Genetic structure of *Vaccinium vitis-idaea* in lowland cool spot and alpine populations: Microrefugia of alpine plants in the mid-latitudes

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Abstract (246 words: 150–250 words)

Local cool spots (wind-holes) in lowland areas of mid-latitudes may act as microrefugia for cold-adapted species outside of their typical alpine habitats. We examined the genetic structure of *Vaccinium vitis-idaea*, a common alpine species in Japan, in eight lowland wind-hole and five surrounding alpine populations. We collected leaf samples and genotyped seven microsatellite loci. Clonal patches (genets) were common in almost all populations. An analysis of annual shoot growth suggested that individuals in the wind-hole populations were long-lived (>500 years old). Genetic diversity (allelic richness) and differentiation (F_{ST}) of the wind-hole populations were lower and higher than those of the alpine populations, respectively. No significant isolation-by-distance trend in the genetic structure was detected for the wind-hole or alpine populations. All wind-hole populations had negative inbreeding coefficients (F_{IS}), suggesting no tendency toward homozygosity due to inbreeding, regardless of the small populations geographically isolated from the large alpine populations. Therefore, wind-holes may harbor genetically isolated but stable populations due to clonal growth, limited gene flow, and abortion of selfed seeds by early acting inbreeding depression. Analysis of molecular variance demonstrated that genetic variations among and within populations contributed more to regional genetic diversity than those between wind-hole and alpine populations, suggesting that the wind-hole and alpine populations are important for maintaining the genetic diversity of mid-latitude *V. vitis-idaea* populations. On

the other hand, Bayesian clustering showed that some wind-hole populations geographically close to the alpine populations had mixed genetic compositions of the alpine and wind-hole populations.

Introduction

Many species inhabiting mountainous areas have upwardly shifted their distributions along with ongoing climate change (Walther et al. 2005; Forister et al. 2010). These elevational shifts are large in alpine plants (Lenoir et al. 2008), and the future distributions of these species are predicted to decline greatly due to the limited area for escape in alpine and geographically isolated habitats (Thuiller et al. 2005; Thuiller 2007). Large-scale range shifts associated with climate change have occurred in past glacial periods, and it has been suggested that small populations persist in local suitable habitats within broadly unsuitable geographical areas (Stewart and Lister 2001; McLachlan et al. 2005; Parducci et al. 2012). These local refugia are called “microrefugia” in contrast to the large, continuous “macrorefugia” (Rull 2009).

Microrefugia are characterized by locally specialized microclimates that are only loosely linked to the surrounding macroclimate. As such, they can provide long-lasting small habitats for specialist species (Dobrowski 2011). In the current interglacial period, alpine habitats are macrorefugia, whereas cool spots situated in topographic depressions, heavily incised valley bottoms, or debris-covered glaciers can be microrefugia (Dobrowski 2011; Gentili et al. 2015). As these small cool spots can be distributed over a broad geographical range (Shimokawabe et al. 2015), microrefugia may play important roles in the response of many species to climate change (Mosblech et al. 2011; Hannah et al. 2014).

Vaccinium vitis-idaea L. (Ericaceae) is a common alpine dwarf shrub inhabiting mid-latitude alpine and subarctic regions. *V. vitis-idaea* also grow in lowland cool spots, including wind-hole micro-topographies (Sato et al. 1993; Sato 1995; Růžička 1999). Wind holes frequently occur at the bottom of talus slopes containing volcanic bedrock (Shimizu 2004), where cool conditions are maintained in the vicinity by the preferential flow of cool air generated in interstitial spaces created by rock fragments or colluvia. We call such cool spots "wind-hole sites." Wind-hole sites harbor various taxa of alpine species that are not found in the surrounding areas (Růžička 1999; Shimokawabe et al. 2015). Shiboi (1975) speculated that lowland *V. vitis-idaea* populations have been maintained in local wind-hole sites since the last glacial period in Hokkaido, northern Japan. If this is true, not only alpine habitats (macrorefugia) but also local wind-hole sites (microrefugia) are important habitats for the maintenance of *V. vitis-idaea* population genetic diversity in mid-latitude regions.

Populations in small and isolated habitats are subject to heightened extinction risks due to inbreeding depression, genetic drift, and demographic stochasticity (Lande 1988; Reed and Frankham 2003). Several mechanisms contribute to the long-term maintenance of microrefugia populations (Hampe and Jump 2011). One exogenous mechanism is migration from the surrounding populations (Mosblech et al. 2011), and one endogenous mechanism is purging deleterious alleles and self-fertilization/compatibility (Mee and Moore 2014). Wind-hole populations are small and isolated from alpine populations, and the mechanisms of

population maintenance may be manifested in their genetic structure.

In the present study, we examined the genetic structure of *V. vitis-idaea* populations in northeastern Hokkaido, including alpine and lowland wind-hole populations. We also measured the annual shoot growth to estimate the age of clonal patches. We discuss the mechanism of population maintenance at wind-hole sites and its ecological significance under ongoing climate change.

Methods

Species studied and field sampling

V. vitis-idaea (2n = 24) is an evergreen dwarf shrub (5–20 cm in height) with creeping stems under the soil surface (Iwatsuki et al. 1993). Based on genetic analyses, Ikeda et al. (2015) suggested that Japanese *V. vitis-idaea* populations have persisted since before the last glacial period. The major habitats in our study area (Engaru and Kitami: 43.7–43.9° N and 143.0–143.4° E, respectively) were alpine zones and lowland wind-hole sites. This species is also known to occur on acid soils in boreal forests and bogs (Garkava-Gustavsson et al. 2005). However, lowland *V. vitis-idaea* populations in Japan other than those at wind-hole sites are limited and fragmentally distributed only in the coastal wetlands of northern/eastern Hokkaido (Umezawa 2007). Flowering occurs in early summer; shrubs are pollinated mainly

98 by bees, and seeds are distributed by animals (Ritchie 1955; Iwatsuki et al. 1993). Upon
99 self-fertilization, partial self-sterility leads to reduced numbers of developed seeds due to
100 early acting inbreeding depression (Guillaume and Jacquemart 1999). *V. vitis-idaea* can form
101 large clonal patches (>30 m) by expanding via underground stems (Persson and Gustavsson
102 2001).

103 The study area was located in northeastern Hokkaido (Fig. 1). Alpine habitats
104 harboring *V. vitis-idaea* cover the western high elevation area (>1,500 m), and wind-hole sites
105 with *V. vitis-idaea* are scattered across the eastern lowland area. This area is topographically
106 complex and includes steep slopes and bedrock dominated by pumice flow deposits
107 (1:200,000 surface geology map by the Ministry of Land, Infrastructure, Transport, and
108 Tourism of Japan). The mean annual temperature during the past 30 years was 5.8°C (mean
109 temperatures of the coldest and warmest months were −8.3 and 19.9°C, respectively) and
110 annual precipitation was 794.5 mm (Japan Meteorological Agency,
111 http://www.jma.go.jp/jma/indexe.html). The temperature at ground level remains 7°C lower
112 than the air temperature during the summer (Shimokawabe et al. 2015).

113 We collected leaves in August–September 2013 from eight wind-hole sites and five
114 separate alpine habitats (Fig. 1). The sample size was 23–31 leaves from each site, totaling
115 393 samples (Table 1). No additional wind-hole sites known to harbor *V. vitis-idaea* are
116 present in our study area (Shimokawabe et al. 2015). We uniformly sampled leaves from all

areas of the wind-hole sites and from the ridge areas in the alpine habitats (sampling interval: 3–10 m for wind-hole sites, 5–10 m for alpine habitats). We recorded the locations of the sampling points using a global positioning system (Garmin GPSMAP 62SJ; Garmin International, Inc., Olathe, KS, USA). Leaf samples were dried in silica gel and preserved at room temperature until analysis.

Microsatellite analysis

We extracted DNA using the cetyltrimethylammonium bromide (CTAB) method. In brief, the sample was crushed in CTAB extraction buffer, chloroform-isoamyl alcohol was added, the aqueous layer containing the DNA was separated, the DNA was purified with isopropanol and ethanol, and the DNA was preserved in Tris-EDTA buffer. Seven microsatellite loci (CA169F, CA236F, NA741, NA800, NA1040, VCC_I2 and VCC_K4: Boches et al. 2005; Appendix A) were amplified and developed for a related species (*Vaccinium corymbosum* L.) using polymerase chain reaction (PCR) and a thermal cycler (Applied Biosystems 2720 Thermal Cycler; Applied Biosystems Inc., Foster City, CA, USA). Following Boches et al. (2005), we used the following PCR program: 3 min at 94°C, followed by 35 cycles of 40 s at 94°C, 40 s at 60°C or 62°C, and 40 s at 72°C with a final rest for 30 min at 72°C. Microsatellite fragments were analyzed using the Applied Biosystems 3130 Genetic Analyzer, and the genotypes were coded using Peak Scanner ver. 2.0 (Applied

Biosystems). GeneScan 500 LIZ Size Standard (Applied Biosystems) was used as the size standard.

Population genetics analysis

A total of 244 multi-locus genotypes in seven loci were identified, and 69 were assigned to more than two different samples (17.6%). Samples with the same genotypes in all loci were treated as the same individual (genet), and the genet was used as the analytical unit. We calculated the probability that two different individuals would have the same genotype (PI: probability of identity) using GenAlEx 6.501 (Peakall and Smouse 2006) to evaluate misassignment of different individuals to genets. We tested departures from Hardy–Weinberg equilibrium based on the chi-square (χ^2) test using GenAlEx 6.501 and obtained the inbreeding coefficient (F_{IS}). We examined the presence of null alleles for each population using GENEPOP 4.3 (Rousset 2008). The Bonferroni correction was used to determine the significance levels of the equilibrium tests.

We obtained F_{ST} values as genetic differentiation indices for each pair of populations. The value of this index can be affected by population history, mutation, gene flow, and genetic drift (Marko and Hart 2011). The correlation between the F_{ST} value and geographic distance (isolation by distance, IBD: Wright 1943) was examined based on the Mantel tests for (i) all populations, (ii) wind-hole and wind-hole pairs, and (iii) alpine and alpine pairs. We also

155 examined the differences in genetic differentiation (F_{ST}) and geographic distances among
156 populations for (i) wind-hole and wind-hole pairs, (ii) wind-hole and alpine pairs, and (iii)
157 alpine and alpine pairs with multiple comparison tests (Steel–Dwass method). We obtained
158 the geographic distance as three-dimensional straight-line distances considering elevational
159 differences. The F_{ST} values were calculated, Mantel tests were conducted using GenAlEx
160 6.501, and multiple comparison tests were performed using R ver. 2.15.2 (R Core Team,
161 Vienna, Austria).

162 We calculated allelic richness and private allelic richness (the number of unique alleles
163 for each population, corrected for sample size) as genetic diversity indices (Kalinowski 2004)
164 using HP-Rare v. June-6-2006 (Kalinowski 2005). The diversity indices were compared
165 between wind-hole sites and alpine habitats using *t*-tests. The correlation coefficient between
166 allelic richness and the index of genetic isolation was determined as the mean F_{ST} value for
167 each population. The mean F_{ST} was obtained from the F_{ST} values of all other populations, and
168 the correlations were examined separately for the wind-hole sites and alpine habitats.

169 We conducted analyses of molecular variance (AMOVA) based on these F_{ST} values to
170 divide genetic variation into those occurring within populations, among populations, and
171 among habitats using GenAlEx 6.501. STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to
172 examine individual based Bayesian clustering to estimate the most likely number of
173 genetically differentiated populations (*K*). An admixture model with the correlated allele

frequencies and informative locations was employed. The optimal number of clusters from 1 to 13 was inferred by 200,000 interactions following a 100,000 step burn-in with 10 replicate runs for each *K*-value. STRUCTURE can identify the highest hierarchical level of a population but often fails to detect hierarchical genetic structure at lower levels (Evanno et al. 2005). Therefore, subsequent runs were executed with the same method to reveal additional hierarchical genetic clusters when $K > 1$ was identified at the initial run. The STRUCTURE results and the online program STRUCTURE HARVESTER ver. 0.6.94 (Earl and vonHoldt 2012) were used to determine the best *K* number. This program infers the best *K* value using the delta *K* based on the rate of change in the log probability of the data (Ln *K*) between successive *K* values (the best *K* has the highest delta *K* value) (Evanno et al. 2005). Finally, the online program CLUMPAK was used to visualize the spatial genetic structure estimated by STRUCTURE (Kopelman et al. 2015).

Annual growth measurements

We measured the annual growth of shoots and estimated their ages to infer the persistence of individuals at the wind-hole sites. We measured annual growth length of the previous year's shoots from 10 individuals each in the six wind-hole populations during May 2015. We thereby obtained a mean value for the 10 individuals in each population. The longest length between ramets was obtained from genetic analyses. We treated this length as

193 the size of the oldest individual in the corresponding population, and estimated its age
194 assuming circular growth: Age = (0.5 × distance between ramets)/annual growth.
195
196 **Results**
197 The mean PI across 13 populations, which indicates the probability of misassignment
198 of different individuals to genets, was very low (2×10^{-4}). Therefore, we treated plants with
199 the same genotype as the same individual (genet). Clones were detected at all alpine and
200 wind-hole sites, except at Mt. Mur (Table 1). The longest distances within clones were 14.3–
201 101.8 m (Table 1). Large ramets had between-ramet lengths > 50 m (Appendix B). The mean
202 annual shoot growth of the six wind-hole populations was 3.90 cm $year^{-1}$. The largest clone
203 was estimated to be 594 years old, and four populations had clones that were > 500 years old
204 (Table 1). Note that our calculations may have underestimated the ages of the clones because
205 we assumed a uniform circular growth pattern. However, this result suggests that *V.*
206 *vitis-idaea* individuals have been maintained at the wind-hole sites for hundreds of years. We
207 observed deviations from Hardy–Weinberg equilibrium at a mean of two populations per
208 locus (adjusted $P < 0.05$). All wind-hole populations had negative F_{IS} values, and six
209 populations differed significantly from zero ($P < 0.05$: Table 1). The existence of null alleles
210 was suggested for multiple loci and populations. However, their frequencies were small, and
211 we used all loci and genets (mean frequency per loci = 0.03).

The Mantel tests did not reveal any significant IBD among populations ($r = -0.12$, $P = 0.23$; Fig. 2) or within habitat types (wind-hole–wind-hole pairs: $r = 0.32$, $P = 0.16$; alpine–alpine pairs: $r = 0.05$, $P = 0.54$; Fig. 2). However, the alpine–alpine pairs and the wind-hole–wind-hole pairs had lower and higher F_{ST} values, respectively, than those obtained among habitat pairs (Steel–Dwass method: $P < 0.05$; Fig. 3a). The wind-hole–alpine pairs had larger geographic distances than the other two pairs (Steel–Dwass method: $P < 0.01$; Fig. 3b). These results suggest that the wind-hole–wind-hole pairs had large F_{ST} values regardless of their short geographic distances. Some wind-hole–alpine pairs had F_{ST} values similar to those of alpine–alpine pairs (Fig. 2).

The wind-hole populations had lower allelic richness (*t*-test: $P < 0.01$; Table 1) and larger variations in allelic richness (Fig. 3c) than the alpine populations. No differences in private allelic richness were detected between the wind-hole and alpine populations, and both populations had unique loci (*t*-test: $P = 0.09$; Fig. 3d, Table 1). Allelic richness and mean F_{ST} values were negatively correlated in the wind-hole populations ($r = -0.87$, $P < 0.01$), but not in the alpine populations ($r = 0.13$, $P = 0.84$; Fig. 4).

All three levels (between habitats, among populations, and within populations) significantly contributed to the genetic variation. Variations between habitat types were smaller than those among and within populations, and within-population variations dominated total genetic variation (Table 2).

231 STRUCTURE revealed two main genetic clusters with the highest delta *K* value (Fig.
232 5a). The alpine populations were assigned to cluster 1, and most genets of the wind-hole
233 populations were assigned to cluster 2. However, some genets of three wind-hole populations
234 (HiH, HiN, and Sin) were assigned to cluster 1. These populations were geographically closer
235 to the alpine sites than the other wind-hole sites (Fig. 1). Therefore, we separated 13
236 populations into two genetic groups with contrasting genetic composition and examined
237 subsequent STRUCTURE runs for each of the two groups (Fig. 5b, c). Subsequent analyses
238 revealed seven genetic clusters within the wind-hole and alpine populations ($K = 4$ for the
239 first group, including five wind-hole populations and $K = 3$ for the second group, including
240 three wind-hole and five alpine populations). Most populations formed unique genetic clusters,
241 indicating high genetic variation among populations, as suggested by AMOVA. However,
242 some populations, which were spatially close to each other, formed similar clusters (e.g., Iga
243 and Mir; HiH, HiN, and Sin; Mt. Hir and Mt. Mur).
244
245
246 **Discussion**
247 Population maintenance at the wind-hole sites
248 The existence of large clonal patches (>50 m spans) in the wind-hole and alpine
249 populations suggests that individual plants can persist for hundreds of years. Such long clonal

lifespans have also been reported in other ericaceous alpine species (Kameyama et al. 2008) and was reported by another genetic study on *V. vitis-idaea* (Persson and Gustavsson 2001). A long lifespan through clonal growth may be important for persistence of small populations, such as wind-hole populations. A low level of heterozygosity is expected for small relict populations if self-fertilization is common (Mee and Moore 2014). However, wind-hole populations had negative F_{IS} values, indicating the existence of mechanisms for avoiding self-fertilization (Wright 1965). This may be because of a very long generation time, which would delay the genetic homogenization caused by inbreeding. On the other hand, early acting inbreeding depression strictly prevents the formation of selfing seeds in *V. vitis-idaea* (Guillaume and Jacquemart 1999). Although restricting seed production by selfing may increase the risk of local extinction by decreasing mating opportunities (Byers and Meagher 1992), the existence of long-lived clones would alleviate the extinction risk.

Genetic diversity was lower in the wind-hole populations than that in the alpine populations. Although we did not find IBD in the wind-hole populations, a negative correlation between the level of genetic isolation (F_{ST}) and genetic diversity suggests that (1) the wind-hole populations are isolated and subject to genetic drift (Hutchison and Templeton 1999) and (2) that gene flow between the wind-hole populations is not dependent on geographic distance. The latter may be partly due to the existence of undiscovered wind-hole populations in the study area. As genetic diversity of a small population can increase by rare

immigration of gametes (Ingvarsson 2001), genetic diversity of a wind-hole population would strongly depend on genetic exchange with other populations. In our study, gene flow was lower in the wind-hole–wind-hole pairs than that in the alpine–alpine pairs. Pollinators and seed dispersers would infrequently visit small wind-hole sites surrounded by forest.

Genetic diversity of the alpine populations

We did not find IBD among the alpine populations, and genetic diversity and differentiation were higher and lower than those in in the wind-hole populations, respectively. Although we selected alpine populations from separate mountains, these sites were structurally connected by alpine habitats. Ikeda et al. (2015) also reported the lack of nationwide genetic differentiation in *V. vitis-idaea* populations in Japan using nuclear and chloroplast DNA markers. This finding suggests long-distance and efficient seed dispersal by birds during the post-glacial period. Therefore, pollinators and seed dispersers may move between alpine populations. High genetic diversity would have been maintained in large-sized alpine populations with small effects from genetic drift and metapopulation structure among populations (Billington 1991).

Ecological significance of wind-hole populations

The AMOVA results suggested that genetic variation within and among populations

made greater contributions to regional genetic diversity than did differences between the alpine and wind-hole populations. We found unique genetic clustering between most of the wind-hole populations and unique loci in almost all populations, suggesting that the alpine and wind-hole populations are important to regional genetic diversity of *V. vitis-idaea*. Although the wind-hole populations had lower genetic diversity than that of the alpine populations, some wind-hole populations had relatively high genetic diversity and were likely subject to gene flow from surrounding populations. Our results also convincingly demonstrate the long-term persistence and genetic isolation of the wind-hole populations.

Microrefugia (wind-hole sites, in our case) are local small habitats, but can be long-lived and span a broad geographical area, at least in our study area (Shimokawabe et al. 2015). Růžička et al. (2015) suggested that wind-hole sites in the Czech Republic will maintain cool conditions even in warmer climates. The *V. vitis-idaea* wind-hole populations geographically close to the alpine populations included the genetic composition of the alpine populations, suggesting that wind-hole habitats act as safe sites for wind-hole and alpine populations of cold-adapted species under global warming conditions. Not all cold-adapted species would benefit from microrefugia (Hylander et al. 2015). Nevertheless, because localized unique geographic features, which can be the bases of many microrefugia (Hjort et al. 2015), occur in economically unproductive sites (Lindenmayer and Franklin 2002), we have suggested previously that identifying and protecting potential microrefugia is a robust

and cost-effective way to mitigate the impact of climate change (Shimokawabe et al. 2015).

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Table 1. Habitat types and characteristics of *Vaccinium vitis-idaea* populations. Size and perimeter were not measured for the high mountain population. Asterisks (*) on the F_{IS} value indicates a significant difference from zero (<0.05) based on mean and standard deviation across seven loci.

Site	Habitat[1]	Ele.[2]	Pop. area[3]	Peri[4]	Dist. alp.[5]	Growth[6]	Dist. ramets[7]	Age[8]	*N*[9]	# genet[10]	AR[11]	Pr. AR[12]	Mean F_{ST}	F_{IS}
Ara	WH	309	2022	278	13.5	6.26 ± 1.66	74.4	594.2	31	10	2.45	0.53	0.19	−0.67*
Goj	WH	519	516	252	18.0	3.46 ± 1.04	32.2	465.3	31	13	2.93	0.05	0.12	−0.45*
Mir	WH	359	555	765	15.2		72.3		31	19	4.07	0.23	0.10	−0.20
Iga	WH	290	866	283	14.5		43.9		30	14	4.35	0.00	0.10	−0.12
HiH	WH	456	248	130	10.8	3.43 ± 1.41	36.2	528.1	31	15	4.81	0.16	0.08	−0.31*
HiN	WH	486	1321	546	10.5	3.62 ± 1.20	37.8	521.4	30	24	4.91	0.25	0.08	−0.17*
Set	WH	635	111	152	19.5	3.16 ± 1.10	14.3	226.3	31	17	4.35	0.38	0.12	−0.33*
Sin	WH	495	671	150	7.2	3.44 ± 0.61	38.8	564.0	31	8	2.43	0.25	0.14	−0.67*

Mt.Ari	HM	1635	-	-	-	55.6	31	27	5.75	0.64	0.08	0.13
Mt.Hir	HM	1771	-	-	-	86.2	31	22	5.39	0.37	0.08	0.04
Mt.Shi	HM	1688	-	-	-	50.7	23	17	5.36	0.42	0.08	−0.03
Mt.Mur	HM	1876	-	-	-	-	31	31	6.64	1.01	0.08	0.21*
Mt.Muk	HM	1759	-	-	-	101.8	31	26	5.57	0.24	0.07	−0.07

[1]Habitat: WH (wind-hole); HM (high mountain). [2]Elevation (m). [3]Population area (m^2). [4]Perimeter of population (m). [5]Distance from alpine vegetation (km). [6]Mean growth rate (± standard deviation; cm $year^{-1}$). [7]Longest distance between ramets (m). We did not detect ramets with the same multi-genotype on Mt. Mur. [8]Estimated age (years). [9]Number of individuals analyzed. [10]Number of genets. [11]Allelic richness. [12]Private allelic richness.

438 Table 2. Analysis of molecular variance for the *Vaccinium vitis-idaea* samples.

Scale	d.f.[1]	SS[2]	Est. var.[3]	% variation	*P*
Between habitat types (wind-holes and high mountains)	1	26.5	0.04	1.4	0.001
Among populations	11	163.0	0.34	12.0	0.001
Within population	475	1129.5	2.48	86.6	0.001

439 [1]Degrees of freedom. [2]Sum of squares. [3]Estimated variance.

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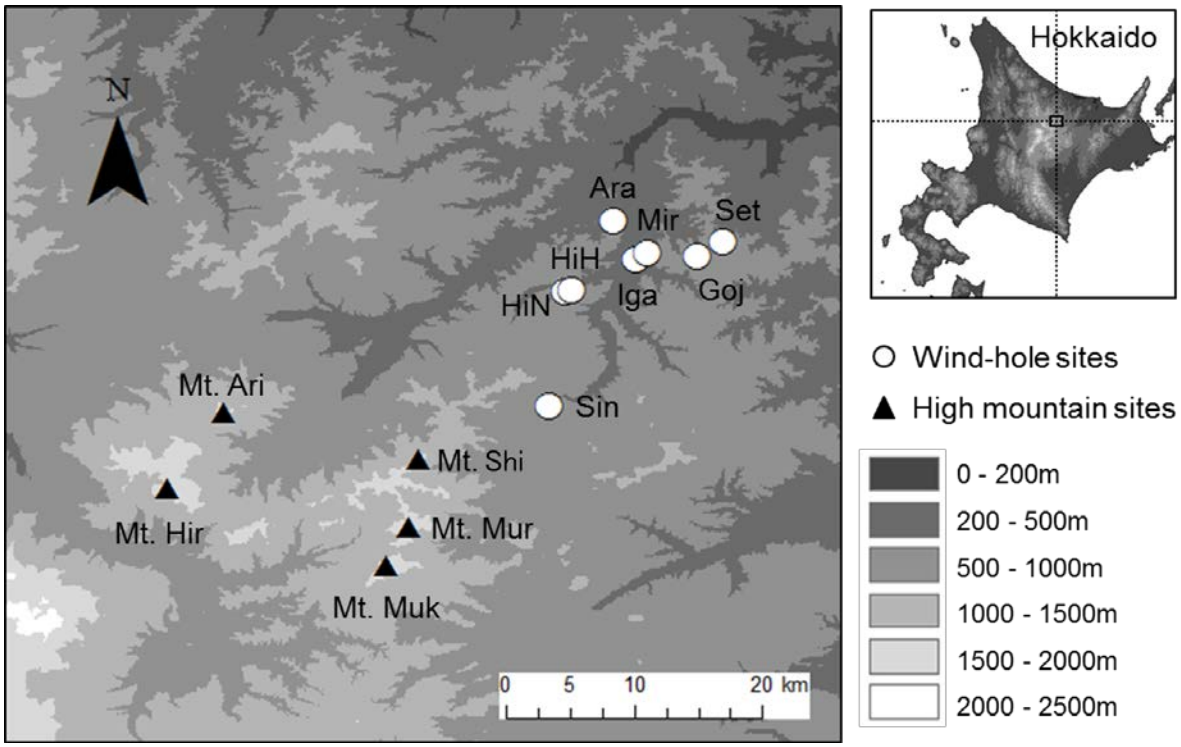

445 Fig. 1. Wind-hole and high mountain population sampling locations.

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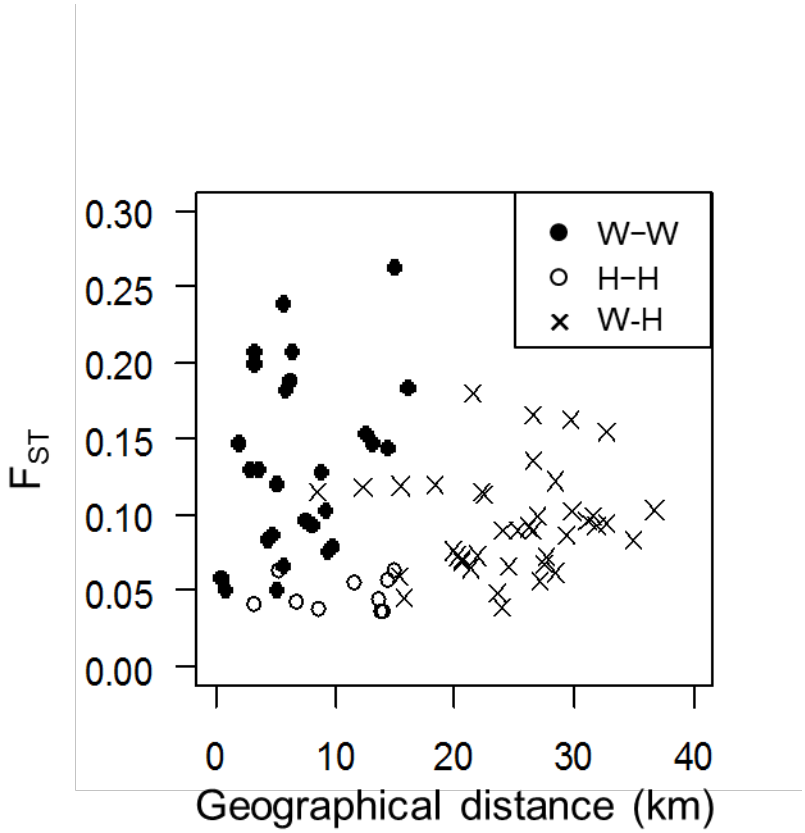

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449 Fig. 2. Relationships between pairwise F_{ST} values and geographical distance. H-H, high

450 mountain pairs; W-W, wind-hole pairs; W-H, wind-hole–high mountain pairs. None of the

451 relationships were significant correlations based on the Mantel test.

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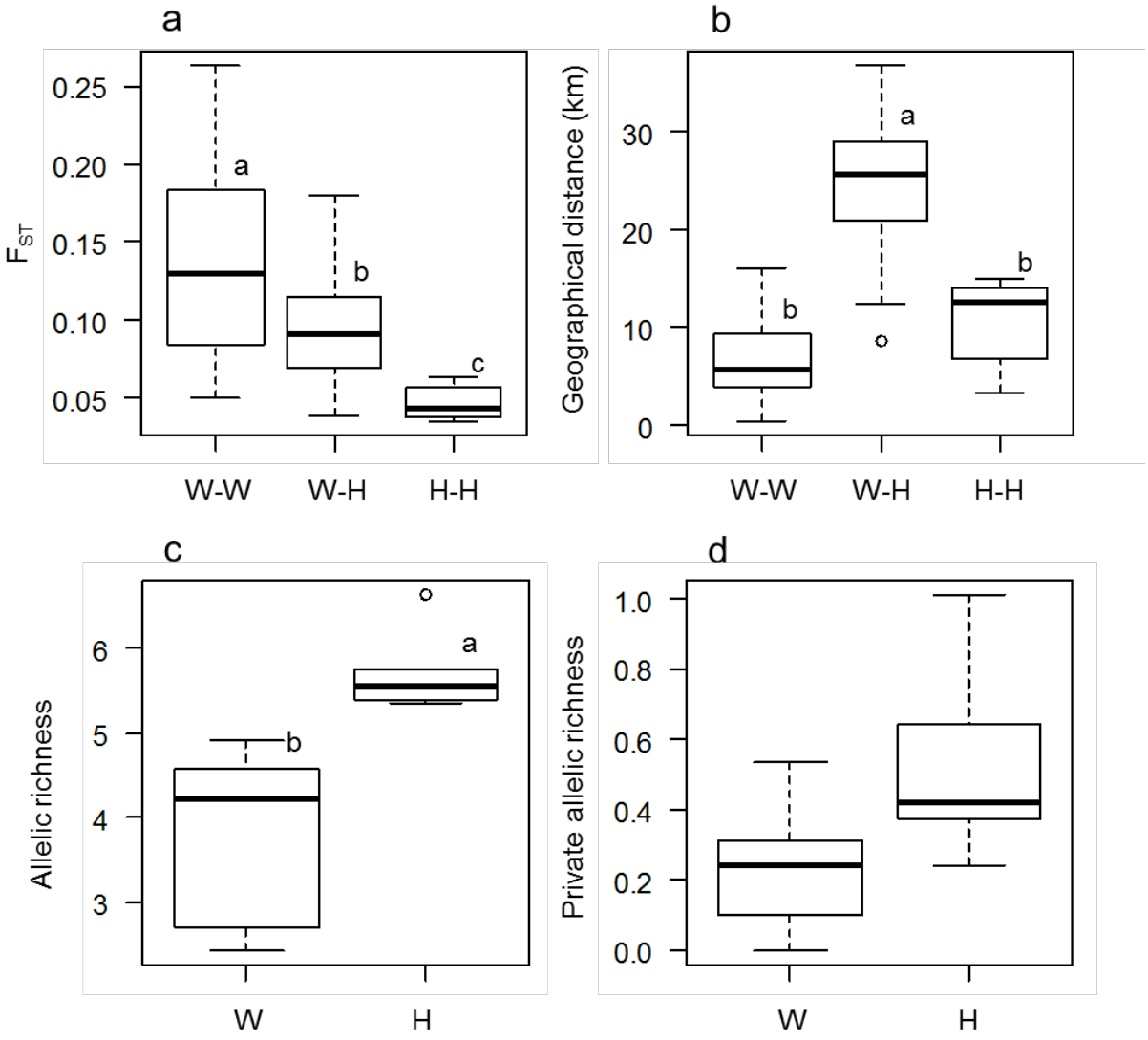

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454 Fig. 3. Genetic distances (a) and geographical distances (b) among habitat types and allelic

455 richness (c) and private allelic richness (d) of *Vaccinium vitis-idaea* populations at the

456 wind-hole and high mountain sites. W, wind-holes; H, high mountains. Different letters within

457 box plots indicate significant differences ($P < 0.05$) based on the Steel–Dwass test for Fig. 3a,

458 b and the *t*-test for Fig. 3c, d.

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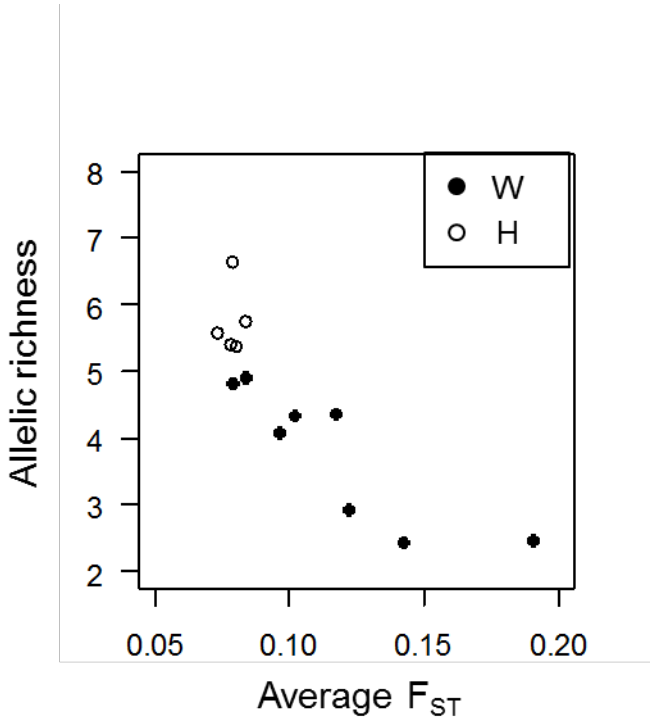

Fig. 4. Relationships between allelic richness and mean F_{ST} values of the wind-hole and high mountain populations. Only the wind-hole populations were significantly correlated based on Pearson's correlation analysis.

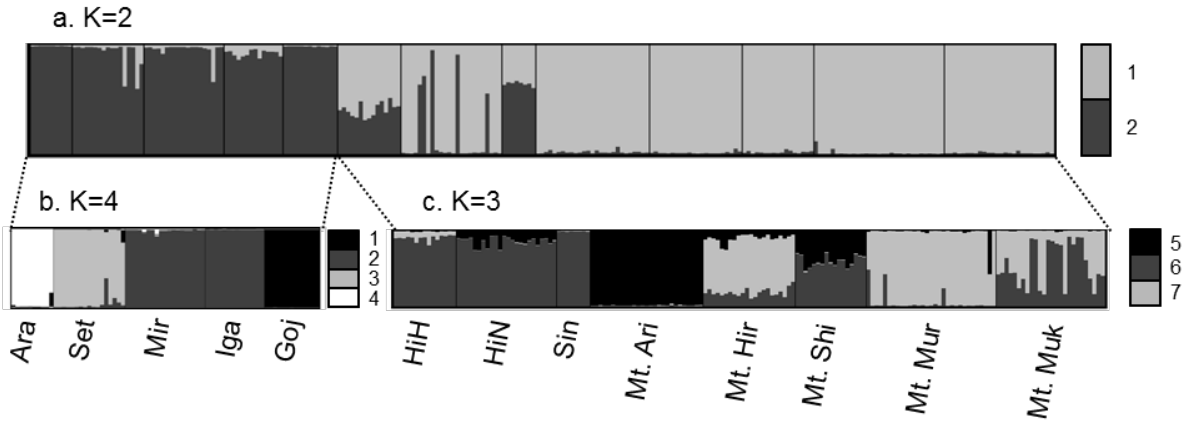

Fig. 5. Genetic clusters of *Vaccinium vitis-idaea* populations in the Engaru and Kitami regions. (a) The initial analysis of all populations showed two genetic clusters (K = 2). (b) A subsequent analysis of five wind-hole populations showed four genetic clusters (K = 4). (c) A subsequent analysis using three wind-hole populations and five alpine populations showed three genetic clusters (K = 3). Colors indicate different genetic clusters within each analysis.