Title: Application of the isolation with migration model demonstrates the Pleistocene origin of geographic differentiation in *Cardamine nipponica* (Brassicaceae), an endemic Japanese alpine plant

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Abstract

The Pleistocene was characterized by a cyclic pattern of cold and warm climatic periods, or climatic oscillations, which caused fluctuations in the distributions of organisms. This resulted in drastic changes in demography, thereby accelerating the genetic divergence of populations. Phylogeographic studies have elucidated the history of populations during the Pleistocene. However, given the lack of model-based analysis of population histories, previous phylogeographic studies could not adequately evaluate the effect of these Pleistocene climatic oscillations on the genetic divergence and migration events between populations. Populations of Japanese alpine plants in central and northern Japan are highly differentiated, and a history of isolation between regions during the Pleistocene was inferred. Using sequences of ten nuclear genes (approximately 7,000 bp in total) from *Cardamine nipponica* (Brassicaceae), we applied an isolation with migration (IM) model to test the significance of the isolation history between central and northern Japan and to assess whether range shifts during the Pleistocene climatic oscillations were involved in the genetic differentiation between regions. The estimated divergence time indicates that the two regions were separated about 100,000–110,000 years ago. The exclusive occurrence of closely related haplotypes within each region (parsimony network) and the high level of genetic differentiation between the regions (mean F_{ST} = 0.417) indicate that genetic divergence occurred following the isolation of the two regions. Therefore, the genetic differentiation between regions was shaped during the Pleistocene, especially during the last glacial and inter- and post-glacial periods. In addition, our multilocus analysis showed that populations in central and northern Japan were completely isolated after they split. Geographic separation and subsequent restricted migration events among mountains could explain this isolation history between regions. Furthermore, genetic drift in the reduced populations would remove evidence of occasional migration, emphasizing the isolation history. Therefore, our application of a demographic model demonstrated the Pleistocene origin of geographic differentiation statistically and provided a plausible migration history for *C. nipponica*.

Introduction

Since the Pleistocene was characterized by a cyclic pattern of cold and warm climatic periods, or climatic oscillations, changes in the global distributions of organisms occurred and populations could have experienced repeated geographic isolation, resulting in drastic changes in demography. Population histories and genetic consequences following these climatic oscillations during the Pleistocene have been elucidated by molecular phylogeographic studies (e.g., Soltis et al. 1997; Taberlet et al. 1998; Petit et al. 2003; Alsos et al. 2007). According to these studies, geographic separation of populations following the climatic oscillations resulted in genetic divergence and population differentiation (Hewitt 1996, 2000; Avise 2000).

However, most phylogeographic studies have been based on the genetic structure of organellar DNA, and therefore reflect only the history of a single gene. Consequently, stochastic variance caused by different rates of mutation and recombination among genes or lineage sorting among regions could result in a different genealogy across the genome (Hey and Machado 2003), biasing the estimated demographic history. Although this single-locus problem was resolved by amplified fragment length polymorphism (AFLP) studies, the anonymous dominant markers do not allow the reconstruction of genealogies, which makes it difficult to estimate sequential relationships among populations. Therefore, most phylogeographic studies cannot reveal a detailed demographic history during the Pleistocene. In addition, due to the lack of appropriate models of population histories and statistical tests for them, the interpretation of population histories has been primarily descriptive and *post hoc* (Knowles and Maddison 2002). Recent parameter-rich population models, such as the isolation with migration (IM) model (Nielsen and Wakeley 2001; Hey and Nielsen 2004), along with multilocus sequence data, could compensate for the deficiencies of previous studies (Hey and Machado 2003), and allow the elucidation of a statistically significant history of populations during the Pleistocene.

Japanese alpine plants are an appropriate study system whose demographic history during the Pleistocene could be tested using model-based analysis. Previous phylogeographic studies of alpine plants in the Japanese archipelago have revealed geographic differentiation between central and northern Japan, suggesting that the populations in central Japan were isolated from more northern regions during the Pleistocene (Fujii et al. 1997, 1999; Fujii and Senni 2006; Ikeda and Setoguchi 2006, 2007, 2009; Ikeda et al. 2006, 2008a, b, c, 2009). However, because previous phylogeographic studies only detected genetic differentiation between regions, they did not evaluate whether this genetic differentiation is related to distribution changes during the Pleistocene statistically. In addition, although high levels of genetic differentiation were detected, whether migration occurred following the range expansion remains unclear. Since current populations of alpine plants in Japan are isolated from one another by uninhabitable areas and restricted to areas around the peaks of high mountains, alpine plants may have been more widely dispersed during the cold periods of the Pleistocene. Nearly half of the species in the Japanese high mountains co-occur in the Arctic (e.g., *Loiseleuria procumbens* and *Diapensia lapponica*), further suggesting dispersal during cold periods. Accordingly, alpine plants may have migrated across their current range during cold periods, although the range of alpine flora was not continuous during the coldest period (ca. 18,000 years ago; Ono and Igarashi 1991). Consequently, model-based analysis of demographic history could demonstrate the migration history between central and northern Japan and the involvement of distribution changes during the Pleistocene in the genetic differentiation of Japanese alpine plants.

Cardamine nipponica Franch. et Savat. (Brassicaceae) is a perennial herb endemic to the Japanese archipelago. It grows near the tops of high mountains and forms very small populations. This plant reproduces annually, mostly by inbreeding. The seed and pollen have no adaptation for long-distance dispersal (Kitakawa 1999; Ikeda personal observation). Based on chalcone synthase

(*CHS*) gene sequences, *C. nipponica* constitutes a monophyletic group with the European species, *Cardamine alpina* and *Cardamine resedifolia*, and the arctic-alpine species, *Cardamine bellidifolia* (Ikeda et al., unpublished data), and probably originated from an arctic-alpine ancestral species that migrated to the Japanese archipelago during cold periods, as did most Japanese alpine plants (Koidzumi 1919; Toyokuni 1981). In addition, the genus *Cardamine* is closely related to *Arabidopsis*, which diverged 13–19 million years ago (Koch, Haubold, and Mitchell-Olds 2001). Therefore, it is appropriate to use the genomic information on *Arabidopsis thaliana* when studying *Cardamine* (Kuittinen et al. 2002).

In this study, we identified polymorphisms in ten nuclear genes of *C. nipponica* from northern Japan (the northern region) and central Japan (the southern region). Based on multilocus nuclear sequence data, we applied the IM model (Nielsen and Wakeley 2001; Hey and Nielsen 2004) to estimate the demographic history of *C. nipponica* as a model case of alpine plants in Japan. Based on the estimated demographic parameters, our goal was to test the significance of the isolation history between regions and to assess whether the genetic differentiation between central and northern Japan was related to the Pleistocene climatic oscillations.

Materials and methods

Sampling and DNA extraction

Previously analyzed DNA samples (Ikeda et al. 2008c) from plants collected from almost the entire distribution of *C. nipponica* were used in this study. Two individuals per location (19 locations in total; Fig. 1, Table 1) were selected randomly for use in this study; this set may contain all of the polymorphisms of this species because a previous study showed little genetic diversity within populations (Ikeda et al. 2008c). To focus on the divergence between the northern and southern regions, between which strong genetic divergence was observed in previous

phylogeographic studies (Fujii et al. 1997, 1999; Fujii and Senni 2006; Ikeda et al. 2006, 2008a, b, 2009), 14 individuals from seven locations in northern Japan and 24 individuals from 12 locations in central Japan were assigned to the northern and southern regions, respectively (Fig. 1). In addition, three individuals each of *C. alpina* and *C. resedifolia* were used as outgroups. DNA was extracted from the outgroups using DNeasy (QIAGEN) and dissolved in 100μL of buffer.

PCR and sequencing

To detect nuclear DNA polymorphisms within *C. nipponica*, 20 pairs of primers previously shown to amplify homologous loci (*COP1*, *DET1*, *GA1*, *TET1*, *PHYA*, *PHYB*, *PHYC*, *PHYD*, *PHYE*, *CAL*, *F3H*, *DER*, *CHI*, *CHS*, *ETR1*, *FAH*, *PGIC*, *FK*, *GS*, and *MAM-L*) in *Arabidopsis thaliana* and *Brassica oleracea* (Kuittinen et al. 2002) were used to amplify nDNA. The compositions of the PCR mixtures were the same as in a previous study (Ikeda et al. 2008c). PCR was performed with 40 cycles of 1 min at 94ºC, 1 min at 52ºC, and 3 min at 72ºC. The PCR products were visualized on 2.0% TAE-agarose gels stained with ethidium bromide, and clear fragments of the expected sizes were detected from 15 of the loci, but not from *PHYB*, *PHYC*, *PHYD MAM-L*, or *FK*. The PCR products were gel-purified with glass powder using GeneClean II (Bio101, Vista, CA, USA). The products were sequenced directly in both directions using the standard methods of the BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA), and the sequences were aligned using Auto Assembler software (Applied Biosystems). Since no amplified products were obtained from five of the 15 loci (*CHI*, *ETR1*, *FAH*, *PGIC*, and *GS*), they were excluded from subsequent analyses. Sequences of the ten remaining loci were determined. If we were unable to clearly obtain the entire sequence of each locus, internal primers were developed based on a conserved region between *C. nipponica* and the outgroup sequences. To avoid erroneous polymorphisms, all sequences with singletons or dual peaks were checked more than twice.

Data analysis

Since clear electropherograms were detected from most analyzed sequenced loci, and the closely related species are diploid (2n = 16; *C. alpina*, *C. resedifolia*, and *Cardamine glauca*; Kučera, Valko, and Marhold 2005), individual sequences without heterozygous positions were treated as homozygous and as the same sequence. Haplotypes were determined manually based on the aligned sequences because two or more heterozygous positions were not obtained from each sequence, and parsimony networks were drawn using TCS1.06 (Clement, Posada, and Crandall 2000). Few sequence polymorphisms would be expected within each population, as shown in our previous investigation (Ikeda et al. 2008c), and STRUCTURE analysis indicated that the significant grouping of individuals corresponded to the two regions; therefore, we lumped populations within each region for neutrality tests. The neutral equilibrium was tested using Tajima's *D* (Tajima 1989) and Fay's *H* (Fay and Wu 2000) tests with outgroup (*C. alpina*) sequences, and significance was determined using 10,000 coalescent simulations. Tajima's *D* was estimated for total (D_{Total}), noncoding (D_{nc}), synonymous (D_s), and nonsynonymous (D_a) sites. In addition, the HKA test (Hudson, Kreitman, and Aguadé 1987) was conducted to test for neutral evolution across loci between each region and outgroup, and between the northern and southern regions. The two outgroup sequences were analyzed separately for Fay's *H* and the HKA test. The minimum number of recombination events (R_M) based on the four-gamete test (Hudson and Kaplan 1985) was estimated for all loci. Nucleotide diversity (π) within regions and genetic divergence (K) between the two regions, *C.nipponica* and *C. alpina*, and *C. nipponica* and *C. resedifolia* for the total (π_{Total}, K_{Total}), noncoding (π_{nc}, K_{nc}), synonymous (π_s, K_s), and nonsynonymous (π_a, K_a) sites were estimated (Nei 1987). The genetic differentiation between regions was estimated using the F_{ST} using the method of Hudson, Slatkin, and Maddison (1992a). Significance was evaluated with S_{nn} (Hudson 2000) based on 1,000 permutations, because these values have the greatest ability to detect

population differentiation (Hudson 2000). All of these analyses were performed using DnaSP 4.00 (Rozas et al. 2003), SITES (Hey and Wakeley 1997), and the HKA program (http://lifesci.rutgers.edu/~heylab/HeylabSoftware.htm#HKA). In addition, the rate heterogeneity among loci was tested by the relative ratio test using HyPhy (Kosakovsky Pond et al. 2005).

Genetic structures among populations throughout the Japanese archipelago and within regions were estimated by Bayesian clustering using STRUCTURE ver. 2 (Pritchard et al. 2000). Clustering of individuals was determined on the basis of their genotypes at multiple loci, where each cluster follows Hardy-Weinberg equilibrium. An admixture model was used that includes linkage among polymorphisms for the ancestry model (Falush, Stephens, and Pritchard 2003) in which polymorphic sites in each locus were treated as a single linkage group and the independence of allele frequencies among populations was assumed. The probability of assigning individuals into clusters was estimated using 5.0×10^5 iterations, following 1.0×10^5 iterations as a burn-in period. The number of clusters (K) was set from 1 to 15, and all runs were replicated 20 times to test the stability of the results. The most likely number of clusters was estimated according to the model value (ΔK) based on the second-order rate of change, with respect to K, of the likelihood function, following the procedure described by Evanno, Regnaut, and Goudet (2005).

Migration rates, effective population sizes, and the population-split time were calculated based on the IM model using the IMa program (Hey and Nielsen 2007). The program served to estimate probability density functions for parameters of the IM model and to assess the posterior probability densities for model parameters using Markov Chain Monte Carlo (MCMC) methods (Hey and Nielsen 2004, 2007). In addition, the likelihood of the isolation model (no migration between populations; $m_1=m_2=0$) was calculated using the estimated functions and compared to the IM model. Running IMa involved two steps (M-mode and L-mode). First, functions of model parameters were

estimated in M-mode based on 5×10^8 MCMC steps following 100,000 burn-in periods, from which genealogies were saved for 200 steps. The M-mode run was repeated three times to check for convergence. Using the functions of model parameters estimated in three independent runs of M-mode, the marginal posterior distribution and the maximum-likelihood estimates (MLEs) of demographic parameters were estimated and the isolation model was assessed in L-mode. An infinite site model of mutation was applied to all loci. Since this analysis assumes that no intra-locus recombination occurred, part of each sequence for which no recombinations were found was used in *GA*1, in which recombination was detected across two regions.

The divergence time was scaled using the geometric mean of the mutation rate per year per locus. Although a synonymous mutation rate of the *CHS* gene in Brassicaceae has been reported (1.5×10^{-8} substitutions per site per year; Koch, Haubold, and Mitchell-Olds 2000), our data included nonsynonymous and noncoding sites as well as synonymous ones. Since the rate heterogeneity among loci was not significantly rejected and evolutionary rates could be consistent among loci, the ratio of the average total genetic divergence to the average synonymous genetic divergence (K_{Total} / K_S) was used to obtain the mutation rate ($\mu = K_{Total} / K_S \times 1.5\times10^{-8}$) for our data. A significant positive correlation between K_{Total} and K_S was detected solely in *C. alpina* ($r = 0.80$, $P < 0.01$; *C. resedifolia*, $r = -0.32$, $P = 0.48$); therefore, we applied genetic divergence only from *C. alpina* and obtained 8.67×10^{-9} substitutions per site per year. Consequently, the geometric mean, 5.585×10^{-6} substitutions per year per locus, was used to scale the divergence time.

Results

Sequence variation

The sequences of ten nuclear DNA loci (7,068bp in total) were obtained from 38 individuals of *C. nipponica* (14 from the northern region and 24 from the southern region; Fig. 1, Table 1) and

from three individuals each of *C. alpina* and *C. resedifolia*, which were used as outgroups. The sequences were deposited in the DNA Data Bank of Japan (DDBJ; AB377085–AB377107, AB378218–AB378290).

The haplotype composition of each population and the relationships among the haplotypes including the outgroups are shown in Table 1 and in parsimony networks (Fig. 2), respectively. All *C. nipponica* haplotypes were distinguished from those of the outgroups. Six of the ten loci had a haplotype that was shared across regions, and these shared haplotypes were the most closely related to the haplotypes of the outgroups. The other haplotypes of these loci were region-specific. The haplotypes of the remaining four loci were not shared between regions, and their geographic distributions were highly structured, except for *TFL1*. Therefore, closely related haplotypes occurred exclusively within each region.

The genetic diversity and divergence are shown in Tables 2 and 3. The average diversity was slightly higher in the northern region (π_{Total} = 0.0015) than in the southern region (π_{Total} = 0.0011), and the level of diversity was highly variable among loci (north π_{Total} = 0.00026–0.00372; south π_{Total} = 0.00008–0.00212). The larger geographic extent of populations in northern Japan, where populations extends to two islands, may have resulted in the higher genetic diversity in that region. As expected from the inbreeding character, the overall level of diversity was low and compatible with that of *A. thaliana* (π = 0.0019–0.0055; Ramos-Onsins et al. 2004), which is also an inbreeding plant. The diversity at noncoding (π_{nc} = 0.0021, 0.0016 in northern and southern regions, respectively) and synonymous (π_s = 0.0024, 0.0007, respectively) sites was higher than that at nonsynonymous sites (π_s = 0.0007, 0.0006, respectively), implying functional constraints on these genes.

The divergences between regions were mostly greater than the diversity (average K_{Total} =

0.00297, ranging from 0.00031 to 0.00686). In particular, compared to diversity, genetic divergence at nonsynonymous sites was high (Tables 2, 3), resulting in high ratios of nonsynonymous to synonymous divergence (π_a/π_s = 0.17, 0.08 in northern and southern regions, respectively; K_a/K_s =0.85). Although the level of genetic divergence between regions was smaller than those between Arabidopsis species (K = 0.05–0.12; Ramos-Onsins et al. 2004, 2008), the ratio of nonsynonymous divergence was much higher between regions than between *Arabidopsis* species (K_a/K_s = 0.08–0.25). However, the neutrality test for synonymous sites (D_a) did not deviate significantly from neutrality. Therefore, although high levels of nonsynonymous divergence were detected, these loci behave in a neutral manner.

Neutrality tests

Although the neutrality equilibrium of loci was rejected in three cases for total sites (*COP1* from the northern region, Tajima's *D*; *COP1* from the southern region, Fay's *H*; and *CAL* from the northern region, Fay's *H*) and in two cases for noncoding sites (*COP1* from the northern region, *TFL1* from the northern region), none showed a consistently significant deviation from neutrality across the two tests (Table 2). Since the haplotype composition and haplotype relationships at these loci showed strong population subdivision within regions (Table 1, Fig. 2), these positive values may be caused by population structure. The HKA test did not reject the neutrality of the polymorphisms or divergence in the intra- and inter-specific analyses (north–south X^2 = 10.038, P = 0.852; north–*C. alpina* X^2 = 10.371, P = 0.870; north–*C. resedifolia* X^2 =9.327, P = 0.925; south–*C. alpina* X^2 = 7.769, P = 0.969; south–*C. resedifolia* X^2 = 7.824, P = 0.970). Therefore, neutral processes may have been important in shaping the genetic variations and geographic structures at these loci.

Genetic differentiation and geographic structure

Significant genetic differentiation between regions was detected for all loci (Table 3). The level of differentiation was variable among loci (F_{ST} = 0.097–0.874) and was mostly higher than for nuclear loci of *A. lyrata* (F_{ST} = -0.235–0.903; Wright, Lauga, and Charlesworth 2003), a species that is distributed widely in the northern hemisphere. This high level of genetic differentiation between regions is consistent with previous phylogeographic studies of other alpine plants (Fujii et al. 1997, 1999; Fujii and Senni 2006; Ikeda et al. 2006, 2008a, b).

STRUCTURE analysis also revealed genetic differentiation between the two regions. The most likely number of clusters for all individuals was two, which corresponded to the northern and southern regions (Fig. 3a, Supplemental Fig. 1). Although the ΔK method is unable to evaluate K=1 and is biased when K=2 (Evanno, Regnaut, and Goudet 2005), significant genetic differentiation was detected between the regions (Table 3). In addition, the genetic differentiation between the regions was conserved when analyzing K>2 (data not shown). Furthermore, as expected from the structured haplotype distributions and the plausible intrinsic genetic structures within regions, unambiguous geographic structures were detected within both the northern and southern regions (Fig. 3b, c, Supplementary Fig. 1). Each population comprised a single cluster, and strong population differentiation constrained to geographic locations of populations was detected. In particular, populations on distinct islands were clearly distinguished by the clusters (Fig. 3b) in the northern region, whereas populations in northern and southern parts of the southern region were clustered together (Fig. 3c).

Demographic history estimated using the IM model

The MLEs and the 90% highest probability density (HPD) of demographic parameters of the IM model are shown in Table 4, and the marginal distributions of the probabilities of the parameters are shown in Fig. 4. Independent runs in M-mode and L-mode gave consistent MLEs, indicating the

robustness of these values for the parameters (Table 4). Regional divergence took place without significant migration (m_1 = 0.005, m_2 = 0.005), and the isolation model (m_1=m_2=0) did not give a worse fit to the data (likelihood; log [P] = 6.493) than the IM model (likelihood; log [P] = 6.491).

The divergence time (t) with maximum likelihood was around 0.6, corresponding to 100,000–110,000 years ago. The effective population size was larger in the southern region than in the northern region (θ_1 = 0.393–0.402, θ_2 = 0.559–0.568), and both were higher than that of the ancestral population (θ_A = 0.310–0.365).

Discussion

Previous phylogeographic studies detected genetic differentiation between populations in central and northern Japan in *C. nipponica* as well as in other alpine plants, and inferred a history of isolation between the two regions during range shifts that occurred in the Pleistocene (Fujii et al. 1997, 1999; Fujii and Senni 2006; Ikeda and Setoguchi 2006, 2007, 2009; Ikeda et al. 2006, 2008a, b, c, 2009). Our multilocus analysis demonstrated the demographic history of *C. nipponica* and suggests that the genetic differentiation between the regions was consistent with the demographic change during the Pleistocene. The estimated divergence time between central and northern Japan was 100,000–110,000 years ago (90% HPD: ca. 38,000-3,500,000; Table 4), corresponding to the last interglacial period (Ono and Igarashi 1991). The polymorphisms at the analyzed 10 loci showed no migration between regions after they split, implying that the two regions remained isolated during the coldest part of the last glacial period (ca. 18,000 years ago; Ono and Igarashi 1991). The exclusive occurrences of closely related haplotypes within each region (Fig. 2) and the high level of genetic differentiation between the regions (Tables 1 and 3, Fig. 3a) indicate that genetic divergence occurred within each isolated region after the split. Therefore, the previously detected genetic

differentiation (Ikeda et al. 2008c) was shaped after the last interglacial period, suggesting a Pleistocene origin of the genetic differentiation.

Although migration may have occurred between the two regions during the last glacial period, the lack of migration in our multilocus analysis would be explained by geographic separation. The floristic distribution in the Japanese archipelago during the coldest part of the last glacial period (Ono and Igarashi 1991) is consistent with this hypothesis of geographic separation. During the coldest period, central and northern Japan were mostly covered by coniferous forest, and the alpine flora was scattered among the high mountains from central to northern Japan. Therefore, the extent of the alpine flora was not continuous throughout the Japanese archipelago, resulting in isolation between central and northern Japan. This would be the case throughout the Pleistocene because the climate was coldest during the last glacial period (Ono and Igarashi 1991). Added to this geographic separation, it may have been difficult for migrating individuals to establish in another region and leave evidence of the migration due to local adaptation and/or genetic drift.

In particular, demographic changes following postglacial global warming would have removed the evidence of migration. Although the present population size within each region is not smaller than the ancestral population (Table 4), each regional population originated from highly isolated small populations. Previous studies have suggested that current populations of alpine plants were shaped by population contraction during the postglacial global warming, and that genetic structures within populations were highly influenced by founder effects due to reduced population sizes (Ikeda et al. 2008b; Ikeda and Setoguchi 2009). Furthermore, the fact that current populations of *C. nipponica* are restricted to areas near the tops of high mountains, together with our finding that all populations harbored a specific haplotype composition across loci (Table 1), is consistent with expectations that founder effects followed population reduction during the postglacial period. Moreover, as shown in previous studies, the within-population genetic diversity might have been removed by bottlenecks

within populations during the postglacial period (Ikeda et al. 2008c), which could remove any evidence of occasional migration from each population. Although the estimated population sizes did not support the bottleneck hypothesis, this discrepancy could be attributed to our analysis method, which pooled all populations within each region that possessed a given population structure. Pooling populations with population structure might result in higher genetic diversity in each region than in each population within the region (Hammer et al. 2003; Moeller, Tenaillon and Tiffin 2007). Therefore, the population sizes of each population were not precisely estimated in this study, resulting in the lack of evidence for population bottlenecks.

As shown in previous phylogeographic studies (Petit et al. 2003; Schönswetter et al. 2005), range shifts during the Pleistocene occurred equally across species. This indicates that species with similar phylogeographic structures would share a similar population history. Since most alpine plants in the Japanese archipelago, including *C. nipponica*, consistently exhibited clear genetic differentiation between central and northern Japan (Fujii et al. 1997, 1999; Fujii and Senni 2006; Ikeda and Setoguchi 2007; Ikeda et al. 2006, 2008a, b, c, 2009), our findings regarding the population history of *C. nipponica* could also explain the demographic histories of other alpine plants in Japan. Accordingly, geographic separation among mountains strongly restricted the dispersal of alpine plants in the Japanese archipelago throughout the Pleistocene, resulting in occasional migrations between disjunct mountains that would have established the current range of alpine flora. In addition, isolation on disjunct mountains caused genetic divergence, accelerating the genetic differentiation between populations in central and northern Japan. Since several alpine plants have different subspecies or varieties in central and northern Japan (e.g., Fujii et al. 2001), genetic differentiation following isolation during the Pleistocene may have caused morphological differentiation. Further model-based approaches to the demographic history of populations could

evaluate the impact of range shifts during the Pleistocene on the evolutionary history of the alpine flora of Japan.

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Literature Cited

Alsos IG, Eidesen PB, Ehrich D, Skrede I, Westergaard K, Jacobsen GH, Landvik JY, Taberlet P, Brochmann C. 2007. Frequent long-distance plant colonization in the changing Arctic. Science 316: 1606-1609.

Avise JC. 2000. Phylogeography: the History and Formation of Species. Massachusetts : Harvard University Press.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657-1659.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 9: 2611-2620.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567-1587.

Fay JC, Wu CI. 2000. Hitchhiking under positive Darwinian selection. Genetics 155: 1405-1413.

Fujii N, Senni K. 2006. Phylogeography of Japanese alpine plants: biogeographic importance of alpine region of central Honshu in Japan. Taxon 55: 43-52.

Fujii N, Ueda K, Watano Y, Shimizu T. 1997. Intraspecific sequence variation of chloroplast DNA in *Pedicularis chamissonis* Steven (Scrophulariaceae) and geographic structuring of the Japanese "Alpine" plants. J. Plant. Res. 110: 195-207.

Fujii N, Ueda K, Watano Y, Shimizu T. 1999. Further analysis of intraspecific sequence variation of chloroplast DNA in *Primula cuneifolia* Ledeb. (Primulaceae): Implication for biogeography of the Japanese alpine flora. J. Plant Res. 112: 87-95.

Fujii N, Ueda K, Watano Y, Shimizu T. 2001. Two genotypes of *Pedicularis chamissonis* (Scrophulariaceae) distributed at Mt. Gassan, Japan: additional genetic and morphological studies. J. Plant Res. 114: 133-140.

Hammer MF, Blackmer F, Garrigan D, Naghman MW, Wilder JA. 2003. Human population structure and its effects on sampling Y chromosome sequence variation. Genetics 164: 1495-1509.

Hewitt GM. 1996. Some genetic consequences of ice age, and their role in divergence and speciation. Biol. J. Linn. Soc. 58: 247-276.

Hewitt GM. 2000. The genetic legacy of the Quaternary ice ages. Nature 405: 907-913.

Hey J, Wakeley J. 1997. A coalescent estimator of the population recombination rate. Genetics 155: 2011-2014.

Hey J, Machado CA. 2003. The study of structured populations – new hope for a difficult and divided science. Nat. Rev. Genet. 4: 535-543.

Hey J, Nielsen R. 2004 Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila psudoobscura* and *D. persimilis*. Genetics 167: 747-760.

Hey J, Nielsen R. 2007. Integration within the Felsenstein equation for improvaed Markov chain Monte Carlo methods in population genetics. Proc. Natl. Acad. Sci. USA 104: 2785-2790.

Hudson RR, Kaplan NL. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. Genetics 111: 147-164.

Hudson RR, Kreitman M, Aguadé M. 1987. A test of neutral molecular evolution based on nucleotide data. Genetics 116: 153-159.

Hudson RR, Slatkin M, Maddison WP. 1992a. Estimation of levels of gene flow from DNA sequence data. Genetics 132: 583-589.

Hudson RR. 2000. A new statistic for detecting genetic differentiation. Genetics 155: 2011-2014.

Ikeda H, Setoguchi H. 2006. Phylogeography of *Arcterica nana* (Maxim.) Makino (Ericaceae) suggests another range expansion history of Japanese alpine plants. J. Plant. Res. 119: 489-495.

Ikeda H, Setoguchi H. 2007. Phylogeography and refugia of the Japanese endemic alpine plant *Phyllodoce nipponica* Makino (Ericaceae). Journal of Biogeography 34: 169-176.

Ikeda H, Setoguchi H. 2009. The homogenous genetic structure and inferred unique history of range shifts during the Pleistocene climatic oscillations of Arcterica nana (Maxim.) Makino (Ericaceae).

J. Plant. Res. in press.

Ikeda H, Senni K, Fujii N, Setoguchi H. 2006 Refugia of *Potentilla matsumurae* (Rosaceae) located at high mountains in the Japanese archipelago. Mol. Ecol. 15: 3731-3740.

Ikeda H, Senni K, Fujii N, Setoguchi H. 2008a. Post-glacial range fragmentation is responsible for the current distribution of *Potentilla matsumurae* Th. Wolf (Rosaceae) in the Japanese archipelago. J. Biogeogr. 35: 791-800.

Ikeda H, Senni K, Fujii N, Setoguchi H. 2008b. Survival and genetic divergence of an arctic-alpine plant, *Diapensia lapponica* subsp. *obovata* (Fr. Schm.) Hultén (Diapensiaceae), in the high mountains of central Japan during climatic oscillations. Pl. Syst. Evol.: in press.

Ikeda H, Senni K, Fujii N, Setoguchi H. 2008c. Consistent geographic structure among multiple nuclear sequences and cpDNA polymorphisms of *Cardamine nipponica* Franch. et Savat. (Brassicaceae). Mol. Ecol: 17: 3178-3188.

Ikeda H, Senni K, Fujii N, Setoguchi H. 2009. High mountains of the Japanese archipelago as refugia for arctic-alpine plants: phylogeography of *Loiseleuria procumbens* (L.) Desvaux (Ericaceae). Biol. J. Linn. Soc. in press.

Kitakawa M. 1999. Brassicaceae 8. *Cardamine.* In: Satake Y, Ohwi J, Kitamura S, Watari S, Tominari T, editors. Wild Flowers of Japan I, Tokyo: Heibonsha. p. 130-132.

Knowles LL, Maddison WP. 2002. Statistical phylogeography. Mol. Ecol. 11: 2623-2635.

Koch MA, Haubold B, Mitchell-Olds T. 2000. Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). Mol. Biol. Evol. 17: 1483-1498.

Koch MA, Haubold B, Mitchell-Olds T. 2001. Molecular systematic of the Brassicaceae: evidence fromo coding plastidic matK and nuclear Chs sequences. Ame. J. Bot. 88: 534-544.

Koidzumi G. 1919. Genetic and floristic phytogeography of the alpine flora of Japan. Bot. Mag. 33: 193-222.

Kosakovsky Pond SL, Frost SDW, Muse SV. 2005. HyPhy: hypothesis testing using phylogenies. Bioinformatics. 21: 676-679.

Kučera J, Valko I, Marhold K. 2005. Online database of the chromosome numbers of the genus *Cardamine* (Brassicaceae). Biologia (Bratislava) 60: 473-476 (database available on-line at http://147.213.100.117/cardamine/index.php/).

Kuittinen H, Aguadé M, Charlesworth D, et al. 2002 Primers for 22 candidate genes for ecological adaptations in Brassicaceae. Mol. Ecol. Notes 2: 258-262.

Moeller DA, Tenaillon MI, Tiffin P. 2007. Population structure and its effects on patterns of nucleotide polymorphism in teosinte (*Zea mays* ssp. *parviglumis*). Genetics 176: 1799-1809.

Nei M. 1987. Molecular Evolutionary Genetics. New York : Columbia University Press.

Nielsen R, Wakeley J. 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158: 885-896.

Ono Y, Igarashi Y. 1991. Natural History of Hokkaido. Sapporo: Hokkaido University Press.

Petit RJ, Aguinagalde I, Beaulieu JL, et al. 2003. Glacial refugia: Hotspots but not melting pots of genetic diversity. Science 300: 1563-1565.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus

genotype data. Genetics 155: 945-959.

Ramos-Onsins SE, Stranger BE, Mitchell-Olds T, Aguade M. 2004. Multilocus analysis of variation and speciation in the closely related species *Arabidopsis halleri* and *A. lyrata*. Genetics 166: 373-388.

Ramos-Onsins SE, Pumerma E, Balañá-alcaide D, Salguero D, Aguadé M. 2008. Multilocus analysis of variation using a large empirical data set: phenylpropanoid pathway genes in *Arabidopsis thaliana*. Mol. Ecol. 17: 1211-1223.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphisms analyses by the coalescent and other methods. Bioinformatics 19: 2496-2497.

Schönswetter P, Stehlik I, Holderegger R, Tribsch A. 2005. Molecular evidence for glacial refugia of mountain plants in the Europe Alps. Mol. Ecol. 14: 3547-3555.

Soltis DE, Gitzendanner MA, Strenge DD, Soltis PS. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. Pl. Syst. Evol. 206: 353-373.

Taberlet P, Fumagalli L, Wust-saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7: 453-464.

Tajima F. 1989. The effect of change in population size on DNA polymorphism. Genetics 123: 597-601.

Toyokuni H. 1981. A preliminary note on the floristic phytogeography of the alpine flora of Japan. J. Fac. Lib. Arts. Shinsyu. Univ. Nat. Sci. 15: 81-96.

Wright S, Lauga B, Charlesworth D. 2003. Subdivision and haplotype structure in natural populations of *Arabidopsis lyrata*. Mol. Ecol. 12: 1247-1263.

Table 1. Haplotype compositions of each population. Letters represent the haplotypes of individuals at each locus, and slashes (/) separate two analyzed individuals.

No.	Location	Coordination			Locus									
					COP1	*DET1*	*GA1*	*TFL1*	*CAL*	*F3H*	*DER*	*CHS*	*PHYA*	*PHYE*
Northern Japan														
1	Kurodake	43°42′N	/	141°55′E	AA/AA	AA/BB	AA/AA	AA/AA	AA/AA	AA/AA	AA/AA	AA/AA	AA/AA	AA/AA
2	Koizumidake	43°40′N	/	141°55′E	AA/AA	AA/BB	AA/AA	AA/AA	AA/AA	AA/AA	AA/FF	AA/AA	AA/GG	AA/AA
3	Shokanbetsudake	43°43′N	/	141°31′E	AA/AA	HH/HH	JJ/JJ	AA/AA	BB/BB	AA/AA	AA/AA	AA/AA	CC/CC	AA/AA
4	Ashibetsudake	43°14′N	/	142°17′E	AA/AA	CC/CC	JJ/JJ	AA/AA	BB/BB	AA/AA	AA/AA	AA/AA	CC/CC	AA/AA
5	Iidesan	37°51′N	/	139°41′E	DD/DD	CC/CC	BB/BB	EE/EE	BB/BB	AA/AA	AA/AA	CC/CC	CC/CC	DD/DD
6	Gassan	38°33′N	/	140°01′E	DD/DD	CC/CC	KK/KK	HH/HH	GG/GG	CC/CC	AA/AA	GG/GG	CC/CC	DD/DD
7	Azumayama	37°45′N	/	140°09′E	DD/DD	CC/CC	II/II	GG/GG	FF/FF	AA/AA	AA/AA	FF/FF	CC/CC	DD/DD
Central Japan														
8	Hakusan	36°09′N	/	136°46′E	CC/CC	DD/DD	EE/EE	DD/DD	CC/CC	AA/AA	AA/AA	AA/AA	CC/CC	CC/CC
9	Tateyama	36°34′N	/	137°37′E	BB/BB	GG/GG	DD/DD	DD/DD	DD/DD	AA/AA	EE/EE	DD/DD	CC/CC	BE/EE
10	Shiroumadake	36°45′N	/	137°45′E	BB/BB	DD/DD	HH/HH	FF/FF	DD/EE	AA/AB	AE/EE	AA/AA	CC/CE	BB/EE
11	Jyounendake	36°19′N	/	137°43′E	BB/BB	EE/EE	DD/DD	DD/DD	CC/CC	AA/AA	EE/EE	EE/EE	CC/CC	FF/FF
12	Ontakesan	36°53′N	/	137°29′E	CC/CC	EE/EE	GG/GG	DD/DD	CC/DD	AA/AA	DD/DD	DD/DD	CC/CC	EE/EE
13	Norikuradake	36°06′N	/	137°33′E	BB/BB	EE/EE	DD/DD	DD/DD	DD/DD	AA/AA	EE/EE	AA/AA	CC/CC	EE/EE
14	Yatsugatake	35°59′N	/	138°22′E	CC/CC	EE/EE	DD/DD	DD/DD	DD/DD	AA/AA	EE/EE	AA/AA	DD/DD	EE/EE
15	Kisokomagatake	35°47′N	/	137°48′E	BB/BB	DD/FF	DD/DD	DD/DD	BB/BB	AA/AA	CC/CC	AA/AA	CC/CC	BB/BB
16	Utsugidake	35°43′N	/	137°49′E	BB/BB	DD/DD	DD/DD	DD/DD	BB/BB	AA/AA	CC/CC	BB/BB	CC/CC	BB/BB
17	Kitadake	35°40′N	/	138°14′E	BB/BB	CC/CC	DD/FF	CC/DD	BB/BB	AA/AA	BB/BB	AA/AA	BB/CC	BB/BB
18	Senjyoudake	35°43′N	/	138°11′E	BB/BB	CC/CC	CC/FF	BB/BB	BB/BB	AA/AA	AA/AA	AA/AA	CC/CC	BB/BB
19	Akaishidake	35°27′N	/	138°09′E	BB/BB	CC/CC	CC/CC	CC/CC	BB/BB	AA/AA	AA/AA	AA/AA	FF/FF	BB/BB

Table 2. Summary of polymorphisms within regions and neutrality tests. The parameters shown are sequence length (bp), the number of sequences analyzed (N), the number of polymorphisms specific to each region (S_x), nucleotide diversity of all sites (π_{Total}) and that of noncoding regions (π_{nc}) and synonymous (π_s) and nonsynonymous sites (π_a), and Tajima's D for all sites (D_{Total}), noncoding regions (D_{nc}), synonymous (D_s), and nonsynonymous sites (D_a), and Fay's H (H) tests. Significance is indicated by asterisks (*P<0.05, **P<0.01).

Locus	bp	Region	N	S_x	π_{Total}	π_{nc}	π_s	π_a	π_a/π_s	D		D_{nc}		D_s	D_a	H	
COP1	529	North	28	2	0.00192	0.00409	0.00000	0.00000	0.0000	2.043	*	2.043	*	NA	NA	-0.0423	
		South	48	1	0.00072	0.00154	0.00000	0.00000	0.0000	0.950		0.950		NA	NA	0.2553	**
DET1	577	North	28	3	0.00176	0.00000	0.00386	0.00306	0.7920	0.763		NA		-0.019	1.009	0.5503	
		South	48	4	0.00186	0.00232	0.00000	0.00182	NA	0.440		-0.171		NA	1.378	-0.3192	
GA1	1225	North	28	6	0.00180	0.00185	0.00332	0.00134	0.4050	1.241		1.699		-0.024	1.009	0.8466	
		South	48	8	0.00107	0.00169	0.00102	0.00055	0.5400	-0.743		-0.606		-0.418	-0.554	1.1844	
TFL1	1009	North	28	5	0.00201	0.00367	0.00000	0.00065	NA	1.615		1.909	*	NA	-0.019	0.5079	
(*LEY*)		South	48	3	0.00064	0.00064	0.00000	0.00086	NA	-0.535		-0.554		NA	0.672	-0.6188	
CAL	791	North	28	5	0.00372	0.00426	0.01270	0.00000	0.0000	1.432		1.262		1.032	NA	1.2275	*
		South	48	3	0.00166	0.00243	0.00000	0.00000	0.0000	0.606		0.606		NA	NA	0.5532	
F3H	770	North	28	0	0.00048	0.00000	0.00000	0.00074	NA	-0.019		NA		NA	-0.019	0.2116	
		South	48	1	0.00008	0.00000	0.00039	0.00000	0.0000	-1.107		NA		-1.107	NA	0.0408	
DFR	526	North	28	1	0.00026	0.00078	0.00000	0.00000	0.0000	-0.741		-0.741		NA	NA	0.1270	
		South	48	4	0.00212	0.00249	0.00000	0.00248	NA	0.519		-0.044		NA	0.860	0.5514	
CHS	534	North	28	3	0.00203	0.00438	0.00000	0.00087	NA	1.290		1.699		NA	-0.019	0.5926	
		South	48	3	0.00102	0.00280	0.00000	0.00000	0.0000	-0.248		-0.248		NA	NA	0.5106	
PHYA	522	North	28	2	0.00062	0.00177	0.00103	0.00000	0.0000	-0.111		0.572		-0.741	NA	0.3810	
		South	48	4	0.00074	0.00217	0.00117	0.00000	0.0000	-0.805		-0.765		-0.418	NA	0.5301	
PHYE	585	North	28	1	0.00066	0.00000	0.00340	0.00000	0.0000	1.557		NA		1.557	NA	-0.1693	
		South	48	3	0.00107	0.00000	0.00446	0.00030	0.0067	0.450		NA		0.860	-0.418	0.2394	
Average	707	North	28		0.00153	0.00208	0.00243	0.00067	0.1710								
		South	48		0.00110	0.00161	0.00070	0.00060	0.0781								

Table 3. Summary of genetic divergences and differentiations. The parameters shown are the total number of polymorphisms (S) and polymorphisms fixed between regions (S_f), genetic divergence between northern and southern regions at all sites (K_{Total}), and that in noncoding regions (K_{nc}), and synonymous (K_s) and nonsynonymous sites (K_a), and population differentiation between northern and southern regions (F_{ST}) and their significance (S_{nn}). Significance is indicated by asterisks (*P<0.05, **P<0.01).

Locus	Pair	S	S_f	K_{Total}	K_{nc}	K_s	K_a	K_a/K_s	F_{ST}	S_{nn}
COP1	North region vs. South region	4	1	0.00427	0.00909	0.00000	0.00000	0.0000	0.689	**
	C. nipponica vs. *C. alpina*			0.00472	0.00601	0.01592	0.00000	0.0000		
	C. nipponica vs. *C. resedifolia*			0.00409	0.00466	0.01592	0.00000	0.0000		
DET1	North region vs. South region	7	0	0.00334	0.00334	0.00217	0.00363	1.6740	0.456	**
	C. nipponica vs. *C. alpina*			0.00701	0.00595	0.00080	0.00978	12.2250		
	C. nipponica vs. *C. resedifolia*			0.00643	0.00595	0.00080	0.00843	10.5375		
GA1	North region vs. South region	16	2	0.00421	0.00462	0.00241	0.00433	1.7980	0.658	**
	C. nipponica vs. *C. alpina*			0.01122	0.01656	0.01422	0.00575	0.4044		
	C. nipponica vs. *C. resedifolia*			0.01232	0.02063	0.00760	0.00634	0.8342		
TFL1	North region vs. South region	8	0	0.00265	0.00358	0.00000	0.00238	NA	0.498	**
	C. nipponica vs. *C. alpina*			0.00654	0.00693	0.00000	0.00568	NA		
	C. nipponica vs. *C. resedifolia*			0.01458	0.02231	0.00794	0.00482	0.6071		
CAL	North region vs. South region	8	0	0.00356	0.00442	0.00857	0.00000	0.0000	0.246	**
	C. nipponica vs. *C. alpina*			0.00542	0.00766	0.00316	0.00000	0.0000		
	C. nipponica vs. *C. resedifolia*			0.01377	0.01996	0.00316	0.00000	0.0000		
F3H	North region vs. South region	2	0	0.00031	0.00000	0.00020	0.00042	2.1130	0.097	*
	C. nipponica vs. *C. alpina*			0.00200	0.01215	0.00012	0.00015	1.2500		
	C. nipponica vs. *C. resedifolia*			0.00356	0.00403	0.01441	0.00015	0.0104		
DFR	North region vs. South region	5	0	0.00170	0.00182	0.00000	0.00210	NA	0.297	**
	C. nipponica vs. *C. alpina*			0.01069	0.01821	0.02752	0.00133	0.0483		
	C. nipponica vs. *C. resedifolia*			0.00939	0.01628	0.02286	0.00133	0.0582		
CHS	North region vs. South region	6	0	0.00204	0.00493	0.00000	0.00049	NA	0.252	**
	C. nipponica vs. *C. alpina*			0.01182	0.01809	0.03773	0.00018	0.0048		
	C. nipponica vs. *C. resedifolia*			0.01124	0.02615	0.01237	0.00018	0.0146		
PHYA	North region vs. South region	6	0	0.00076	0.00224	0.00116	0.00000	0.0000	0.105	**

	C. nipponica vs. *C. alpina*			0.00588	0.01132	0.01067	0.00219	0.2052		
	C. nipponica vs. *C. resedifolia*			0.00631	0.00961	0.01066	0.00365	0.3424		
PHYE	North region vs. South region	8	4	0.00686	0.00000	0.01457	0.00590	0.4030	0.874	**
	C. nipponica vs. *C. alpina*			0.00702	0.01038	0.01505	0.00414	0.2751		
	C. nipponica vs. *C. resedifolia*			0.00603	0.00344	0.01445	0.00414	0.2865		
Average	North region vs. South region			0.00297	0.00340	0.00291	0.00193	0.8554	0.417	
	C. nipponica vs. *C. alpina*			0.00723	0.01133	0.01252	0.00292	1.60142		
	C. nipponica vs. *C. resedifolia*			0.00877	0.01330	0.01102	0.00290	1.26908		

Table 4. Maximum likelihood estimates (MLEs) and the 90% highest posterior density (HPD) intervals of demographic parameters. θ_1, θ_2, and θ_A represent the effective diversity in the northern region, the southern region, and the ancestral population, respectively; m_1 and m_2 represent the migration rate from the north to the south and from the south to the north, respectively; and t and T indicate the time of divergence and the scaled divergence time, respectively. The results of L-mode and three independent M-mode runs are shown.

	θ1	θ_2	θ_A	m_1	m_2	t		T (ca. year)
L-mode								
MLE	0.3932	0.5682	0.3469	0.005	0.005	0.59		106,000
HPD90Lo	0.2267	0.3649	0.0046	0.005	0.005	0.21	*	38,000
HPD90Hi	0.6707	0.8453	7.5072	1.325	0.285	19.91	*	3,565,000
M-mode1								
MLE	0.3839	0.5589	0.3562	0.005	0.005	0.59		106,000
HPD90Lo	0.2267	0.3649	0.0046	0.005	0.005	0.21	*	38,000
HPD90Hi	0.6614	0.8361	7.6552	1.355	0.285	19.99	*	3,579,000
M-mode2								
MLE	0.3932	0.5682	0.3099	0.005	0.005	0.61		109,000
HPD90Lo	0.2267	0.3649	0.0046	0.005	0.005	0.21	*	38,000
HPD90Hi	0.6707	0.8453	7.424	1.315	0.275	19.99	*	3,579,000
M-mode3								
MLE	0.4024	0.5682	0.3654	0.005	0.005	0.59		106,000
HPD90Lo	0.2267	0.3649	0.0046	0.005	0.005	0.21	*	38,000
HPD90Hi	0.6707	0.8453	7.424	1.315	0.285	19.99	*	3,579,000

Legends for figures

Figure 1. (a) Location of the Japanese archipelago. (b) Locations of sampling sites in the Japanese archipelago. Crosses and black circles represent the northern and southern regions, respectively.

Figure 2. Parsimony networks of haplotypes of *Cardamine nipponica* from ten nuclear loci. Gray and white circles represent haplotypes from the northern and southern regions, respectively. Haplotypes found in both regions are shown in halftone. Single haplotypes of *C. alpina* (*C.a.*) and *C. resedifolia* (*C. r.*) are included.

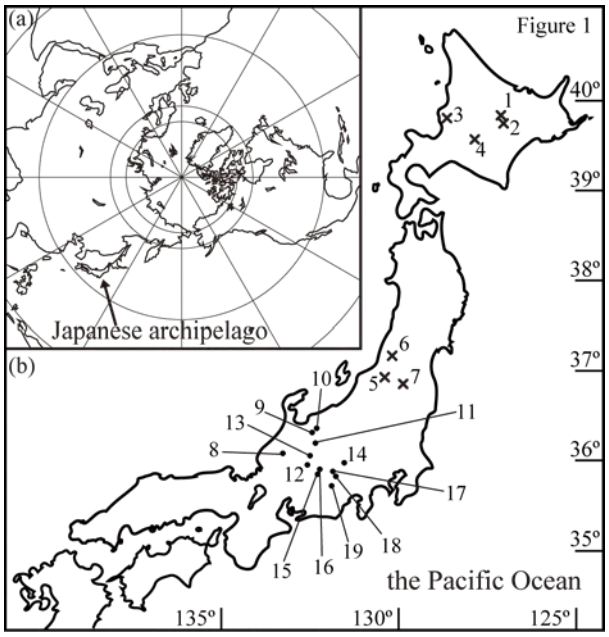
Figure 3. Individual assignment to the clusters with the highest likelihood (K=2) across (a) all populations and populations within (b) the northern region and (c) the southern region by STRUCTURE analysis. Bold lines distinguish populations, and gray and white sections within each population represent the probability of assigning the individuals to each cluster. The number below the bar indicates the population number.

Figure 4. Marginal distribution of the posterior probability of six demographic parameters estimated by the IM model.

Supplementary Fig. 1

The distribution of likelihood values (K=1-15; a, c, e) and model parameters (ΔK; b, d, f) estimated following Evanno *et al*. (2005). (a, b) all populations, (c, d) populations within the northern region, and (e, f) populations within the southern region.

Figure 1


(a)
Figure 1
Japanese archipelago
(b)
40º
39º
38º
37º
36º
35º
135º
130º
125º
the Pacific Ocean
1
2
3
4
5
6
7
8
9
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19

Figure 2

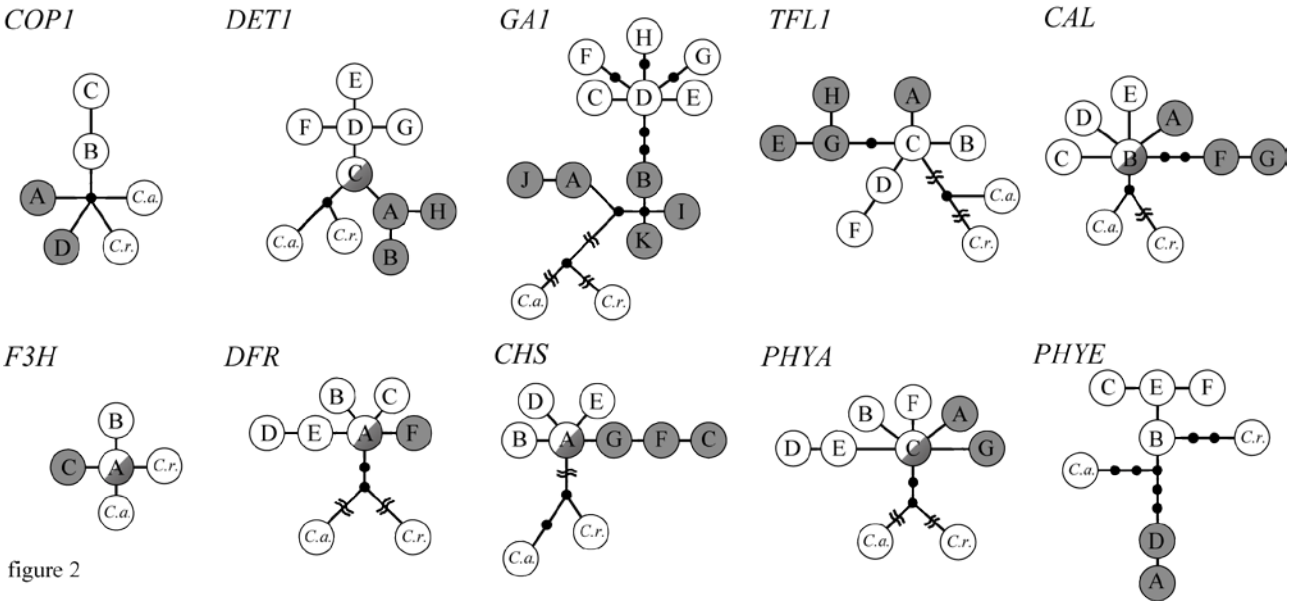


figure 2

Figure 3

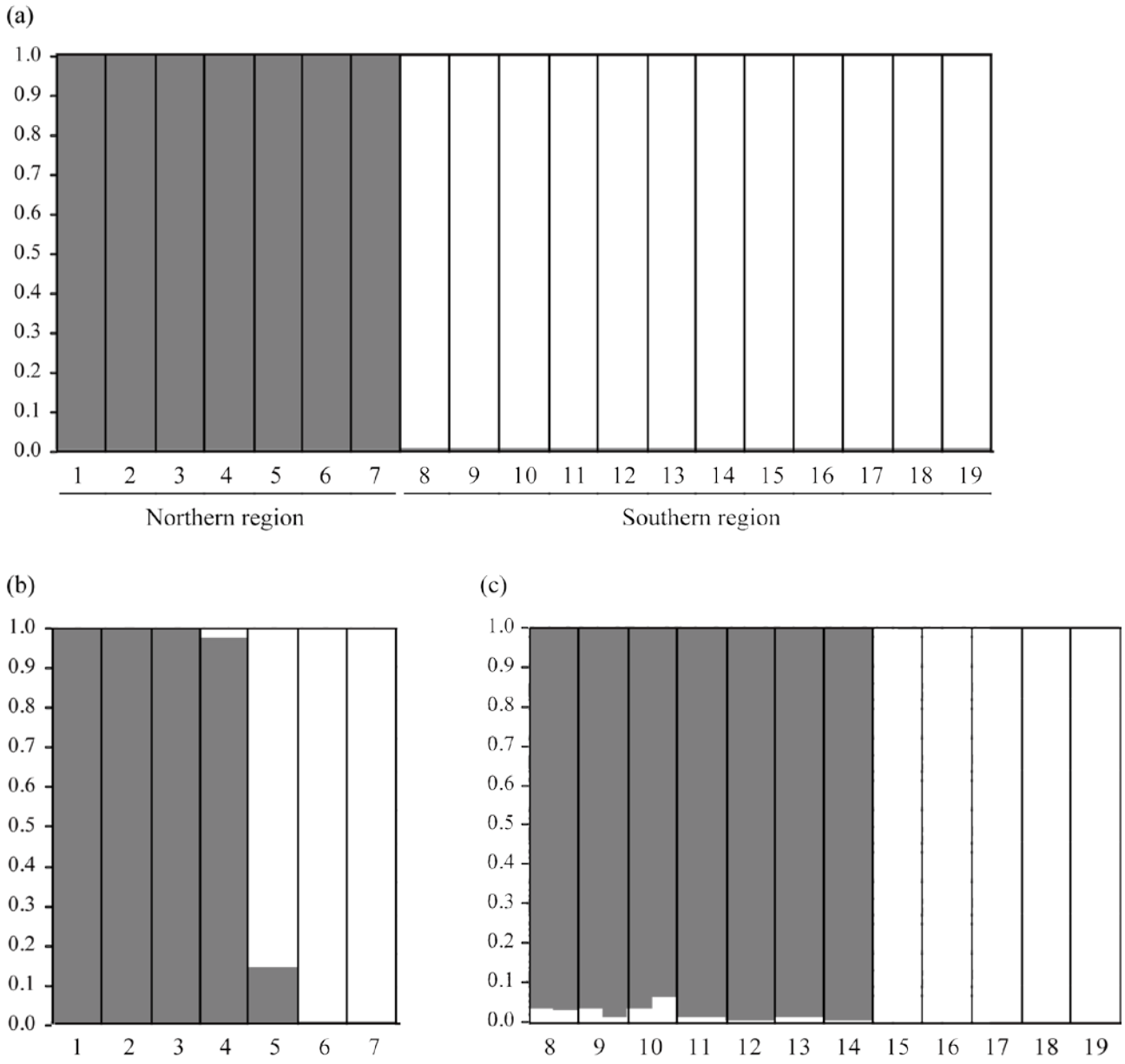
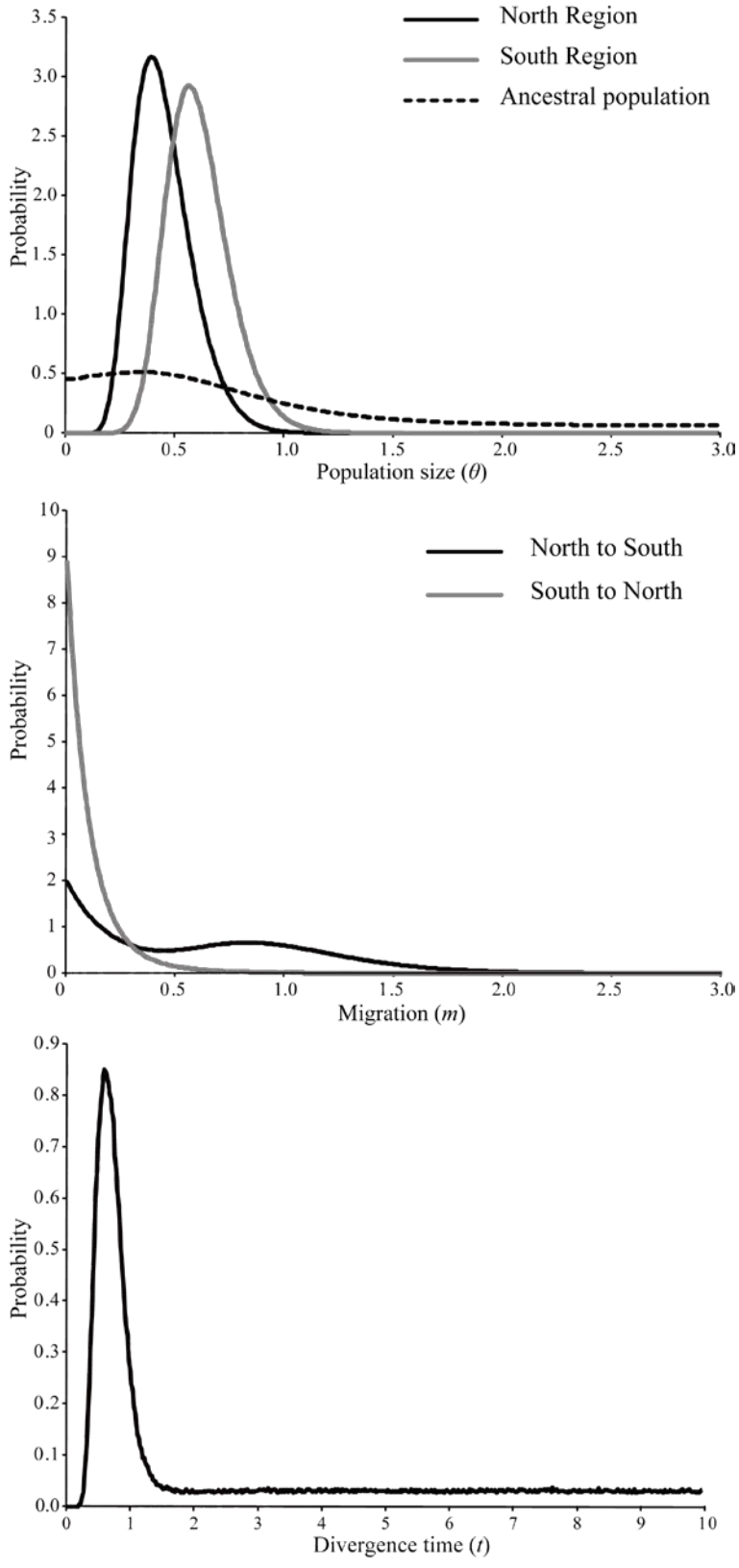

(a)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Northern region
Southern region
(b)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1 2 3 4 5 6 7
(c)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
8 9 10 11 12 13 14 15 16 17 18 19

Figure 4


North Region
South Region
Ancestral population
Probability
Population size (θ)
North to South
South to North
Probability
Migration (m)
Probability
Divergence time (t)