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Comparison of genetic and phenotypic variations in disjunct populations of highly polyploid *Betula davurica* in the Japanese Archipelago with continental populations in a region of northeast Asia

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Abstract

Betula davurica Pallas is widely distributed in continental regions of northeast Asia but is disjunctly distributed in the Japanese Archipelago. To examine the lower diversity and higher differentiation in genetic and phenotypic variations expected in these disjunct populations compared to the continental populations, genome-wide single-nucleotide polymorphism (SNP) genotypes, chloroplast (cp) DNA sequences, and leaf morphology were investigated in seven disjunct populations in Hokkaido and Honshu in comparison to three populations in Primorye, where *B. davurica* is continuously distributed. SNP genotypes of putative octaploid individuals indicated that genetic diversity was higher in Honshu ($0.158 \leq H_E \leq 0.176$) than in Hokkaido and Primorye ($0.145 \leq H_E \leq 0.149$). Genetic differentiation between Honshu and the other regions ($0.011 \leq F_{ST} \leq 0.035$) was larger than that between Hokkaido and Primorye ($0.002 \leq F_{ST} \leq 0.009$) and within regions ($0.001 \leq F_{ST} < 0.007$). CpDNA (*trn*L–*trn*F) sequences in Honshu were different from those in Hokkaido and Primorye. Variations in leaf shape and size overlapped among populations in the three regions. These results from *B. davurica* in the Japanese Archipelago are not consistently congruous with the general trends expected in disjunct populations.

KEYWORDS

chloroplast DNA sequences, disjunct distribution, double-digest restriction-site associated DNA (ddRAD), leaf morphology, polyploidy

1 | INTRODUCTION

Disjunct distributions are characterized by discontinuous distributional ranges separated by geographic gaps and barriers that prevent dispersal and gene flow (Thorne, 1972). Disjunct distributions of plants occur not only in intercontinental scales (Villaverde et al., 2017; Wen et al., 2016) but also in regional scales, for example, among islands along the edge of a continent (Rinaldi et al., 2019). In these regional scales, the formation of sea

straits and land bridges as well as the alteration of suitable habitat ranges under climate change in the Quaternary are likely to result in disjunct distributions (Comes & Kadereit, 1998; Hewitt, 2004). Sea strait formation creates a barrier, and local extinction makes a gap, between disjunctly distributed ranges. Migration through past land bridges and colonization of islands by long-distance dispersal lead to disjunct distributions.

The Japanese Archipelago along the eastern edge of the Eurasian continent harbors disjunctly distributed plants (Ohsawa & Ide, 2011). Plant populations disjunctly distributed on different islands of the Japanese Archipelago show various phylogeographic patterns reflecting their unique history. First, boreal plants from arctic continental regions colonized Hokkaido and the central mountains of Honshu through the Kuril and Sakhalin Islands (Fujii & Senni, 2006). Multiple colonization events in glacial periods and subsequent isolation at alpine zones in post-glacial periods created a genetic divergence between central Honshu and Hokkaido plus northern Honshu (Ikeda, 2022). This phylogeographic pattern has been found in arctic-alpine plants, for example, *Cardamine nipponica* (Ikeda et al., 2012) and *Salix arbutifolia* (Nagamitsu, Hoshikawa, et al., 2014). Second, temperate plants migrated between continental regions in East Asia and the Japanese Archipelago through the Korean Peninsula and a land bridge across the East China Sea (Jin et al., 2016). *Viola orientalis*, which is disjunctly distributed in temperate grasslands, originated in continental regions of northeast Asia and colonized the Japanese Archipelago through the Korean Peninsula (Sata et al., 2021). Third, cool-temperate plants from continental regions in northeast Asia colonized both northern and southern parts of the Japanese Archipelago through the Sakhalin Island and the Korean Peninsula, respectively. In *Picea jezoensis* (Aizawa et al., 2007, 2009) and *Lychnis wilfordii* (Tamura et al., 2019), disjunct populations in Hokkaido and Honshu are genetically divergent probably due to independent colonization through the north and south migration routes.

Betula davurica Pallas is a canopy tree species found in cool-temperate deciduous broad-leaved forests in northeast Asia (Krestov et al., 2006; Okitsu, 2009). This species is widely and continuously distributed throughout the Russian Far East (Primorye), eastern Mongolia, northeastern China, and the Korean Peninsula in the Eurasian continent (Li & Skovortsov, 1999), but is found only in central Honshu and eastern Hokkaido in the Japanese Archipelago (Figure 1), exhibiting clear disjunct distributions (Shitara et al., 2018). Disjunct populations in central Honshu are reduced and restricted due to the specific preference for regeneration habitats of this species (Otsu et al., 2023). Species distribution modeling revealed alteration of potential habitats after the last glacial period. In the last glacial maximum, the potential habitats were continuously distributed around eastern China, the terrestrialized shelf of the East China Sea, the Korean Peninsula, and the Japanese Archipelago. In the mid-Holocene, these potential habitats retreated from eastern China and southwestern Japan but remained in the Korean Peninsula and central Honshu mountains, whereas additional potential habitats expanded to continental regions in northeastern China, eastern Mongolia, and Primorye and occurred in the Sakhalin and Hokkaido Islands (Shitara et al., 2018).

Generally, marginal disjunct populations tend to have lower diversity and higher differentiation in genetic variations than central continuously-distributed populations although species-specific biogeographic history can affect these trends (Eckert et al., 2008; Hamilton & Eckert, 2007; Meeus et al., 2012). Phenotypic variations also tend to be divergent in disjunct populations due to heterogeneous environments at the range margins (Aguilar et al., 2020; Jones et al., 2013). In *C. nipponica*, divergent selection between the southern (central Honshu) and northern (Hokkaido and northern Honshu) populations results in amino acid replacement in a light receptor protein, which is involved in phenological traits of flowering or seed germination (Ikeda et al., 2009; Ikeda & Setoguchi, 2010). In *Betula ermanii* in the Japanese Archipelago, isolated populations in the southern boundary show different ploidy and morphology of leaves and seeds (Aihara et al., 2024). These general trends in genetic and phenotypic variations may be applied to disjunct populations of *B. davurica* in the Japanese Archipelago. Thus, we sampled multiple local populations from each of the disjunctly distributed regions in Hokkaido and Honshu as well as those from a continuously distributed region in Primorye using a similar sampling design.

Genetic analysis of *B. davurica* is difficult due to the high ploidy levels of this species. Both hexaploids and octaploids were found in each of the continental and insular *B. davurica* specimens (Wang et al., 2016, 2021). Recent advances in sequencing and analysis enable us to genotype polyploids at high ploidy levels (Gerard et al., 2018; Zohren et al., 2016). Thus, we inferred ploidy using allele read counts obtained from double-digest restriction-site associated DNA (ddRAD) sequencing and confirmed the ploidy using flow cytometry. Then, we assigned genome-wide single-nucleotide polymorphism (SNP) genotypes to individuals with the same ploidy level. In addition to the SNP genotypes, we examined chloroplast (cp) DNA sequences and leaf morphology. Because leaf morphology in birches is known to geographically change with different climate conditions (Migalina et al., 2010), we expected that phenotypes of leaf morphological traits varied among the three regions, Hokkaido, Honshu, and Primorye. Based on these genetic and phenotypic variations, we tested lower diversity and

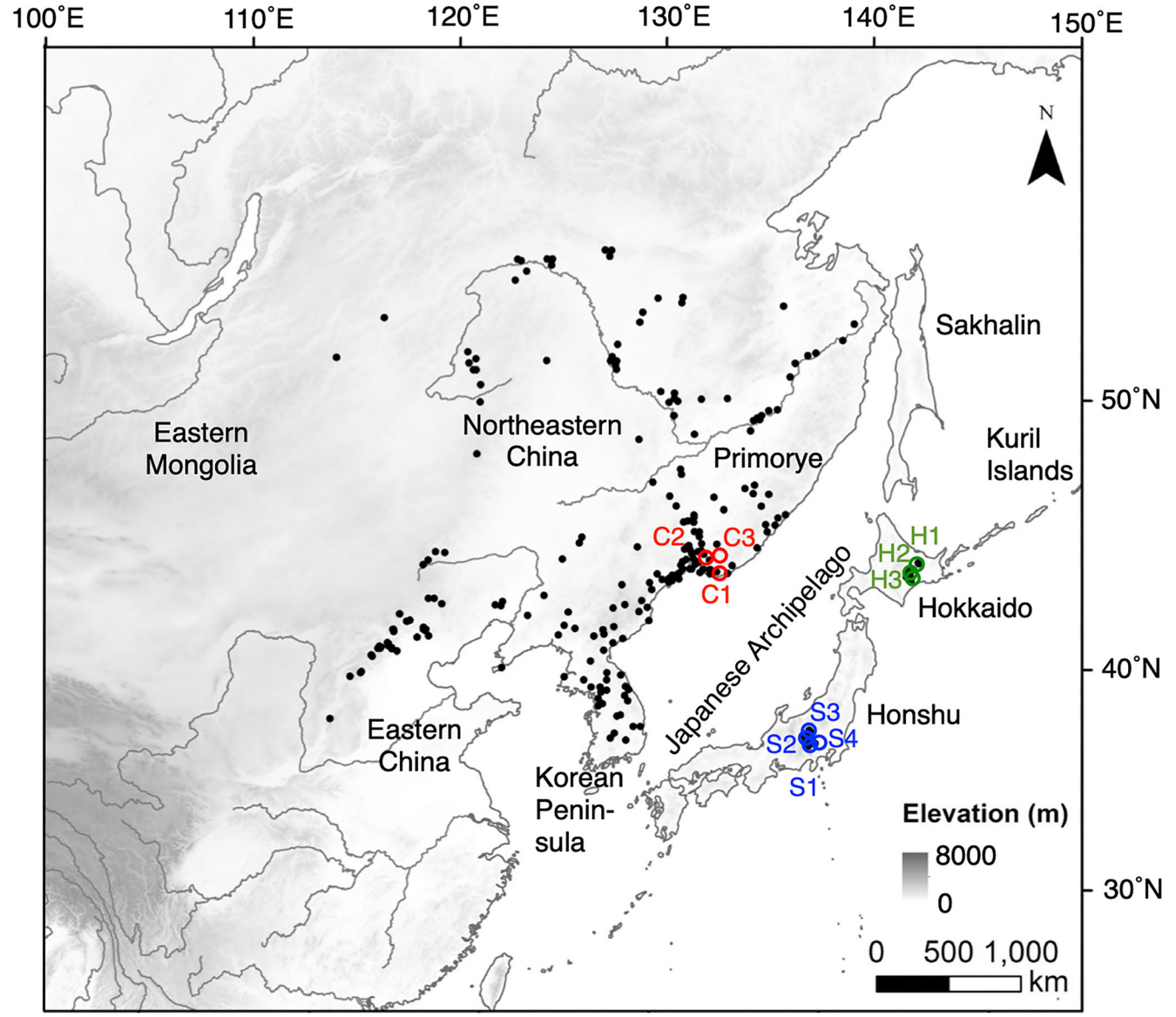

FIGURE 1 Distributional ranges of *Betula davurica* (dots: 251 occurrence records compiled by Shitara et al., 2018) and locations (open circles) of study populations. Colors indicate regions (red: Primorye, green: Hokkaido, blue: Honshu), and letters indicate population codes.

higher differentiation in local populations in Hokkaido and Honshu than those in Primorye.

2 | MATERIALS AND METHODS

2.1 | Species and sampling

Betula davurica Pallas was used as the species name in this study although there are multiple orthographical variants for this species (IPNI, 2024). Ploidy assessment using flow cytometry reported not only five octaploid specimens collected in Primorye, Korea, Hokkaido, and Honshu, but also two hexaploid specimens collected in Primorye and Hokkaido (Wang et al., 2016). It is not clear whether *B. davurica* is autopolyploid, allopolyploid, or segmental allopolyploid with homoeologous replacement between subgenomes. In a phylogenomic study, most sequence reads of octaploid *B. davurica* were mapped to the reference sequences of diploid *Betula humilis*, suggesting that most subgenomes of *B. davurica* were derived from this diploid progenitor (Wang et al., 2021).

We selected three populations (C1–C3) around Vladivostok in Primorye, three populations (H1–H3) in eastern Hokkaido, and four populations (S1–S4) in central Honshu (Figure 1 and Table 1). These populations were apart from one another over 30–80 km in each region. We collected a branch with several leaves from each of 32–50 trees, which were apart from one another over 10 m, in each population located within an area spanning 1–2 km

TABLE 1 Locations, sample sizes of SNP genotypes at ddRAD loci, cpDNA sequences, and leaf morphology, and genetic diversity at ddRAD loci of putative octaploid individuals of *Betula davurica* populations.

Population			Latitude	Longitude	Elevation	No. of sampled individuals			Genetic diversity		
Code	Region	Locality name	(°N)	(°E)	(m)	ddRAD	cpDNA	Leaf	H_E	H_O	F_{IS}
C1	Primorye	Sestra	42.830	132.998	100	16	2	32	0.148	0.153	−0.0365
C2	Primorye	Shkotovo	43.325	132.395	200	16	2	39	0.148	0.153	−0.0329
C3	Primorye	Novaya Moskva	43.355	132.634	200	15	2	50	0.145	0.148	−0.0274
H1	Hokkaido	Tsubetsu	43.693	143.972	120	16	1	35	0.146	0.150	−0.0317
H2	Hokkaido	Ashoro	43.251	143.486	230	15	2	32	0.145	0.149	−0.0293
H3	Hokkaido	Honbetsu	43.127	143.453	200	15	2	34	0.149	0.153	−0.0268
S1	Honshu	Yamanashi	35.799	138.632	1690	12	2	36	0.167	0.175	−0.0481
S2	Honshu	Hokuto	35.939	138.420	1530	11	2	36	0.167	0.175	−0.0501
S3	Honshu	Sakuho	36.107	138.411	1250	16	2	35	0.158	0.165	−0.0441
S4	Honshu	Chichibu	36.003	139.143	740	8	2	36	0.176	0.186	−0.0540
					Total	140	19	365	0.139	0.139	0.0019

Abbreviations: cpDNA, chloroplast DNA; ddRAD, double-digest restriction site associated DNA; F_{IS}, inbreeding coefficient; H_E, expected heterozygosity, H_O, observed heterozygosity; SNP, single-nucleotide polymorphism.

in radius. The collections were conducted in Primorye in June 2006, in Hokkaido in August 2009, and in Honshu in September 2014.

We stored some fresh leaves of a collected branch at −20°C and extracted DNA from the leaf tissue of each sampled tree using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). We selected a single leaf, which had a typical shape and size in a collected branch, from each sampled tree. We pressed and dried the selected leaves for measurement of leaf morphology.

2.2 | ddRAD sequencing

We assessed the quality of extracted DNA using a Varioskan LUX (Thermo Fisher Scientific, Waltham, MA, USA) and selected 16 individuals with sufficient DNA concentration (>20 ng/μL) from the sampled trees in each population, except for eight individuals from population S4 (Table 1). We prepared the ddRAD library using the standard protocol (Peterson et al., 2012). DNA of the selected individuals was double digested using *Pst*I and *Sau*3AI restriction enzymes (Invitrogen, Waltham, MA, USA), ligated with Y-shaped adaptors, and amplified using a polymerase chain reaction (PCR) with KAPA HiFi polymerase (KAPA Biosystems, Boston, MA, USA). After PCR amplification with adapter-specific primer pairs (Access Array Barcode Library for Illumina, Fluidigm, South San Francisco, CA, USA), an equal amount of DNA from each individual was mixed and size-selected using BluePippin 2% agarose gel (Sage Science, Beverly, MA, USA). We retrieved library fragments between 450 and 600 bp and checked the quality of the library using KAPA library quantification kits on a LightCycler 480 Instrument (Roche, Basel, Switzerland). Finally, the libraries were sequenced using a high-throughput Illumina Hi-Seq X Ten platform (Macrogen, Inc., Seoul, South Korea) to generate paired-end reads with 150 bp lengths.

We conducted read mapping and variant calling for the obtained sequences using dDocent 2.7.8 (Puritz et al., 2014). In the read mapping, the obtained reads were aligned to the high-quality whole genome sequence of an inbred individual of diploid *Betula pendula* (Salojärvi et al., 2017). We used the default settings of dDocent, following the procedure shown in the tutorial, except for the filtering of loci based on an allele balance and the Hardy–Weinberg equilibrium, which seemed to be unsuitable for polyploids. Multistep filtering was used to remove false positive calls and to keep sufficient SNP loci. In the first filtering, we selected polymorphic loci that were biallelic without indels, with <50% missing data across all individuals, with >5% minor allele frequency, with >4 read depth, and with >30 quality value, using VCFtools 0.1.14 (Danecek et al., 2011). In the second filtering, we selected loci with >64 mean read depth and <5% missing data across all individuals, when data were missing at loci with <32 read depth. After the multistep filtering of loci, we removed individuals with missing data at >15% filtered loci.

We extracted read counts of reference and alternative alleles from the vcf file after the filtering of loci and

individuals using the function extract.gt of the package vcfR (Knaus & Grünwald, 2017) in R 4.3.1 (R Core Team, 2023). To infer the ploidy level of each individual from the ddRAD data, we examined a frequency distribution of allele ratios from read counts of minor alleles at heterozygous loci with >80 read depth (Zohren et al., 2016). In these frequency distributions, a polymorphic octaploid individual should have peaks at $k/8$ ratios ($k = 1$–7). We selected putative octaploid individuals with a peak at the lowest ratio close to 1/8 and removed other individuals. We assigned genotypes at the selected ddRAD loci with >240 mean read depth for the putative octaploid individuals using the function flexdog (model = "norm") of the R package updog (Gerard et al., 2018). We described genotypes at each locus of each individual as the copy number of minor alleles.

2.3 | Flow cytometry

To confirm ploidy, we examined the bud tissues of six *B. davurica* individuals that originated from the population H1 in eastern Hokkaido. In addition, we examined three individuals of each species of diploid *Betula maximowicziana* Regel, diploid *Betula platyphylla* Sukaczev, tetraploid *B. ermanii* Chamisso, and tetraploid *Betula ovalifolia* Ruprechit (Shiotani et al., 2020; Wang et al., 2016). These individuals were planted in the Arboretum of the Hokkaido Research Center, Forestry and Forest Products Research Institute.

An appropriate amount of tissues in fresh buds was added to 50 μL 4′,6-diamidino-2-phenylindole (DAPI) solution (supplemented with 2% polyvinylpyrrolidone K30 and 2 μL/mL 2-mercaptoethanol) and chopped with the internal reference standard *Epipremnum aureum*. The isolated nuclei were then stained with 950 μL DAPI solution, and incubated for 15–30 min at room temperature. The samples were filtered through a 30-μm nylon mesh to remove tissue debris. The fluorescence intensity of 5000 particles was measured using a Quantum P Flow Cytometer (Quantum Analysis, Germany). A histogram of the fluorescence intensity was obtained using CyPAD software version 1.3 (Quantum Analysis, Germany). We estimated the ploidy levels of the 18 individuals from their peak positions of the fluorescence histograms as the peak position of *E. aureum* was initially set at 700 in CyPAD.

2.4 | Genetic analysis

We conducted principal component analysis (PCA) for putative octaploid individuals based on SNP genotypes using the function dudi.pca of the R package ade4 (Dray & Dufour, 2007). We calculated the Nei's genetic distance between the individuals using the function dist.prop of the R package ade4 and made a neighbor-joining (NJ) tree using the function nj of the R package ape (Paradis & Schliep, 2019).

We obtained multi-locus estimates of population genetic diversity indices, the observed and expected heterozygosity (H_O, H_E), and the inbreeding coefficient (F_{IS}) for each population from octaploid SNP genotypes using the program POLYGENE (Huang et al., 2020). H_O is the population average probability that two alleles randomly chosen from eight alleles of an individual are different. H_E is $1 - \Sigma_j p_j^2$, where p_j is the frequency of jth allele ($j = 1, 2$) in a population. F_{IS} is $1 - H_O/H_E$. We estimated pairwise genetic differentiation (F_{ST}, G'_{ST}) between populations (Hedrick, 2005; Hudson et al., 1992) using POLYGENE. These estimates vary depending on not only mutation, migration, drift, and selection in populations but also inheritance mode and subgenome structure in octaploid individuals (Huang et al., 2020).

2.5 | CpDNA sequencing

To examine cpDNA sequences, we selected 1–2 individuals from each population (Table 1). We determined nucleotide sequences in a region, *trn*L (UAA) 3′ exon—*trn*F (GAA), using primer pairs B49873 and A50272 (Taberlet et al., 1991), a BigDye Terminator Sequencing Kit, and a 3100 Genetic Analyzer (Thermo Fisher Scientific, Waltham, USA). We assembled and manually edited the obtained sequences using Sequencher 10.4.1 (Gene Codes Corporation, Ann Arbor, USA). We compared the assembled sequences among populations with reference to previously published sequences of *B. davurica* in this cpDNA region (the accession numbers, FJ012053 and KY199634).

2.6 | Leaf morphology

We scanned the selected leaf of each individual. From the scanned leaf image, we calculated the area (cm^2) and extracted an outline (closed contours) of the leaf blade. The contours were described by a chain-code and transformed to coefficients of normalized elliptic Fourier descriptors using the program SHAPE (Iwata & Ukai, 2002). To summarize the coefficients of Fourier descriptors, we conducted PCA based on the coefficients and visualized the variation in leaf shape explained by each PC using SHAPE.

3 | RESULTS

3.1 | Ploidy

We obtained read count data at 39468 filtered ddRAD loci of 150 filtered individuals in the 10 populations. Among the 150 individuals, frequency distributions of the read count ratios of minor alleles at heterozygous loci of 140 individuals demonstrated a peak of the lowest ratio close to 1/8 expected in octaploids (Figure S1). We selected the 140 individuals as putative octaploids and removed 10 individuals: 1 in the population H2, 4 in S1, and 5 in S2, which showed a peak of the lowest ratio close to 1/6 or 1/7 rather than 1/8 (Figure S1).

Flow cytometry demonstrated that all six individuals that originated from the population H1 were octaploids, in comparison to diploid *B. maximowicziana* and *B. platyphylla* and tetraploid *B. ermanii* and *B. ovalifolia* (Figure S2).

3.2 | SNP genotypes

We conducted PCA for the 140 putative octaploid individuals based on the SNP genotypes. Contributions of the first to fifth PCs to total genotypic variation were 6.37%, 2.33%, 2.03%, 1.72%, and 1.38%, respectively. The first PC demonstrated genetic divergence of the Honshu populations from both Hokkaido and Primorye populations, and the second PC showed genetic differentiation between the Hokkaido and Primorye populations with overlaps in some individuals (Figure 2). An NJ tree based on Nei's genetic distance calculated from the octaploid SNP genotypes also demonstrated the genetic divergence of the Honshu populations from both Hokkaido and Primorye populations (Figure 3).

We estimated the genetic diversity indices for the 10 populations of putative octaploid individuals from the SNP genotypes. The observed and expected heterozygosity was higher in the Honshu populations ($0.165 \leq H_O \leq 0.186$, $0.167 \leq H_E \leq 0.176$) than in both Hokkaido and Primorye populations ($0.148 \leq H_O \leq 0.153$, $0.145 \leq H_E \leq 0.149$; Table 1). The inbreeding coefficient of every population was slightly negative and was lower in the Honshu populations ($-0.0540 \leq F_{IS} \leq -0.0441$) than in both Hokkaido and Primorye populations ($-0.0365 \leq F_{IS} \leq -0.0268$; Table 1). The pairwise genetic differentiation between the Honshu populations and both Hokkaido and Primorye populations ($0.0108 \leq F_{ST} \leq 0.0347$, $0.0163 \leq G'_{ST} \leq 0.0228$) was larger than that between the Hokkaido and Primorye populations ($0.0020 \leq F_{ST} \leq 0.0092$, $0.0083 \leq G'_{ST} \leq 0.0132$) and between populations within each region ($0.0005 \leq F_{ST} \leq 0.0072$, $0.0073 \leq G'_{ST} \leq 0.0130$; Table 2).

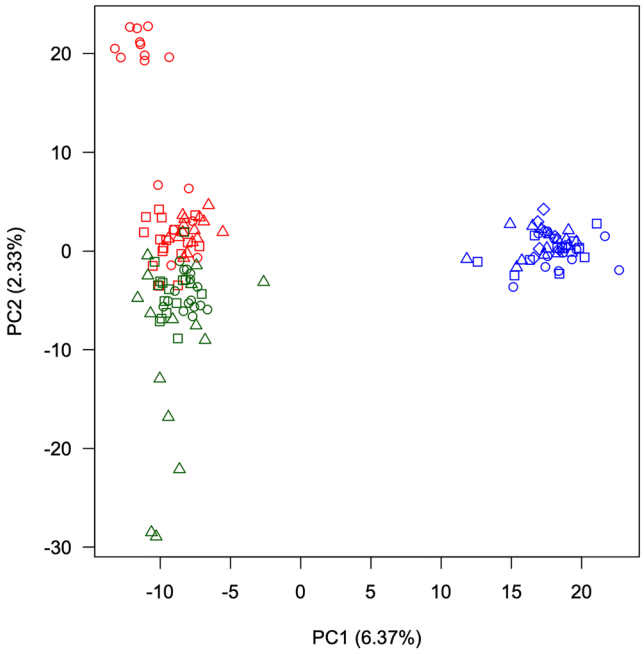

FIGURE 2 Principal component analysis for putative octaploid individuals of *Betula davurica* populations based on single-nucleotide polymorphism (SNP) genotypes. Contributions (%) of the first and second principal components to total genotypic variation are shown. Red, green, and blue symbols indicate individuals in Primorye, Hokkaido, and Honshu populations, respectively. Circles (C1, H1, and S1), squares (C2, H2, and S2), triangles (C3, H3, and S3), and diamonds (S4) indicate different populations in each region.

3.3 | cpDNA sequences

In cpDNA sequences in the *trn*L (UAA) 3′ exon—*trn*F (GAA) determined in 19 individuals (Table 1), we found four polymorphic sites, including a deletion with up to 52 bp length, two substitutions, and a simple sequence repeat (SSR). In the range between the end of *trn*L gene and the intergenic spacer of *trn*L and *trn*F, 1–9 bp deletions were found in both Hokkaido and Primorye populations, while 41–52 bp deletions were found in the Honshu populations (Figure 4). In the intergenic spacer, two individuals in population C3 exhibited substitutions at two sites and extension to 11 bp length at the SSR (Figure 4). Except for the two individuals, the SSR was 9 bp long in both the Hokkaido and Primorye populations but 10–11 bp long in the Honshu populations.

3.4 | Leaf morphology

We conducted PCA for 365 individuals based on the coefficients of Fourier descriptors for leaf shape (Table 1). Contributions of the first to fifth PCs to total morphological variation were 52.6%, 12.7%, 7.3%, 6.7%, and 4.6%,

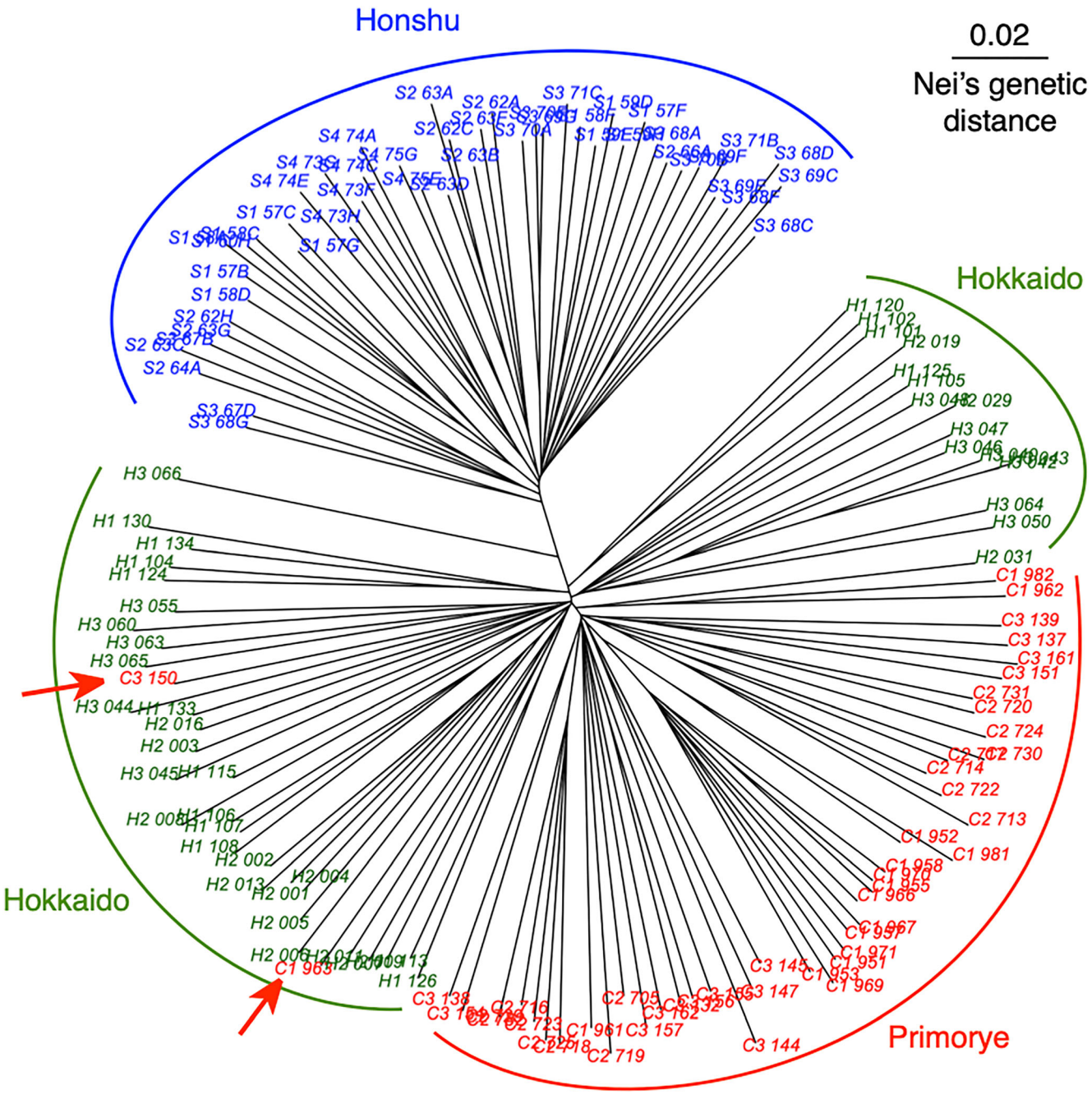

FIGURE 3 Neighbor-joining trees of putative octaploid individuals of *Betula davurica* based on the Nei's genetic distance calculated from single-nucleotide polymorphism (SNP) genotypes. Red, green, and blue letters indicate individuals in Primorye (C1–C3), Hokkaido (H1–H3), and Honshu populations (S1–S4), respectively. Red arrows indicate individuals in Primorye, which are included in clades of individuals in Hokkaido.

respectively. The first PC represented the width of leaf blades, and the second PC reflected the variation between elliptic and ovate shapes (Figure S3). The first and second PCs overlapped among the Primorye, Hokkaido, and Honshu populations (Figure 5a). The area of a leaf blade was also overlapped among these populations (Figure 5b).

4 | DISCUSSION

The observed read count ratios in ddRAD sequencing indicated that most individuals in the studied *B. davurica* populations are octaploids, some of which were confirmed by flow cytometry. Several individuals in the Honshu populations showed read count ratios different from

TABLE 2 Pairwise genetic differentiation (upper diagonal: F_{ST}, Hudson et al., 1992; lower diagonal: G'_{ST}, Hedrick, 2005) between *Betula davurica* populations.

Population	C1	C2	C3	H1	H2	H3	S1	S2	S3	S4
C1	0.0000	0.0072	0.0044	0.0071	0.0067	0.0092	0.0271	0.0284	0.0178	0.0347
C2	0.0121	0.0000	0.0018	0.0047	0.0043	0.0058	0.0219	0.0224	0.0154	0.0270
C3	0.0097	0.0082	0.0000	0.0021	0.0020	0.0041	0.0191	0.0205	0.0108	0.0260
H1	0.0120	0.0100	0.0083	0.0000	0.0008	0.0028	0.0207	0.0210	0.0135	0.0260
H2	0.0116	0.0103	0.0083	0.0073	0.0000	0.0025	0.0215	0.0220	0.0126	0.0285
H3	0.0132	0.0109	0.0100	0.0084	0.0088	0.0000	0.0196	0.0196	0.0139	0.0240
S1	0.0228	0.0210	0.0189	0.0194	0.0207	0.0209	0.0000	0.0032	0.0021	0.0042
S2	0.0219	0.0201	0.0184	0.0181	0.0194	0.0198	0.0115	0.0000	0.0006	0.0028
S3	0.0205	0.0185	0.0163	0.0169	0.0178	0.0183	0.0100	0.0088	0.0000	0.0005
S4	0.0214	0.0203	0.0183	0.0182	0.0198	0.0205	0.0130	0.0129	0.0098	0.0000

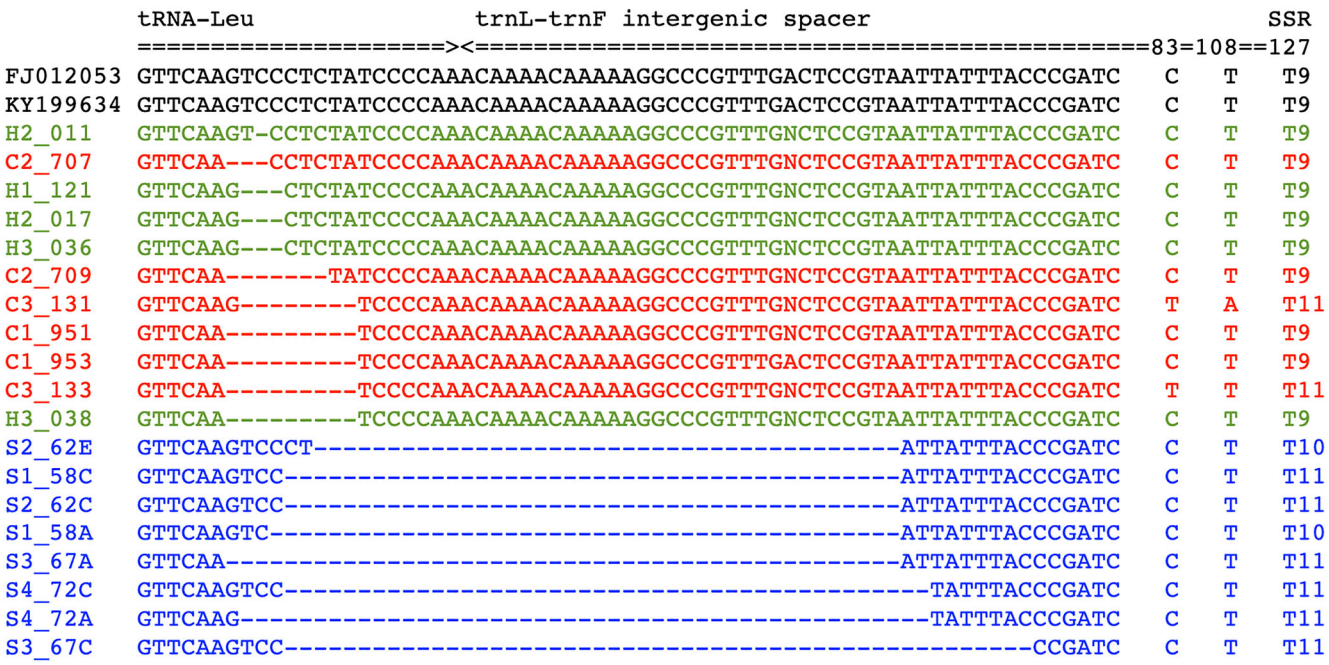

FIGURE 4 Polymorphisms in cpDNA region, *trn*L (UAA) 3′ exon—*trn*F (GAA), among 19 individuals in Primorye (red), Hokkaido (green), and Honshu populations (blue) and two accessions of reference sequences (black) of *Betula davurica*. Numbers in the upper row indicate nucleotide positions in intergenic spacer. Numbers in the simple sequence repeat (SSR) column indicate the number of nucleotide repeats.

those expected from octaploidy, suggesting ploidy variation within these populations. This result is consistent with the previous report of both hexaploids and octaploids in *B. davurica* (Wang et al., 2016). In mixed ploidy species, various factors cause ploidy variation (Kolář et al., 2017). Hybridization with tetraploid species can produce hexaploids in octaploid populations. A tetraploid species, *B. ermanii*, is common at subalpine zones in the Hokkaido and Honshu regions and potentially hybridizes with other birch species (Nagamitsu et al., 2006; Tsuda et al., 2017). Thus, hybridization with *B. ermanii* may be responsible for the ploidy variation in *B. davurica*. Other factors, such as fertilization by irregularly reduced gametes are also able to result in the ploidy variation (Kolář et al., 2017). In future studies, mixed ploidy in *B. davurica* can be investigated in the Honshu populations.

Subgenome structure and inheritance mode are not known in octaploid *B. davurica*. In tetraploid birches, polysomic inheritance has been estimated from segregation patterns (Brown & Al-Dawoody, 1979; Nagamitsu, Kawahara, & Kanazashi, 2014), suggesting that genetic

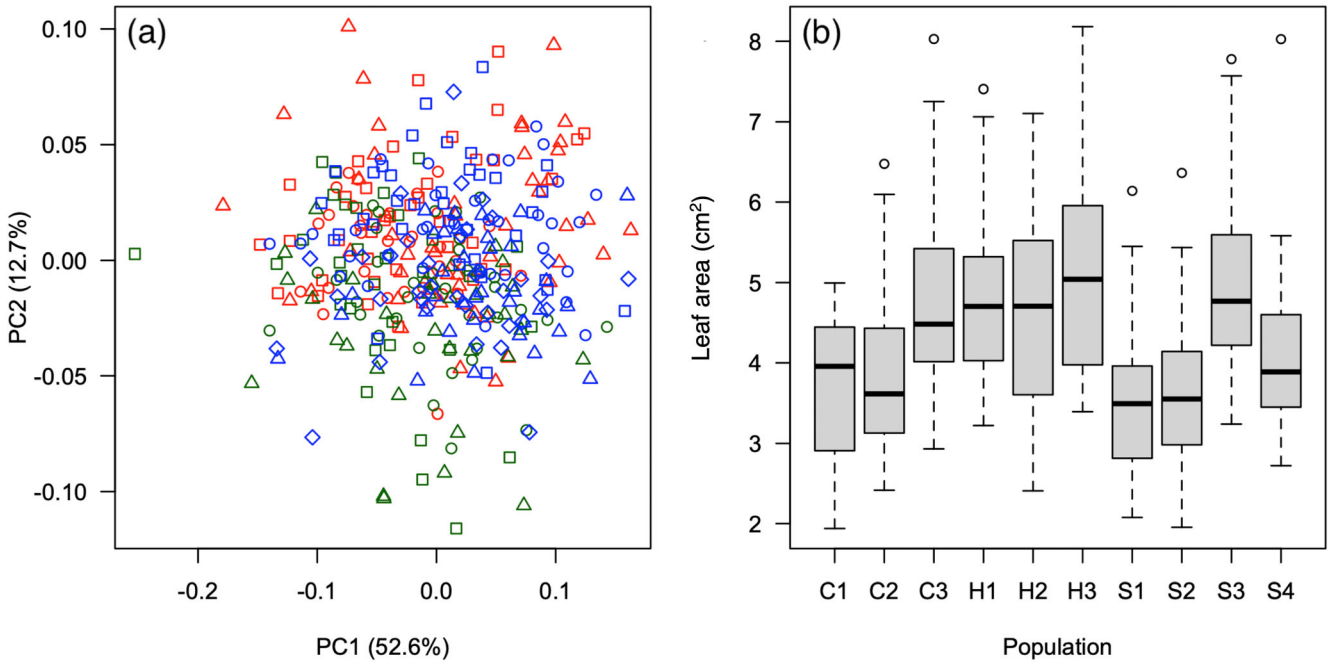

FIGURE 5 Leaf morphological variation in individuals of *Betula davurica* populations. (a) Principal component analysis based on the coefficients of Fourier descriptors for outlines of leaf shape. Contributions (%) of the first and second principal components to total morphological variation are shown. Red, green, and blue symbols indicate individuals in Primorye, Hokkaido, and Honshu populations, respectively. Circles (C1, H1, and S1), squares (C2, H2, and S2), triangles (C3, H3, and S3), and diamonds (S4) indicate different populations. (b) The area of a leaf blade in Primorye (C1–C3), Hokkaido (H1–H3), and Honshu (S1–S4) populations. Median (lines), quantiles (boxes), 95 percentiles (whiskers), and outliers (circles) are shown.

recombination can occur between homoeologous chromosomes associated with different subgenomes. Such recombination results in complex subgenome structures in polyploid birches (Leal et al., 2024). In this study, simple indices of genetic diversity were calculated from octaploid genotypes at genome-wide SNP loci, most of which are thought to be evolutionary neutral. The expected heterozygosity and its derived indices (H_E, F_{ST}, G'_{ST}) are likely to mainly depend on demographic factors (mating system, effective population size, gene flow, and so on), and the observed heterozygosity and its derived index (H_O, F_{IS}), which are affected by allelic compositions within individuals, are likely to depend on both demographic factors and subgenome structure. Based on these indices as well as the patterns of PCA and NJ tree, the differences in cpDNA sequences, and the phenotypic variations in leaf morphology, we tested general trends in disjunct populations (Eckert et al., 2008; Hamilton & Eckert, 2007; Meeus et al., 2012), that is, lower diversity and higher differentiation in the Hokkaido and Honshu populations in disjunctly distributed regions than in the Primorye populations in a continuously distributed region.

The results from the genetic differentiation indices (F_{ST}, G'_{ST}), the patterns of PCA and NJ tree, and the differences in cpDNA sequences indicated higher genetic divergence of the Honshu populations from both Hokkaido and Primorye populations, which is congruent with our expectation. This genetic divergence can be a result of the post-glacial isolation of potential habitats in central Honshu, which had been a part of potential habitats around eastern China, the terrestrialized shelf of the East China Sea, the Korean Peninsula, and the Japanese Archipelago in the last glacial period (Shitara et al., 2018). The F_{ST} values ($0.010 < F_{ST} < 0.035$) between the Honshu populations and both Hokkaido and Primorye populations are lower than those ($0.084 < F_{ST} < 0.098$) between British populations and are similar to those ($0.013 < F_{ST} < 0.024$) between Scandinavian populations of diploid *Betula nana*, using the same genetic marker system (Borrell et al., 2018). These *B. nana* populations are thought to have recolonized the British and Scandinavian regions after the last glacial period, and the British populations have been reduced and fragmented over the past centuries (Borrell et al., 2018). These findings suggest the relatively low genetic divergence of *B. davurica* populations in Honshu despite their disjunct distributions in northeastern Asia, in comparison to diploid *B. nana* populations in Europe. Genetic variations maintained in highly polyploid genomes, large sizes of isolated populations, and/or recent

isolation from the continental regions may be responsible for the relatively low genetic divergence.

The results from SNP genotypes and cpDNA sequences did not indicate genetic divergence of the Hokkaido populations from the Primorye populations, which has not been expected from their disjunct distributions. The genetic similarity between the Hokkaido and Primorye populations suggests migration between Hokkaido and continental regions in northeast Asia but does not suggest migration between Hokkaido and central Honshu. To clarify the migration process between Hokkaido and the continental regions, it is necessary to investigate geographic genetic structure and demographic history in multiple continental regions across the current distributional range. Although potential habitats of *B. davurica* in Sakhalin were estimated in the mid-Holocene, its current distributional records are absent there (Shitara et al., 2018). Thus, if the migration between Hokkaido and the continental regions had occurred, there is a possibility of long-distance dispersal over the sea or stepwise dispersal through Sakhalin Island, where *B. davurica* has been locally extinct. Long-distance seed dispersal in *B. davurica* is unlikely because the dispersal distance of birch seeds with wings is relatively short, in comparison to other wind-dispersed seeds, such as willow seeds with hairs (Tiebel et al., 2020).

The results from the expected heterozygosity (H_E) indicated higher genetic diversity in the Honshu populations than in both Hokkaido and Primorye populations, which is inconsistent with the general trends in disjunct populations. The H_E values in the Honshu populations ($0.15 < H_E < 0.18$) and in both Hokkaido and Primorye populations ($0.14 < H_E < 0.15$) are higher than those in British and Scandinavian populations ($0.10 < H_E < 0.12$) of diploid *B. nana* (Borrell et al., 2018) and are lower than those in Japanese populations ($0.23 < H_E < 0.28$) of tetraploid *B. ermanii*, except for its southernmost isolated population ($H_E = 0.06$) (Aihara et al., 2023), using the same genetic marker system. These findings suggest that the disjunct *B. davurica* populations maintain genetic diversity comparable to other birch populations. The slightly higher genetic diversity in the Honshu populations than in both Hokkaido and Primorye populations implies genetic variations accumulated in stable populations, which is suggested by the past potential habitats of *B. davurica* have been persistent in central Honshu during both glacial and post-glacial periods (Shitara et al., 2018).

The results from the expected and observed heterozygosity did not indicate a positive inbreeding coefficient (F_{IS}) in the studied *B. davurica* populations, suggesting predominant outcrossing and little sub-structuring in local populations. The F_{IS} values in the Honshu populations ($-0.054 < F_{IS} < -0.044$) were consistently lower than those in both Hokkaido and Primorye populations ($-0.037 < F_{IS} < -0.026$). The higher heterozygote excess in central Honshu can result from two factors, different allele frequencies between subgenomes within an individual and different allele frequencies between female and male gametes in mating within a population (Balloux, 2004).

The shape and size of leaves did not show obvious differentiation among the studied populations. This result suggests that low genetic differentiation is responsible for the leaf morphological traits and/or similar environmental conditions in the disjunctly distributed regions. In *Betula nigra* in North America, phenotypic variances in leaf morphology tended to be larger in continuous populations than in disjunct populations, implying the effects of genetic drift in the disjunct populations (Coyle et al., 1982). Such effects on phenotypic variations in leaf morphology are not found in *B. davurica*.

In conclusion, the disjunct *B. davurica* populations in Honshu are genetically divergent from continental populations in a continuously distributed region in northeast Asia although the disjunct populations in Hokkaido are not divergent. These disjunct populations in the Japanese Archipelago maintain genetic diversity comparable to the continental populations. These findings are not consistently congruous with the general trends expected in disjunct populations. The genetic differentiation between the Hokkaido and Honshu populations implies a possibility that *B. davurica* independently colonized northern and southern parts of the Japanese Archipelago from continental regions in northeast Asia, as was reported in *Picea jezoensis* and *Lychnis wilfordii* (Aizawa et al., 2007, 2009; Tamura et al., 2019). The north and south connections between the Japanese Archipelago and the continental regions lead to a circular genetic structure in *Abies nephrolepis* (Kwak et al., 2024). In future studies, it is necessary to investigate multiple continental populations around the north and south migration routes to verify this possibility.

AUTHOR CONTRIBUTIONS

Study design and sampling: TN, VB, and CO. *Genetic analysis*: KU and TN. *Leaf morphology analysis*: KS. *Writing manuscript*: TN and TS.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

CpDNA sequences are deposited in the DNA Data Bank of Japan (DDBJ) with accessions: LC804785–LC804785. Octaploid SNP genotypes and leaf morphology data are provided in Supporting Information (Data S1 and S2).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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