Recovery process of genetic diversity through seed and pollen immigration at the northernmost leading-edge population of *Fagus crenata*

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**Abstract**
The range expansion of a plant species begins with colonization of ecological empty patches from posterior source populations. This process involves stochastic loss of genetic diversity. However, the founder population could restore genetic diversity by gene flow from posterior populations via seeds and pollen and its recovery affects evolutionary potential for species expansion. To clarify the recovery process of genetic diversity during species range expansion, gene flow via seeds and pollen was investigated at the expansion front of *Fagus crenata*. Based on eight nuclear microsatellite genotypes of a total of 150 individuals and 225 seeds at the northernmost leading-edge population, genetic diversity, fine-scale spatial genetic structure (FSGS), and genetic differentiation from other five northern populations were investigated. Moreover, both seed and pollen immigration and their effects on genetic diversity at different successional stages were analyzed. The leading-edge population showed lower genetic diversity and substantial genetic differentiation, reflecting its strong genetic drift. Non-significant FSGS and a negative inbreeding coefficient for mature trees may indicate that the earliest generation consisted of founders from foreign seed sources. The significant proportion of seed and pollen immigration increased the number of different alleles for later successional stages. The effective number of pollen parents from foreign sources (20.8) was markedly higher than that from the local source (2.1). These results indicated that pollen immigration incorporated new and rare alleles and increased the genetic diversity of the population. However, the proportion of foreign gene flow decreased during succession, probably due to the increased reproductive success of local individuals as they reached maturity and grew in size.

**Keywords**
colonization, Fagaceae, founder effect, geographical distribution, neighborhood model
long-lived tree species. Furthermore, Shi and Chen (2012) reported that the process of recovery of genetic diversity by pollen immigration should affect the evolutionary potential for the range shift or expansion of plant species. However, such a recovery process in the leading-edge population, which should affect further expansion of long-lived tree species, has not been confirmed through an examination of temporal changes in gene flow and its genetic effects. Such an investigation could be conducted in the early founder phase in the leading-edge population, where a substantial proportion of the gene flow and subsequent increase in genetic diversity is occurring at the moment.

Temperate and boreal forests in the Northern Hemisphere have been expanding their range northward after southern retreat during the last glaciation (Cheddadi et al., 2006; Liepelt et al., 2009; Magri et al., 2006; McLachlan & Clark, 2004; McLachlan, Clark, & Manos, 2005; Petit et al., 2002). Likewise, temperate tree species in the Japanese archipelago, including Fagus crenata, have spread northward for the last 10,000 years of post-glacial warming (Hara, 1996; Yasuda & Miyoshi, 1998). The fossil pollen record revealed the northward range expansion of F. crenata (Tsukada, 1982a, 1982b) to its present northern range limit approximately 1,000 years ago (Bradshaw, Kito, & Giesecke, 2010; Kito, 2003; Sakaguchi, 1989). At the moment, the density of F. crenata in the forests shows a progressive decrease from the southwestern to the northeastern leading-edge margin (Figure 1) (Kito, 2003; Tatewaki, 1948).

One of the well-known northernmost populations of F. crenata, Tsubamenosawa (population 5 in Figure 1), is estimated to have been established about 400 to 680 years ago, and the species is considered to be expanding further northward at present (Kobayashi & Watanabe, 2003). Kitamura et al. (2015) revealed that the genetic diversity declined and strong genetic differentiation occurred among the northern range margin of this species. In 2013, one small population of F. crenata was discovered in the Niseko mountain range, 12 km northeast from the previously established northernmost distributional margin and 14 km from the high-density continuous distribution boundary (population 1 in Figure 1, Tanaka et al., 2016).

This population is assumed to be the most recently colonized founder population at the expansion front (Tanaka et al., 2016). First, field observations revealed that the population consisted of individuals with a wide variety of sizes, with most of the individuals of smaller size instead of showing a bimodal size distribution as in other northern populations (Kobayashi & Watanabe, 2003). For this reason, the population is
considered to have been established recently and is still growing in size at the moment (Tanaka et al., 2016). Second, the population did not include dead or super huge *F. crenata* individuals, which are usually observed in matured or overly matured natural forests. Third, the oldest individual in the Niseko population was 131 years of age, which is younger than ca. 250 years in mature forests on the northern range (Kitamura, Kobayashi, & Kawahara, 2007). Therefore, this population may include the earliest founder individuals.

Even though simulation studies using several climate scenarios have estimated the future northward range expansion of *F. crenata* (Matsui et al., 2009; Nakao et al., 2013), restrictions to the accumulation of genetic diversity by the decreasing gene flow with population growth might hamper further expansion of long-lived tree species (Elleouet & Aitken, 2019; Lesser et al., 2013) as high genetic diversity is necessary for adaptation to less favorable (Honjo, Ueno, Tsumura, Washitani, & Ohsawa, 2004) or future environmental conditions (Shi & Chen, 2012). In this regard, it is important to study the ongoing recovery process of genetic diversity at the current expansion front of *F. crenata* to ensure the future range shift or expansion of this species. The aim of this study was to clarify the recovery process of genetic diversity by seed and pollen immigration to the founder population at the species range expansion front. In this study, we investigated the genetic drift and estimated the gene flow via seeds and pollen at the northernmost leading-edge population of *F. crenata*. Further, we evaluated the effects of foreign gene flow on genetic diversity during different successional periods.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

The study site is located in the Niseko mountain range in southern Hokkaido (population 1 in Figure 1). It is a small (ca. 0.1 ha; Figure 2), isolated population of *F. crenata* at the leading edge of the northernmost expansion front of the geographical distribution (hereafter, the Niseko population). The Niseko population was discovered in 2013 and, to date, no population or individual has been identified within a 12 km radius (Tanaka et al., 2016) (Tanaka et al. personal investigation). The entire population consists of 150 individuals, including current-year seedlings, with one of the oldest individuals estimated to be 131 years of age in 2014 (Tanaka et al., 2016). We measured the stem diameter at breast height (DBH) for individual trees higher than 130 cm,
and the GPS position of each the 150 individuals was also collected (Tanaka et al., 2016).

We categorized the entire population of 150 individuals into three successional stages; mature, juvenile, and seedling (Table 1) (Kitamura, Shimada, Nakashima, & Kawano, 1997). Twenty-five mature individuals were determined to be reproductive trees, based on confirmed flowering in the mast-flowering year of 2015. The size of the mature individuals was characterized by a DBH > 15 cm. Ten extracted mature trees with a DBH from 17.4 to 67.2 cm ranged from 79 to 131 years old. Thus, we approximated the age of mature trees in the study site to be ca. 80 to 130 years. Seventy juveniles were determined to be in the vegetative phase, having not flowered in the mast-flowering year of 2015. The juveniles had a DBH < 14 cm and were considered to be younger than the mature trees; i.e., less than ca. 80 years old. The youngest juvenile was determined to be 4 years old by bud-scale scar counts. Fifty-five current-year seedlings were identified by field observation. The locations of all individuals in the study site are mapped in Figure 2. In addition, in September of the mast-fruiting year of 2017 (Table 1), we collected 225 seeds directly from branches of four mature trees (m1 to m4 in Figure 2; 99, 15, 61, and 50 seeds, respectively).

2.2 | Simple sequence repeat analyses

We collected winter buds or fresh leaves from all 150 individuals comprising the Niseko population in April and July, 2014. Total DNA was extracted from ca. 50 mg of winter buds, fresh leaves, or cotyledons from seeds using a DNeasy Plant Mini Kit (Qiagen K. K., Tokyo, Japan). We used 8 simple sequence repeat loci; mfc2 (Tanaka, Nakamura, & Tsumura, 1999), FS1-03, FS4-46 (Pastorelli et al., 2003), sfc7, sfc18, sfc161, sfc378, and sfc1063 (Asuka, Tani, Tsumura, & Tomaru, 2004). Polymerase chain reaction (PCR) analysis was performed by Multiplex PCR Kit (Qiagen K. K., Tokyo, Japan). The length of the amplified fragments was analyzed using GeneScan 600 LIZ as the size standard, an ABI 3130-xl Genetic Analyzer, and GENESCAN software (Thermo Fischer Scientific Ltd., Tokyo, Japan). Extracted DNA of randomly chosen mature trees from five already known posterior northern populations (populations 2 to 6 in Figure 1 and Table 2) from our previous study (Kitamura et al., 2015) was also genotyped and included in the analyses.

2.3 | Estimation of genetic diversity, genetic differentiation, and fine-scale spatial genetic structure

We used GenoDive ver. 2.0b17 (Meirmans & Van Tienderen, 2004) to calculate the following genetic parameters of the Niseko and five other northern populations: the number of different alleles (N_A), the effective number of alleles (N_EF = 1 / \sum p_i^2, where p_i is the frequency of the i-th allele (Kimura & Crow, 1964)), observed heterozygosity (H_O), gene diversity (H_E), and the inbreeding coefficient (F_IS). Principal component analysis (PCA) was carried out to explicate the genetic relationship among the six populations using the same software.

To examine gene dispersal for each successional stage in the Niseko population, we analyzed the fine-scale spatial genetic structure (FSGS) using SPAGeDi ver. 1.5a (Hardy & Vekemans, 2002). As barochore species generally show FSGS caused by the aggregation of maternal siblings around the seed parents due to limited seed dispersal (Hamrick & Nason, 1996; Kawano & Kitamura, 1997; Kitamura et al., 2003), the individuals originating from local reproduction and first colonizers derived from various foreign seed sources would show significant and non-to weakly significant FSGS, respectively (Hamrick & Trapnell, 2011). The FSGSs of mature trees, juveniles, and seedlings were investigated by regression slopes (b_F) of coancestry, F_ij (Loiselle, Sork, Nason, & Graham,
1995), as a kinship coefficient against the natural logarithm of the spatial distance between individuals. In addition, to generate $F_{ij}$ correlograms for the three successional stages, the average $F_{ij}$ value was calculated for each of the five continuous distance classes equally allocated a number of pairs of individuals by the program. The significance of both the $b_F$ and average $F_{ij}$ values was tested by 1,000 permutations, in which spatial distances were permuted randomly among pairs of individuals.

### 2.4 Neighborhood model approach

To estimate the patterns of seed and pollen dispersal, parameters for the function of reproductive successes as seed and pollen parents, and the most likely parent pairs for offspring, i.e., seeds, seedlings, and juveniles, in the Niseko population, we used a neighborhood model approach (Burczyk, Adams, Birkes, & Chybicki, 2006) based on multilocus genotypes of the mature trees and offspring in the Niseko population by NM$\pi$ software (Chybicki, 2018).

We regarded all 25 mature trees as neighbors and assumed the seed- and pollen-parental reproductive success of each mature tree as exponential functions of the distance to the offspring and to a seed parent, respectively, as well as the standardized basal area of the mature tree. We estimated maximum-likelihood parameters, i.e., selfing rate ($s$), frequency of immigration ($m$), mean dispersal distance ($d$), and selection gradient (effect of the standardized basal area of the mature tree on its reproductive success, $g$) for seed and pollen dispersal, and genotyping errors. As seeds were collected directly from known seed parents, estimations for seeds were made only for pollen dispersal.

The NM$\pi$ software estimates the most likely parent pairs for each offspring (parentage reconstruction) based on the estimated maximum-likelihood model, in which each offspring could have (a) both parents in the local (Niseko) population, (b) one parent in the local population and the other from a foreign source (outside of the Niseko population), or (c) both parents from foreign sources. Results of the most likely parent pair were used for later analyses.

### 2.5 Effects of seed and pollen immigration on the genetic diversity of offspring

We analyzed how seed and pollen immigration affect the genetic diversity of offspring in the Niseko population. First, we categorized seedlings and juveniles into three categories of parentage such as local or foreign sources of seed and/or pollen parents by parentage reconstruction based on the maximum-likelihood model as follows: (i) individuals originated from local reproduction (both parents in the Niseko population), (ii) individuals originated from local seed parent regardless of pollen parent source, and (iii) all individuals. Seeds were classified into two categories, (i) and (ii).

Second, we calculated genetic diversity parameters (mean values of $N_A$, $N_{EF}$, and $H_E$ over the 8 loci) for each category. We also calculated increases in each parameter from (i) to (ii) and from (ii) to (iii), for seedlings and juveniles, and from (i) to (ii) for seeds. Increases from (i) to (ii) and from (ii) to (iii) indicate the effects of pollen and seed immigration on the genetic diversity of the offspring, respectively.

Third, to test the increases in parameters while excluding the effects of sample sizes, we used bootstrapping tests. To test the increases from category (i) to (ii), we randomly extracted the same number of individuals as (i) from (ii) without replacements, and calculated increases in the parameters from the extracted sample to

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**Table 2: Genetic diversity of mature trees in the Niseko and five posterior northern populations of Fagus crenata**

| Population ID in Kitamura et al., 2015 | Population | Location       | $n$ | $N_A$ (SE) | $N_{EF}$ (SE) | $H_O$ (SE) | $H_E$ (SE) | $F_{IS}$ (SE) |
|---------------------------------------|------------|----------------|----|-----------|---------------|------------|------------|--------------|
|                                       | 1          | Niseko         | 25 | 8.9 (0.6) | 3.8 (0.2)     | 0.725 (0.029) | 0.668 (0.028) | −0.085 (0.004) |
|                                       | 2          | Gorobeisawa    | 24 | 10.3 (0.7) | 6.2 (0.5)     | 0.771 (0.027) | 0.776 (0.022) | 0.007 (0.018) |
|                                       | 3          | Horobetsugawa  | 20 | 9.1 (0.5)  | 4.8 (0.3)     | 0.781 (0.022) | 0.752 (0.021) | −0.039 (0.019) |
|                                       | 4          | Horobetsugawa  | 24 | 10.5 (0.6) | 4.6 (0.3)     | 0.672 (0.034) | 0.735 (0.018) | 0.086 (0.046) |
|                                       | 5          | Tsubamenosawa  | 27 | 10.0 (0.5) | 4.7 (0.2)     | 0.773 (0.015) | 0.766 (0.014) | −0.009 (0.014) |
|                                       | 6          | Sannosukesawa  | 24 | 10.1 (0.6) | 5.5 (0.3)     | 0.771 (0.017) | 0.785 (0.019) | 0.018 (0.014) |

*Note: Standard errors are shown in parentheses. Abbreviations: $F_{IS}$, inbreeding coefficient; $H_E$, gene diversity; $H_O$, observed heterozygosity; $n$, number of analyzed individuals; $N_A$, number of different alleles; $N_{EF}$, effective number of alleles.*
(ii). This resampling was repeated 2000 times, and each aforementioned observed value of the increase was compared with the corresponding 2000 simulated values from the resampling to test the significance. The same procedure was employed for the increase from category (ii) to (iii). The significance of the increases was tested with Bonferroni correction for the number of examined parameters (3). These calculations were made using R ver. 3.6.3 (R Core Team, 2020) [Correction added on 14 May 2021, after first online publication: ‘R Development Core Team, 2012’ has been updated to ‘R Core Team, 2020’ in text citation and Reference list.].

2.6 Two-generation analysis

To estimate genetic variation in pollen pools among seed parents and genetic differentiation in pollen pools between pollen sources, that is, local or foreign sources for the Niseko population, we carried out two-generation (two-gener) analysis (Smouse, Dyer, Westfall, & Sork, 2001). We used genotypes of four seed parents (m1 to m4) and their 225 seeds, the pollen sources for which were determined by parentage reconstruction. The following three model approaches were made by analysis of molecular variance (AMOVA) (Excoffier, Smouse, & Quattro, 1992) using the GENEALEX 6.5 software package (Peakall & Smouse, 2006).

First, we used model 1 (equation (1)) to estimate the genetic differentiation in global pollen pools between local and foreign sources (ΦIO) averaged for all seed parents and genetic variation in pollen pools among seed parents nested within the sources (ΦM/IO for both sources):

\[ G_{ijk} = \mu + f_{ij} + \omega_{ijk} \]  

(1)

where \( G_{ijk} \) is a haplotype vector of the \( k \)th pollen gamete from the \( i \)th source and \( j \)th seed parent, \( \mu \) is a mean vector of all pollen gametes, \( f_{ij} \) is the average effect of the \( i \)th source, \( \omega_{ijk} \) is the replication error associated with the \( k \)th pollen gamete from the \( i \)th seed parent and the \( j \)th source.

Second, we used model 2 (equation (2)) to estimate the genetic variation in pollen pools among seed parents (ΦM) and genetic differentiation in pollen pools between sources nested within seed parents (ΦIO/M for all seed parents):

\[ G_{ijk} = \mu + f_{ij} + \omega_{ijk} \]  

(2)

where \( G_{ijk} \) is a haplotype vector of the \( k \)th pollen gamete from the \( i \)th seed parent and the \( j \)th source, \( f_{ij} \) is the average effect of the \( i \)th seed parent, \( \omega_{ijk} \) is the replication error associated with the \( k \)th pollen gamete from the \( i \)th seed parent and the \( j \)th source.

\( \omega_{ijk} \) is the replication error associated with the \( k \)th pollen gamete from the \( i \)th seed parent and the \( j \)th source.

Third, we used the standard model (equation (3)) of Smouse et al. (2001) to estimate the genetic variation among seed parents, of local (ΦMI) and foreign (ΦMO) pollen pools, and of total pollen pools (ΦMT), separately:

\[ G_{ij} = \mu + f_{ij} + \omega_{ij} \]  

(3)

where \( G_{ij} \) is a haplotype vector of the \( j \)th pollen gamete from the \( i \)th seed parent, \( f_{ij} \) is the average effect of the \( i \)th seed parent, and \( \omega_{ij} \) is the replication error associated with the \( j \)th pollen gamete from the \( i \)th seed parent.

We also used ΦMI, ΦMO, and ΦMT to calculate the effective number of pollen parents (Neq; Smouse et al., 2001) by \( N_{eq} = 1/(2\Phi) \).

3 RESULTS

3.1 Genetic diversity and differentiation in the Niseko population

Three genetic-diversity parameters, \( N_A \), \( N_{EF} \), and \( H_E \), for the Niseko population were 8.9 ± 0.6 (standard error), 3.8 ± 0.2, and 0.668 ± 0.028, respectively, and lower than those for the 5 posterior northern populations (Table 2). The \( F_{IS} \) value for the Niseko population (−0.085 ± 0.004) was also lower than those for the five northern populations.

The PCA results showed substantial genetic differentiation from five northern populations in all successional stages of the Niseko population (Figure 3). The contribution ratio of the first (Co1) and second components (Co2) were 44.5% and 22.1%, respectively. Co1 distinguished three successional stages in the Niseko population as negative and other five northern populations as positive scores. Co2 isolated 4-Horobetsugawa as positive and 3-Horobetsugawa and 5-Tsubamenosawa as negative scores. Population 2-Gorobeisawa and 6-Sannosukesa were relatively large populations in the northern range (Kitamura et al., 2015) and those showed similar Co2 scores to the Niseko population.

3.2 FSGS for the three successional stages of the Niseko population

The results from the FSGS analyses of the three successional stages of the Niseko population are shown in Figure 4 and Supporting Information Table S1. The regression slope was significantly negative for juveniles (\( b_p = -0.013, p < 0.001 \); Supporting Information Table S1) but not for mature trees or seedlings. As a
result, FSGS was detected among juveniles, but not among mature trees or seedlings. Also, the correlogram of juveniles showed significantly positive and negative values for mean $F_g$ at the first and third distance classes, but mature trees and seedlings did not show significant mean $F_g$ values at any distance class.

### 3.3 | Gene flow and reproductive success parameters

The results from the neighborhood model approach are shown in Table 3. For juveniles, the parameters $s$, $m$, $d$, and $g$ in relation to pollen dispersal were $0.240 \pm 0.076$, $0.488 \pm 0.097$, $16.8$ m, and $0.827 \pm 0.224$, respectively. The parameters $m$, $d$, and $g$ in relation to seed dispersal were $0.461 \pm 0.075$, $5.0$ m, and $0.722 \pm 0.202$, respectively. The 95% confidence intervals of parameter $s$ and parameters $m$ and $g$ for both seed and pollen dispersal were higher than zero.

For seedlings, the parameter $s$ and parameters $m$, $d$, and $g$ in relation to pollen dispersal were $0.000$, $0.479 \pm 0.086$, $13.3$ m, and $0.629 \pm 0.179$, respectively. The values of $m$, $d$, and $g$ for seed dispersal were $0.095 \pm 0.049$, $10.3$ m, and $0.633 \pm 0.144$, respectively. The 95% confidence intervals of the parameters $m$ for pollen dispersal and $g$ for both seed and pollen dispersal were higher than zero.

For seeds, the parameter $s$ and parameters $m$, $d$, and $g$ in relation to pollen dispersal were $0.000$, $0.235 \pm 0.032$, $6.5$ m, and $1.494 \pm 0.125$, respectively. The 95% confidence intervals of parameters $m$ and $g$ for pollen dispersal were higher than zero.

### 3.4 | Effects of seed and pollen immigration on the genetic diversity of offspring

Based on the results from the parentage reconstruction, three categories of seed and pollen sources were determined for offspring. Of all the 70 juveniles, 33 (47.1%) and 37 (52.9%) were reproduced by foreign and local seed parents, respectively. Among the 37 juveniles with local seed parents, 17 (45.9%) and 20 (54.1%) were pollinated by foreign and local pollen parents, respectively. Of all 55 seedlings, 5 (9.1%) and 50 (90.9%) were reproduced by foreign and local seed parents, respectively. Among the 50 seedlings from local seed parents, 26 (52.0%) and 24 (48.0%) were pollinated by foreign and local pollen parents, respectively. Of all 225 seeds, 50 (22.2%) and 175 (77.8%) were pollinated by foreign and local pollen parents, respectively.

Genetic diversity parameters for categories (i), (ii), and (iii) at the 3 successional stages are shown in Figure 5. For juveniles, $N_A$, $N_{EF}$, and $H_E$ for category (i), consisting of 20 individuals, were 5.4, 3.493, and 0.634, respectively.
respectively. The values for category (ii), consisting of 37 individuals, were 9.3, 3.740, and 0.648, respectively. The values for category (iii), consisting of all 70 individuals, were 12.8, 4.030, and 0.663, respectively. The increases in $N_A$, $N_{EF}$, and $H_E$ from category (i) to (ii) were 3.9, 0.247, and 0.013, respectively, with the increase in $N_A$ being significant ($p < 0.005$) although the increases in the other two parameters were not. The corresponding increases from category (ii) to (iii) were 3.5, 0.290, and 0.016, respectively, with the increase in $N_A$ being significant ($p < 0.05$) although the increases in the other two parameters were not.

For seedlings, the values of $N_A$, $N_{EF}$, and $H_E$ for category (i), consisting of 24 individuals, were 5.8, 2.980, and 0.582, respectively. The values for category (ii), consisting of 50 individuals, were 9.0, 3.312, and 0.618, respectively. The values for category (iii), consisting of all 55 individuals, were 9.9, 3.412, and 0.621, respectively. The increases in $N_A$, $N_{EF}$, and $H_E$ from category (i) to (ii) were 3.3, 0.332, and 0.036, respectively, with only the increase in $N_A$ being significant ($p < 0.05$). The increases from category (ii) to (iii) were 0.9, 0.100, and 0.003, respectively, with none of the increases being significant.

For seeds, the values of $N_A$, $N_{EF}$, and $H_E$ for category (i), consisting of 175 individuals, were 5.4, 2.775, and 0.530, respectively. The corresponding values for category (ii), consisting of all 225 individuals, were 11.1, 2.973, and 0.556, respectively. The increases in $N_A$, $N_{EF}$, and $H_E$ from category (i) to (ii) were 5.8, 0.199, and 0.027, respectively, with the increases in all three parameters being significant ($p < 0.005$).

### 3.5 Genetic variation in pollen pools among seed parents and differentiation between local and foreign pollen pools

We categorized 225 seeds into two groups for two-gener analyses: those having local (175) and foreign (50) pollen parents based on parentage reconstruction. Two-gener
analyses based on model 1 showed significant genetic differentiation ($\Phi_{IO} = 0.009, p < 0.05$) between local and foreign sources of the global pollen pool averaged for all seed parents. The analysis also showed significant genetic variation among seed parents nested within sources ($\Phi_{M/O} = 0.180, p < 0.005$). The analysis based on model 2 revealed significant genetic variation in pollen pools among seed parents ($\Phi_M = 0.046, p < 0.005$) and genetic differentiation between local and foreign sources of pollen pools nested within seed parents ($\Phi_{DOM} = 0.149, p < 0.005$). Two-gener analyses based on model 3 showed significant genetic variation among seed parents for local ($\Phi_{MI} = 0.235, p < 0.005$), foreign ($\Phi_{MO} = 0.024, p < 0.05$), and total pollen pools ($\Phi_{MT} = 0.140, p < 0.005$). $N_{IS}$ values calculated by $\Phi_{MI}$, $\Phi_{MO}$, and $\Phi_{MT}$ values were 2.1, 20.8, and 3.6, respectively.

4 | DISCUSSION

4.1 | Genetic drift in the initial colonizing process of range expansion

Geographical range expansion of temperate forest species during post-glacial warming has been revealed by fossil pollen and molecular markers (Cheddadi et al., 2006; Liepelt et al., 2009; Magri et al., 2006; McLachlan et al., 2005; McLachlan & Clark, 2004; Petit et al., 2002). In regard to genus Fagus, the European (Comps et al., 2001) and North American (Kitamura & Kawano, 2001; McLachlan et al., 2005) beech species expanded their distribution northward, resulting in a loss of genetic diversity in northern populations. Our previous study of F. crenata revealed a decline in genetic diversity toward the northern range margin (Kitamura et al., 2015). Moreover, the present study revealed a further reduction in genetic diversity at the expanding front-edge population, i.e., the Niseko population (Table 2).

In addition, our present results provided circumstantial evidence that the present mature trees might be the earliest founders of the Niseko population. First of all, strong genetic differentiation was observed by the PCA, which distinguished the Niseko population from posterior northern populations by CoI scores (Figure 3). This may be because of the population isolation and a bottleneck effect, in which the limited number of colonizers results in a stochastic loss of genetic diversity, especially when populations are small (Nei, Maruyama, & Chakraborty, 1975). Also, isolation by the fragmented landscape occupied by the Niseko population prevented genetic homogenization with the posterior northern populations.

Second, the estimated $F_{IS}$ of the Niseko population was negative and lower than those of the other five northern populations (Table 2). This result indicated that the Niseko population underwent a recent bottleneck event. When a population experiences a reduction in size and remains small in size, allelic loss occurs more rapidly than that of heterozygosity, resulting in heterozygosity excess (Maruyama & Fuerst, 1985). The heterozygosity excess is only observed for a short period after the bottleneck event and is lost in later generations (Luikart & Cornuet, 1998). A negative $F_{IS}$ value was observed in all 8 loci for the Niseko population (data not shown), which indicates a heterozygosity excess by the recent bottleneck of a founder event. For this reason, the negative $F_{IS}$ indicates that the mature trees in the Niseko population might have originated from a small number of individuals derived from large seed sources by long-distance dispersal.

Third, the non-significant FSGS among the mature trees (Figure 4a) suggests that many of these individuals did not originate from local seed parents as barochore species like genus Fagus generally show significant FSGS among reproductive individuals (Kitamura, O’Neill, Wigham, & Kawano, 1998; Linhart, Mitton, Sturgeon, & Davis, 1981; Oddou-Muratorio et al., 2010) due to the aggregation of seed-parental siblings around the seed parents (Hamrick & Nason, 1996; Inanaga, Nakanishi, Torimaru, Nishimura, & Tomaru, 2014; Kawano & Kitamura, 1997; Kitamura et al., 2003). The agents of long-distance seed dispersal of F. crenata were likely birds such as nutcrackers (Kobayashi & Watanabe, 2003; Vander Wall & Balda, 1977) and jays (Johnson & Adkisson, 1985; Tanaka et al., 2016), which are capable of carrying seeds over several kilometers. As long-distance dispersal practically contributes to erasing local FSGS (Jordano, 2017), the first colonizers would not show FSGS, indicating that they originated from other unrelated sources (Pluess, 2011; Troupin, Nathan, & Vendramin, 2006).

4.2 | Gene flow via seeds and pollen to the leading-edge population

The mean distance of seed and pollen dispersal (5.0—10.3 and 6.5—16.8 m, respectively) within the Niseko population was shorter than those of other studies on larger populations of Fagus species (Asuika et al., 2004; Hanaoka et al., 2007; Inanaga et al., 2016; Kitamura, Kobayashi, Kodani, & Yada, 2008), which may be due to the population area being extremely small. However, we detected significant proportions of pollen (0.235—0.488 for all examined stages) and seed (0.461 for juvenile) immigration to the Niseko population (Table 3). Due to the anemophily and barochory, long-distance pollen flow and relatively short-distance seed dispersal are commonly observed among F. crenata and other Fagus species.
Long-distance pollen flow over many kilometers should be general for anemophilous species (Kremer et al., 2012), and previous studies on isolated populations reported long-distance gene flow via pollen exceeding 7 km for *F. crenata* (Inanaga et al., 2016) and 5 km for holm oak (Hampe, Pemonge, & Petit, 2013). However, this study indicated long-distance gene flow not only via pollen but also via seeds exceeding 12 km, as no other populations or individuals were found within a 12 km radius of the Niseko population.

The neighborhood model approach estimated a 24.0% selfing rate for juveniles despite the allogamy of *F. crenata* (Table 3). Similar results were obtained from previous experiments of imperfect self-incompatibility among individual trees of *Fagus* species. For example, Terazawa (1997) detected a 2.2% to 10.4% selfing rate in *F. crenata*. *Fagus sylvatica* showed a 0% to 13% selfing rate in artificial crossing (Nielsen & Muckadeli, 1954; Wang, 2003) and an even higher rate of 48% at a marginal population, which might be attributable to pollen limitation (Gauzere, Klein, & Oddou-Muratorio, 2013). These findings indicated that not all, but a small number of individuals are partially self-compatible. It might be the case in small, isolated populations where the number of mating individuals is limited that the reproductive success of partially self-compatible plants is higher than that of self-incompatible ones. Therefore, it is possible to speculate that partially self-compatible plants reproduce more progeny in early successional phases.

The immigration rates of seeds and pollen declined in the later successional stages, i.e., the pollen immigration rate fell from 0.488 for juveniles to 0.235 for seed stages, and the seed immigration rate from 0.461 for juveniles to 0.095 for the seedling stage (Table 3). This may be due to population growth. We detected positive effects of the basal area of mature trees on seed- and/or pollen-parental reproductive success in all successional stages, which indicated that larger individuals contribute more to reproduction. Although early regeneration largely depends on foreign seed and pollen when founders are immature or small, the proportion of the founders’ contributions increases as the size and the number of mature individuals increases. Subsequently, the effective population size increases and foreign gene flow decreases.

These changes in effective population size may also be supported by the FSGS being significant in juvenile but not in seedling stages. Juveniles showed a significant FSGS (Figure 4b, Supporting Information Table S1), indicating the aggregation of seed-parental siblings within the population due to limited seed dispersal within the population (5.0 m estimated by the neighborhood model approach). Within a couple of decades of colonization, a small number of initial colonizers might reach reproductive maturity (Kitamura et al., 2015). The neighborhood model approach estimated more than half of the juveniles reproduced by local seed parents (Table 3). Furthermore, as a few initial colonizers gain reproductive dominance in early successional forests (Hamrick & Trapnell, 2011), the overlap of seed shadows might be small, which also contributes to the induction of FSGS (Oddou-Muratorio & Klein, 2008). Thus, the significant FSGS among juveniles might reflect earlier regeneration by local seed dispersal.

The non-significant FSGS among seedlings (Figure 4c, Supporting Information Table S1) appears to indicate an increasing number of seed parents, promoting overlapping seed shadows (Hamrick & Trapnell, 2011), as more than 90% of seedlings were estimated to originate from local seed parents based on the neighborhood model approach (Table 3). Despite the limited seed dispersal distance within the population (10.3 m; Table 3), a high density of mature trees (25 mature trees in a ca. 0.1 ha population area) and the small population area itself should induce overlapping seed shadows and weaken the FSGS (Oddou-Muratorio & Klein, 2008).

The small sample sizes might be due to the non-significant FSGS among mature trees and seedlings (Figure 4a,c); however, the circumstances underlying this might differ between the mature trees and seedlings. Most of the mature trees would be founder individuals of this population (Tanaka et al., 2016), which colonized the area from different unrelated outside seed sources. Therefore, the FSGS could not be observed among mature individuals (Figure 4a). In contrast, the seedlings were mainly reproduced by local seed parents as indicated by the neighborhood model approach (Table 3). However, due to the increased reproductive success of local individuals as they reached maturity and grew in size, the overlapping seed shadows produced by the high density of reproductive trees could weaken the FSGS among seedlings (Figure 4c). Changes in the FSGS in different successional stages may indicate the process of initial colonization gradually reaching reproductive maturity, thus increasing the effective population size. Similar changes for pollen sources, from foreign to local, was also observed in the expanding front populations of Sitka spruce (Elleouet & Aitken, 2019).

### 4.3 Effects of foreign gene flow via seeds and pollen on the genetic diversity of the leading-edge population

Seed immigration significantly increased the $N_A$ value of juveniles but not that of seedlings in the Niseko population.
Seeds from foreign sources introduced new or rare genetic variations into the population and increased the genetic diversity of the juveniles, but the seed immigration rate later decreased, probably with increases in seed-parental reproductive successes of the local mature trees (Table 3). On the other hand, pollen immigration significantly increased the $N_a$ values of all offspring stages as well as the $N_{EF}$ and $H_E$ values of the seed stages (Figure 5). Furthermore, two-gener analyses indicated genetic differentiations in pollen pools between foreign and local sources, and the $N_{EF}$ of foreign sources (20.8) was markedly higher than that of the local source (2.1). Although both of the pollen and seed immigration rates decreased in later successional stages (Table 3), the effect of pollen immigration may have persisted for a longer period than that of seed immigration. As a consequence, the Niseko population is currently recovering its genetic diversity by effective long-distance pollen flow from foreign sources. Also, previous studies of marginal populations indicated rapid recovery of the genetic diversity by gene flow, but this recovery became saturated with population growth (Elleouet & Aitken, 2019; Lesser et al., 2013). Moreover, the recovery was incomplete due to a shift from foreign to local pollen flow (Elleouet & Aitken, 2019). In fact, the recovery of genetic diversity by gene flow and subsequent decreases in pollen immigration rates are consistent with our present results. Thus, the increase in genetic diversity will become saturated eventually due to decreases in pollen immigration in later successional stages. This might retard accumulating the genetic diversity to those of posterior populations and hinder evolutionary potential for the further range expansion of *F. crenata*.

5 | CONCLUSIONS

The recently discovered leading-edge population of *F. crenata* was revealed to be a founder population. It showed lower genetic diversity and higher differentiation from other populations than did the other northern marginal populations. Mature individuals in this population might be the first colonizers, which established the population and have grown to reproductive maturity at present. A significant proportion of pollen and seed immigration contributed to increase the genetic diversity of the offspring. The effective number of pollen parents for seeds from foreign sources was markedly higher than that from the local population. Thus, pollen immigration continuously supplies genetic variations to this leading-edge population.

However, when the size and the number of reproductive individuals in the local population increases, local reproductive success increases and the proportion of immigrant genes will decrease in the future (Elleouet & Aitken, 2019; Sezen, Chazdon, & Holsinger, 2005, 2007). Lesser et al. (2013) pointed out that the accumulation of alleles would be saturated when the population size grows to a certain number of individuals. We should be careful with respect to the accumulation of genetic diversity at the leading-edge population to understand how its level of genetic diversity allows it to adapt to the new environment and expand the further geographic range.

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