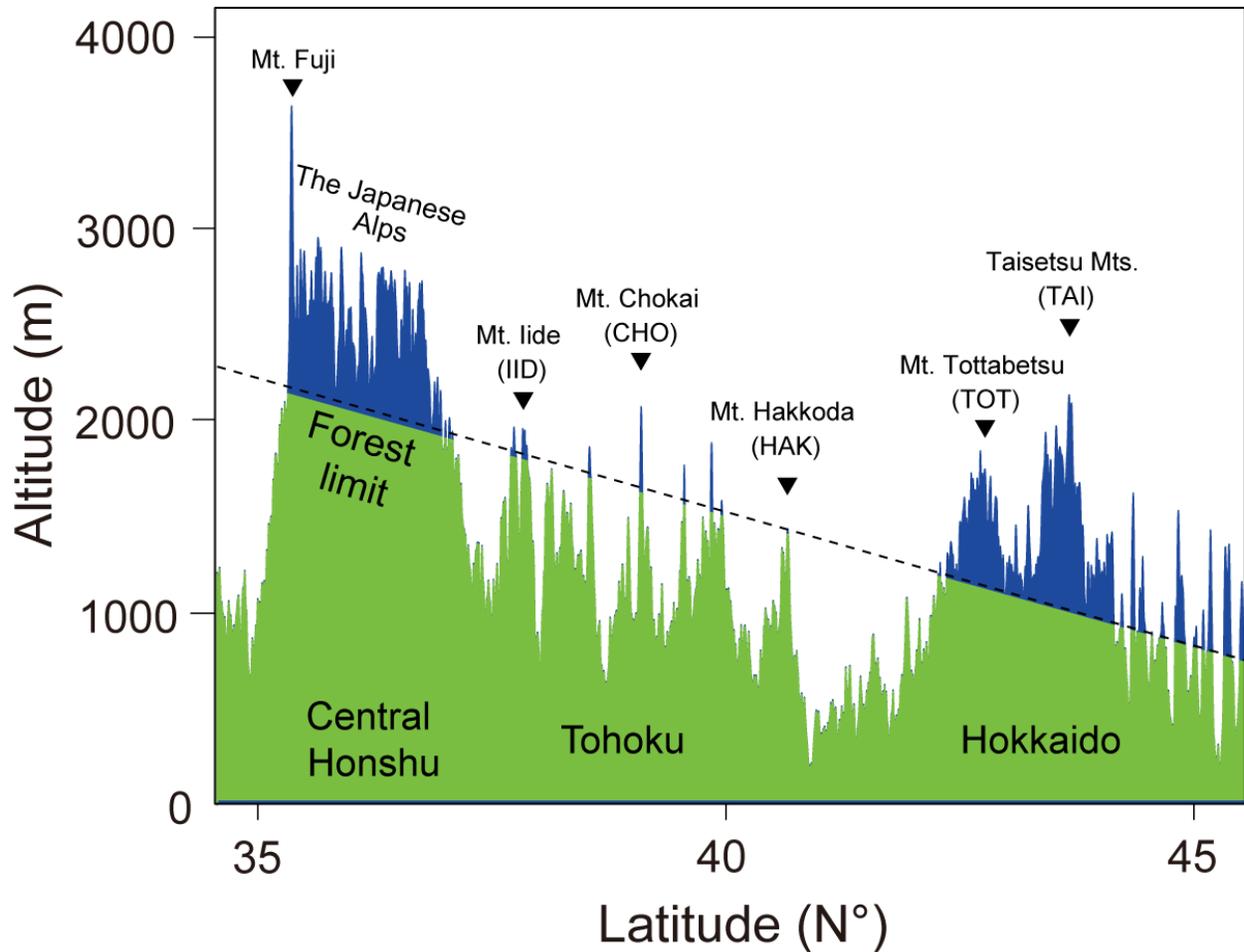
Appendix S8. Distribution of (A) fellfield vegetation and (B) snowbed vegetation on high mountains of the Japanese archipelago.



Appendix S9. DDBJ/EMBL/GenBank accession numbers of newly obtained cpDNA haplotype sequences for *Potentilla matsumurae*

Haplotype	<i>rpl20-rps12</i>	<i>trnT-trnL</i>
A1	AB241660	LC378556
A2	LC378550	LC378556
A3	AB241660	LC378557
A4	AB241660	LC378558
A5	AB241660	LC378559
A6	AB241660	LC378560
A7	AB241660	LC378561
A8	LC378551	LC378556
A9	AB241661	LC378556
A10	AB241660	LC378562
C1	AB241660	LC378563
G1	AB241660	LC378564
G2	AB241660	LC378565
H1	LC378552	LC378566
I1	AB241660	LC378567
I2	LC378553	LC378567
K1	LC378554	LC378568
K2	AB241660	LC378568
L1	AB241660	LC378569
N1	AB241660	LC378570
O1	LC378555	LC378571
P1	AB241662	LC378572
P2	AB241662	LC378573
R1	AB241660	LC378574

Appendix S10. Diagram of alpine zones (blue) and the forest limit (dashed line) along latitudinal and altitudinal gradients in northern and central Japan. Altitudes were obtained from a digital elevation model with a horizontal resolution of 1 km × 1 km from the Geographical Survey of Institute, Japan. The forest limit has been adapted from Kaizuka *et al.* (1986). See Table 1 for more information on the mountain regions in Hokkaido and Tohoku.



LITERATURE CITED

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Appendix S11. Flowering plants of *Potentilla matsumurae* grown in the head of the snowy valley, “Senjya-dani”, on Mt. Chokai, Thohoku region, Japan (left photo taken on 26 August, 2007). The summit of Mt. Chokai and the remaining snow on the “Senjya-dani” valley (right photo).

