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Landscape genetics of a threatened maple, *Acer miyabei*: implications for restoring riparian forest connectivity

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Abstract

Because forest fragmentation affects ecological connectivity, establishing corridors is increasingly important in conserving biodiversity. Conserving the connectivity of riparian forests should be a priority because they often support rich and unique biota but are greatly modified by humans. *Acer miyabei* is a threatened maple which grows in floodplain ecosystems in northern Japan. We examined the effects of forest fragmentation on its genetic connectivity and identified candidate areas to be restored as riparian forest corridors. We collected leaf samples from 290 of *A. miyabei* individuals in 13 populations and determined pairwise genetic distances among the populations using 12 microsatellite loci. We also calculated geographic and resistance distances; the latter was quantified by least-cost path and circuit theory models by designating forested or riparian forested areas as having lower resistance than other types of land use. According to multiple regression analyses, genetic distance showed significant positive relationships with resistance distance but was not significantly related to geographic distance. The results indicate that forest fragmentation impedes gene flow of the species. Genetic differentiation among populations was greater in the smaller tree group than in larger one, suggesting that more recently established individuals are exposed to greater genetic isolation than the mature individuals owing to increasing forest fragmentation over time. Reduction of genetic connectivity was conspicuous in and around deforested areas. Such areas can be targeted for promoting connectivity of riparian habitats in future landscape planning.

Key words: circuit theory, corridor, endangered species, gene flow, habitat fragmentation, isolation by resistance

1. Introduction

Forest fragmentation affects ecological connectivity (Radford and Bennett, 2007; Wilcove et al., 1998). Thus, establishing corridors and new habitats has increasing significance in conserving biological diversity (Beier and Noss, 1998). Although the number and coverage of natural areas are limited in urban and agricultural regions, effective networks in such regions facilitate movement, dispersal, and gene flow of organisms, contributing to long-term persistence of natural populations (Gilbert-Norton et al., 2010; Tewksbury et al., 2002). Yet few practical models are available for the implementation of habitat networking (Brodie et al., 2016; Lacher and Wilkerson, 2014). Such knowledge is particularly restricted for riparian forest ecosystems, although rivers intrinsically have a high potential to function as corridors owing to their linear characteristics, as well as their rich and unique biota (Lees and Peres, 2007; Rouquette et al., 2013).

Historically, the utility of rivers in transportation has induced intensive urbanization and development along them. In addition, large levees have been built, and river channels have been regulated for flood prevention (Nakamura et al., 2006; Washitani, 2001). This trend is becoming common in many places across the world, making the conservation of river floodplain ecosystems one of the great challenges of the 21st century (Gergel et al., 2002; Richardson et al., 2007; Tockner and Stanford, 2002). Japan has numerous rivers owing to its abundant precipitation under a monsoon climate and wide elevational gradients. But the flat land alongside these rivers has been heavily altered by development into agricultural and residential areas.

To facilitate networking of habitats, examination of genetic connectivity among extant populations is important because it helps with identifying the spatial features impeding gene flow (Dyer and Nason, 2004; Mech and Hallett, 2001; Storfer et al., 2007). In this framework, landscape genetics, which integrates population genetics and landscape ecology (Manel et al., 2003), is a powerful tool and is increasingly being applied to a wide range of conservation projects (Holderegger and Wagner, 2008; Manel and Holderegger, 2013). Since the research field emerged, animals have been a major target of study (Storfer et al., 2010); in particular, large mammals and birds are well studied because information on their movement is often essential in landscape-level conservation planning (e.g., Epps et al., 2013; Pavlova et al., 2012). In contrast, plants

have drawn less attentions. Yet their gene exchange occurs via pollen and seeds, which is difficult to measure by direct observation in the field. Thus, genetic data are useful in evaluating ecological connectivity of their natural populations (e.g., Dyer et al., 2012; McRae and Beier, 2007; Sork et al., 2010). Furthermore, many plant species are strictly associated with specific ecosystem types, making them excellent, easily observable indicators of threatened ecosystems.

Here, we analyzed the effects of forest fragmentation on gene flow of an endangered plant, *Acer miyabei* Maxim. (Sapindaceae), a riparian maple inhabiting lowland floodplain ecosystems in northern Japan (Ogata, 1965; van Gelderen et al., 1994). The species is designated as Vulnerable (VU) in the national Red List due to recent population declines resulting from habitat loss and fragmentation (Ministry of Environment, Japan, 2012). We focused on this species because of its conservation concern and strict indication of rare and undisturbed riparian forest ecosystems. Its long lifespan allows us to compare the degree of genetic differentiation in young (small) and mature (large) individuals and thus examine how recent landscape changes have affected the genetic diversity of the species.

Our general objective was to characterize the effects of habitat fragmentation on patterns of genetic variation in *A. miyabei* as a basis for landscape connectivity planning of riparian forest ecosystems. The specific objectives were (i) to examine fragmentation effects in recent years by comparing genetic differentiation between small and large individuals; (ii) to test the hypothesis that forest fragmentation interferes with gene flow by using the isolation by resistance model (Adriaensen, et al., 2003; McRae, 2006); and (iii) to identify candidate areas to be prioritized for future restoration projects to promote riparian forest connectivity in the landscape.

2. Materials and methods

2.1 Study species

Acer miyabei is a deciduous tree species that grows in temperate forests in East Asia (Ogata, 1965; van Gelderen et al., 1994). Mature trees often grow more than 15 m tall with a diameter at breast height (DBH) of 40 cm. Its occurrence is strongly associated with river floodplain ecosystems, occurring on both first and second terraces and on the slopes along river valleys. *Acer miyabei* consists of three intraspecific taxa: (i) ssp.

miyabei f. *miyabei* in central to northern Japan; (ii) ssp. *miyabei* f. *shibatae* (Nakai) K. Ogata in a small area of Honshu; and (iii) ssp. *miaotaiense* (Tsoong) A. E. Murray in China. All of these taxa are listed in national or IUCN Red Lists because of their limited range and habitat decline caused by agricultural and residential development (Ministry of Environment, Japan, 2012; IUCN, 2016). This study focused on natural populations of *A. miyabei* ssp. *miyabei* f. *miyabei* (hereafter, *A. miyabei*). This taxon covers a relatively wide area of southwestern Hokkaido, enabling us to assess genetic connectivity at the landscape level. Sexual expression is polygamo-dioecious, characterized by dichogamous hermaphrodite, female, and male flowers (Hotta, 2004). Flowers are yellow, and the pollinators are Diptera (Bibionidae) (Hotta, 2004; Nagamitsu et al., 2014), Coleoptera (Cerambycidae), and Hymenoptera (Tenthredinidae) (pers. obs.), which are generally known to be more abundant in natural forests than in urbanized areas. Samaras are dispersed by wind and occasionally by water when trees grow beside rivers and streams.

2.2 Field collection

Leaf tissues of *A. miyabei* were collected from 290 individuals at 13 sites (i.e., populations) in southwestern Hokkaido (42.42°–42.80°N, 141.59°–142.48°E; Fig. 1, Table 1). The sampled populations lay within an area of approximately 80 km × 100 km including seven major river basins. The average elevation of collection sites was 83.8 m a.s.l. (range, 7–204 m).

For population genetic analyses, we collected foliage from 18–45 individuals per population and recorded their DBH (ranging, 0.7–54.1 cm; Table 1). We noted that individuals with DBH ≥ 15 cm tended to be of reproductive age and consisted of 33% of our samples. Although the individual can be used as the operational unit in landscape genetics (Manel et al., 2003), we used the population because *A. miyabei* typically grows within discrete forest patches on floodplains. Collection was made from September 2013 to September 2014. The foliage samples were dried in silica gel immediately after collection and stored at room temperature until DNA extraction.

2.3 Laboratory procedures

DNA was extracted with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Each

DNA sample was assigned an identification number (Lab ID), which is linked to information on geographic location, herbarium label information, or labels for trees sampled in the field. Voucher specimens of representative samples are stored at the Makino Herbarium (MAK) of Tokyo Metropolitan University, Japan (App. 1).

To assess genetic diversity, genotypes of 12 microsatellite markers (Saeki et al., 2015) were scored at the Sugadaira Montane Research Center, University of Tsukuba, Japan. DNA (ca. 10 ng) was placed into each well of a 96-well plate and dried at room temperature over several hours, followed by multiplex PCR in 2 μL (Kenta et al., 2008) containing 1× TYPE-IT Microsatellite PCR Kit Master Mix (Qiagen) and 0.2 μM each primer, with 6 μL of mineral oil overlaid. Each forward primer was labeled with either FAM, HEX, PET, or NED fluorescent dye. We also prepared unlabeled forward primers and mixed them with fluorescent ones, following Suyama (2012). The ratio was initially set at 1 fluorescent to 24 unlabeled, but was changed later for optimizing fluorescent signals (see Saeki et al., 2015 for details). The thermal-cycler program was 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 90 s, and 72 °C for 30 s; and a final 72 °C for 30 min. The PCR products were directly mixed with 0.25 μL of GeneScan-500 LIZ size standards (Applied Biosystems, Foster City, CA, USA) and 9.25 μL of Hi-Di formamide (Applied Biosystems). Samples were run on an ABI 3130 Genetic Analyzer (Applied Biosystems), and PCR products were examined in GeneMapper v. 4.0 software (Applied Biosystems).

2.4 Data analyses

To compare genetic diversity among the 13 populations, we calculated the average number of alleles (A) and observed and expected heterozygosity (H_O and H_E) for each population in GenAlEx v. 6.502 software (Peakall and Smouse, 2006, 2012). The fixation index within a population (F_{IS}) and allelic richness (R) were calculated in FSTAT v. 2.9.3 software (Goudet, 1995). Null allele frequencies were estimated with CERVUS v. 3.0.7 software (Kalinowski et al., 2007; Marshall et al., 1998).

To compare genetic characteristics between young and mature trees, we divided samples of each population into a small-DBH group (n = 141) and a large-DBH group (n = 149). The individuals were divided at the median to make the sample sizes nearly equal. For those individuals having the median-size DBH, we randomly assigned them

194 into either small or large tree group. The maximum DBH of the small tree group per
195 population ranged from 2.3 to 15.5 cm (Table 1); ages were estimated as about 10–40
196 years old.
197 For evaluation of genetic connectivity among the populations, we calculated the
198 pairwise Cavalli-Sforza and Edwards' (1967) chord distance *D*c and the pairwise
199 fixation index F_{ST} (Weir and Cockerham, 1984) among the 13 populations in MSA 4.05
200 (Dieringer and Schlötterer, 2003) and the HIERFSTAT package (Goudet, 2005) in R v.
201 3.2.3 (R Development Core Team, 2015), respectively. Then we calculated the average
202 *D*c and F_{ST} of each population for the large and small tree groups. We assumed that the
203 difference in *D*c and F_{ST} between groups provided a reasonable estimate of change in
204 genetic differentiation from the past to recent times. Analyses of molecular variance
205 (AMOVA; Excoffier et al., 1992) were performed for both groups with 999
206 permutations to estimate genetic variation within and among populations. To determine
207 whether the two size classes yield different levels of genetic differentiation among
208 populations, we used a randomization test: We shuffled individuals within each
209 population and divided them randomly into two groups without replacement. In this
210 process, the two sets of global F_{ST} (Weir and Cockerham, 1984) were calculated from
211 the shuffled data using the HIERFSTAT package (Goudet, 2005) in R v. 3.2.3 (R
212 Development Core Team, 2015). The difference between the two global F_{ST} values was
213 computed with 9999 replacements. Then the observed value was compared with this
214 null distribution for the hypothesis (i.e., F_{ST} in the small class > F_{ST} in the large class) to
215 assess the significance with a one-tailed test.
216 To examine the relationship between genetic distance and forest fragmentation, we
217 calculated geographic and resistance distances. The geographic distance was obtained in
218 Arc Map v. 10.3.1 software (ESRI, Redlands, CA, USA) with the Euclidean distance
219 calculation function and was used for testing the isolation by distance model (Wright,
220 1943). To obtain resistance distances, we first developed resistance surfaces by
221 designating forested areas along rivers as having lower resistance than the other land-
222 use areas; we set the ratio of resistance values at 1:10, presuming that areas lacking
223 forests or rivers extremely impede gene flow of *A. miyabei*. This means that pollination
224 and seed dispersal occur much better in forests along rivers than in all other types of
225 land use. In other words, the abundance of pollinators decreases in non-forested areas

(e.g., Taki et al., 2010), and *A. miyabei* can establish by seed only at sites along rivers. We call this the RF-model (riparian forest versus others). In this model, cells containing river and forested areas were assigned low resistance values. Cell sizes were set at different spatial scales, as described below. We also tested a model that designates forested areas as having lower resistance than non-forested areas, regardless of distance from rivers. We call this model the FN-model (forest versus non-forest). There are numerous ways to develop resistance surfaces for examination of genetic connectivity (Spear et al., 2010). We selected a limited number of models (i.e., hypotheses) based on the prior knowledge that habitat suitability of *A. miyabei* is clearly high in riparian floodplain forests, and that gene flow by pollination is dependent on flies (Diptera) (Hotta, 2004; Nagamitsu et al., 2014) and other flying insects known to be abundant in forests. In terms of resistance values, we also tested 1:100 models, but because 1:10 models provided a better fit, we selected 1:10 values.

The resistance surfaces were prepared in a GIS raster format with digital maps of forest cover and river channels that were obtained from the open database of MLITT (2016). The cell size was set at scales of 500, 1000, 2000, and 3000 m for validation of results. These scales were selected *a priori* based on a spatial genetic structure of 2600 m between *A. miyabei* trees (Nagamitsu et al., 2014), as well as the general resolution of land-use heterogeneity in our study area. In total, eight types of resistance surfaces were created from the combinations of the two models (RF-model, FN-model) × four spatial scales (500, 1000, 2000, and 3000 m pixel sizes).

With the above resistance surfaces, we quantified resistance distances using two algorithms, least-cost path (LCP; Adriaensen et al., 2003) and circuit theory (CT; McRae, 2006). Resistance distance by LCP was determined in Linkage Mapper v. 1.1.0 software (McRae and Kavanagh, 2016). That by CT was obtained in Circuitscape v. 4.0 software (McRae et al., 2013). The main difference between these algorithms is that LCP represents a single optimal pathway, whereas CT accounts for multiple pathways. In the CT-based modeling, maps with cumulative current among the studied populations were also produced for each resistance surface to identify areas with higher current density, or that are expected to contribute most to connectivity (McRae et al., 2008; Fig. 2). Consequently, we obtained 16 resistance distances based on the two algorithms and eight sets of resistance surfaces.

We performed multiple regression analyses on distance matrices (MRM, Legendre et al., 1994; Lichstein, 2007) to examine the relationship between genetic, geographic, and resistance distances. Genetic distance was quantified by *D*c, following Dyer et al. (2010) and Séré (2017), who reported that *D*c shows relatively better sensitivity performance when it is used to examine isolation by distance as well as isolation by resistance models. We tested the significance of regression by using 2000 permutations. Then we modeled a linear regression of genetic distance with geographic and resistance distances. For each regression model, Akaike's information criterion (AIC) was determined to assess the goodness-of-fit of each landscape model. These analyses were performed in R with the MRM function in the library ecodist (Goslee and Urban, 2008). The analyses were performed for three sample sets: all, large, and small tree groups. When forest fragmentation reduces gene flow, we expect that genetic distances would show higher correlations and better model fit with resistance distances than with geographic distances (McRae, 2006).

3. Results

3.1 Genetic diversity of *Acer miyabei*

For all individuals, the genetic diversity indices were similar across populations (Table 2). The mean number of alleles per locus (*A*) ranged from 2.92 (Baro) to 4.75 (Shizunai-North), and the average heterozygosity indices were $H_O = 0.52$ (range, 0.42–0.62) and $H_E = 0.51$ (range, 0.40–0.60) (Table 2). No loci consistently showed a high null allele frequency estimate per population (>0.1).

The average allelic richness (*R*) was 1.51 after rarefaction to 2 (range, 1.40–1.60). The average fixation index within a population (F_{IS}) ranged from –0.10 to 0.09, none of which was significantly different from 0 ($p > 0.05$). The average pairwise Cavalli-Sforza and Edwards' (1967) chord distance (*D*c) varied greatly among populations: the largest *D*c for all individuals occurred in the Bibi population (0.39), followed by Baro (0.36) and Rankoshi (0.34), and the smallest in the Toyonukabashi and Shizunai-North populations (0.27). The average pairwise F_{ST} showed patterns similar to those of *D*c: the highest was 0.17 (Baro), and the smallest was 0.07 (Toyonukabashi).

In comparisons between small and large tree groups, there were no significant differences in *A*, H_O, H_E, F_{IS}, or *R* (pairwise *t*-test, $P > 0.1$). The values of these indices

290 are similar to those of all individuals in each population. In contrast, average pairwise
291 genetic distances among the populations (*D*c and F_{ST}) were higher in the small tree
292 group than in the large tree group. By population, 10 and 11 of the 13 populations
293 showed greater genetic distances in the small tree group as assessed by *D*c and F_{ST},
294 respectively. The greatest difference between the groups was found in the Nioi, Biu, and
295 Shizunai-south populations by *D*c (0.05) and in the Shizunai-South population by F_{ST}
296 (0.06). To illustrate spatial patterns of differences in genetic distances between small
297 and large tree groups, we drew a Voronoi diagram for the 13 sampled populations and
298 calculated differences of genetic distances between the small and large tree groups. We
299 selected the Voronoi algorithm by assuming that gene flow most likely occurs through
300 nearest neighbors (Dupanloup et al., 2002). In general, marked increases in genetic
301 distance were detected in the populations in and around the non-forested areas (Fig. 3).
302 Marked differences in *D*c (>30%) between the large and small tree groups were found
303 in pairs between Biu, Shizunai-North, and Shizunai-South (44%, 39%, and 34%).
304 Similar results were obtained for F_{ST} (App. 2). By AMOVA, the large tree group
305 contained 17% of variation among populations ($P < 0.002$) and 83% within populations,
306 and the small tree group contained 22% among and 78% within populations ($P < 0.002$).
307 The observed difference in global F_{ST} between small and large tree sample sets (0.029)
308 was significantly higher than the average difference obtained by randomization ($P <$
309 0.05). This result indicates that there is likely more differentiation than expected from a
310 random process.
311
312 3.2 Effects of forest fragmentation on gene flow
313 Among all individuals, there were significant relationships between genetic and
314 resistance distances, but no significant relationships between genetic and geographic
315 distances (Table 3). The highest correlation with genetic distance (*D*c) was obtained
316 from the RF-model based on CT with a 3000-m spatial scale ($r^2 = 0.348$, $P < 0.01$) with
317 the lowest AIC among the tested models. Yet the other three models (FN-models with
318 1000-, 2000-, and 3000-m scale based on CT) also showed similar goodness-of-fit with
319 similar AIC values (i.e., delta AIC $\leq$ 2.0). In the large tree group, the same RF-model
320 was best supported ($r^2 = 0.334$, $P < 0.01$), followed by the FN-model with 3000-m scale
321 based on CT. In the small tree group, the RF-model based on CT with a 2000-m scale

was supported by the highest correlation and the lowest AIC value (r^2 = 0.354, P < 0.01). The RF-model with 1000-m scale (CT) and the FN-model with 2000-m scale (CT) also showed similar goodness-of-fit with similar AIC values.

4. Discussion

In the pre-modern era, forests in Hokkaido were relatively well preserved because the human population density remained low. The indigenous Ainu people depended on hunting and gathering, and thus retained a large amount of natural forests. In the late 19th century, however, the rapid decline of forests started when the number of immigrants from Honshu dramatically increased (Miura, 2011). Most of the flat lowlands, such as those in our study region, were converted to agricultural and residential uses. Over the past 50 years, natural forests were also largely converted to urban and agricultural uses (Himiyama, 1995). We aimed to determine the effects of this landscape change by examining genetic connectivity of *A. miyabei*, a species characterizing the riparian forest ecosystem. Our results indicate that forest fragmentation reduces its genetic connectivity (Tables 2, 3; Fig. 3; App. 2).

According to the MRM analyses, the genetic distances were significantly related to resistance distances, whereas they were not or were much less significantly related to geographic distance (Table 3). Comparisons of pairwise genetic distances between the large and small tree groups showed an increasing trend of genetic isolation among populations (Table 2; Fig. 3), which suggests that more recently established individuals are exposed to greater isolation than mature individuals owing to recent forest fragmentation. The results of AMOVA and randomization tests for global F_{ST} support this pattern.

The strong effects of forest fragmentation on gene flow in *A. miyabei* may be explained by its reproductive traits. *Acer miyabei* is pollinated mainly by insect Diptera (Hotta, 2004; Nagamitsu et al., 2014), which reproduce in litter on the forest floor. Other potential pollinators depend on forest habitats as well. Thus, loss of forests likely reduces gene flow via pollination. In addition, *A. miyabei* produces larger and heavier samaras than other species of *Acer* (van Gelderen et al., 1994). The typical dispersal range is limited to 50 m (Hotta, 2004). Such limitations likely resulted in acceleration of genetic isolation by fragmentation.

354 The spatial genetic structure of riparian plants has been reported. Mosner et al.
355 (2012) examined the genetic structure of a basket willow (*Salix viminalis* L.) that
356 dominates river floodplains in Germany. In spite of severe fragmentation, its population
357 genetic structure was weak, which indicates that gene flow among local populations was
358 active. In contrast, studies of *Populus nigra* L. in France and *P. fremonti* S. Watson in
359 the USA revealed significant genetic differentiation among populations (Cushman et al.,
360 2014; Imbert and Lefèvre, 2003). Meta-analyses of more than 20 riparian plant species
361 found that more than half did not show significant spatial (i.e., Mantel) correlations
362 (Honnay et al., 2010). These patterns seem dependent on the species' dispersal ability
363 and spatial scales of study populations. Yet we infer that gene flow of *A. miyabei* is
364 more restricted than that of *Salix* and *Populus*, widely occurring riparian tree groups
365 whose pollen and seeds are dispersed by wind for long distances.
366 We frequently obtained significant and relatively good model fit between genetic
367 and resistance distances at spatial scales ≥1000 m, whereas the models at the 500-m
368 scale were not supported well in any sample sets (Table 3). This result agrees with an
369 earlier study by Nagamitsu et al. (2014) that gene flow in *A. miyabei* is maintained
370 between fragmented forests separated by up to 500 m around the Neshikoshi population.
371 Negative effects of fragmentation on genetic connectivity of *A. miyabei* are likely more
372 conspicuous at larger spatial scales, and the threshold is probably located between 500
373 and 1000 m. Although *A. miyabei* is predominantly distributed in riparian floodplains,
374 both the RF- and NF-models showed significant correlations with genetic distances. We
375 interpret this result from two perspectives. One is that river floodplain ecosystems are
376 suitable habitats for *A. miyabei*, but not only forests along rivers but also surrounding
377 forests are important for maintaining gene flow. The other is that the topography of the
378 study region is gently hilly with numerous rivers and streams (Fig. 1). Under this
379 condition, many cells at a large scale, such as ≥1000 m × ≥1000 m, tend to include
380 streams and rivers intrinsically (Fig. 2). Consequently, the occurrence of forest appears
381 to be a substantially more important factor than the occurrence of rivers, although river
382 systems are critically important for *A. miyabei*.
383 Unlike genetic variation among populations, genetic diversity within a population
384 did not show major differences between the large and small tree groups (Table 2). The
385 results indicate that seedling generations maintain a certain amount of diversity despite

ongoing forest fragmentation. We are unable to determine the exact mechanism behind this. As Kramer et al. (2008) suggested, the time since subdivision of populations may be too short at present, and thus study populations have not yet experienced a severe bottleneck. In this case, a decrease of genetic diversity within populations can be more conspicuous later when genetic drift is repeated over several generations. The other possibility is that natural selection favors heterozygous genotypes so that a certain level of within-population genetic diversity is maintained in the young generation (Hedrick, 2012). Examining these hypotheses is beyond the scope of this study, but a high ratio of heterozygous individuals in young generations in a fur seal population in Antarctica was reported, and was considered adaptive to severe environments caused by climate change (Forcada and Hoffman, 2014).

When patterns of genetic differentiation among landscape features are examined, it is often difficult to identify whether such patterns were constructed by a historical process with a long time scale or by habitat fragmentation in modern times (Schwartz et al., 2009). We aimed to overcome this issue by comparing genetic structure of mature and young individuals. This approach can be applied to other long-lived plants as well.

4.1 Conservation implications

Remnant populations of *A. miyabei* are important as reservoirs of genetic diversity. Habitats supporting these populations surely should be conserved without further loss; in particular, those in highly fragmented areas are important for their role as stepping stones of genetic connectivity. In addition, our results suggest that not only forests along rivers but also forests surrounding them contribute to maintaining gene flow. This indicates that establishing protected areas within river floodplain ecosystems may not be sufficient for promoting connectivity. Rather, we recommend preserving riparian ecosystems with adjacent natural areas integrally with as large a spatial scale as possible. Because the models with cell sizes over 1000 m were supported, avoiding fragmentation over this spatial scale is especially important. Finally, establishment of new habitats and riparian corridors seems helpful in restoring genetic connectivity for *A. miyabei*. The area around Neshikoshi, Biu, Shizunai-North, and Shizunai-South should be given priority because these populations were genetically more isolated than the others (Fig. 3; App. 2). In our data sets, although genetic diversity within populations

418 did not show decreasing trends (Table 2), its change should be followed over the long
419 term.
420
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Table 1. Locations, elevation, sample size, and tree size of 13 populations of *Acer miyabei* in southern Hokkaido, Japan.

Site No.	Site Name	Lat. (N)	Long. (E)	Elev. (m)	Sample size	DBH [1)] (cm)		
						Average	Median	Range
1	Rankoshi	42.81	141.59	69	18	12.8	9.9	3.0–33.5
2	Neshikoshi	42.86	141.66	7	19	14.2	6.8	2.0–47.6
3	Bibi	42.80	141.72	21	22	9.2	5.4	1.0–31.5
4	Kyouwa	42.88	141.76	28	23	12.2	5.0	2.4–54.1
5	Oiwake	42.87	141.81	55	21	6.1	2.3	1.5–30.0
6	Baro	42.60	142.05	88	22	12.8	8.8	2.4–52.9
7	Kuratomizawa	42.55	142.19	61	20	13.4	9.4	2.9–34.0
8	Hobetsuhiraoka	42.77	142.20	89	19	12.5	9.4	3.4–35.3
9	Nioi	42.69	142.21	73	19	15.7	15.3	2.0–39.0
10	Biu	42.50	142.35	168	21	9.7	7.3	0.7–26.7
11	Toyonukabashi	42.70	142.41	204	19	8.9	8.3	2.8–17.6
12	Shizunai-North	42.45	142.48	140	45	16.1	14.9	3.5–48.4
13	Shizunai-South	42.42	142.47	87	22	18.5	15.5	5.5–50.3
	Average	42.68	142.07	83.8	22.3	12.5	9.1	–

1) DBH, diameter at breast height. Average, median, and range were calculated from the DBH of the largest stem of each tree.

Table 2. Genetic summary statistics of 13 populations of *Acer miyabei* in southern Hokkaido, Japan.

Site No.	Locality	*A*			H_o			H_e			F_{IS}			*R[2]*			*D*c			F_{ST}		
		all	large	Small	all	large	small	all	large	small	all	large	small	all	large	small	all	large	small	all	large	small
1	Rankoshi	3.33	2.92	2.92	0.47	0.41	0.53	0.52	0.45	0.51	0.09	0.16	0.03	1.52	1.48	1.54	0.34	0.36	0.36	0.16	0.19	0.14
2	Neshikoshi	4.17	3.83	4.00	0.62	0.54	0.70	0.60	0.51	0.60	-0.04	0.00	-0.12	1.60	1.54	1.63	0.32	0.33	0.36	0.09	0.07	0.10
3	Bibi	3.42	3.08	3.25	0.58	0.61	0.55	0.55	0.53	0.53	-0.05	-0.09	0.01	1.55	1.56	1.56	0.39	0.39	0.41	0.15	0.12	0.16
4	Kyouwa	3.67	3.58	2.75	0.47	0.53	0.41	0.47	0.48	0.42	0.00	-0.06	0.07	1.47	1.50	1.44	0.33	0.33	0.36	0.12	0.10	0.14
5	Oiwake	4.50	4.33	3.58	0.62	0.63	0.61	0.58	0.55	0.55	-0.08	-0.10	-0.05	1.58	1.57	1.58	0.30	0.31	0.33	0.08	0.07	0.08
6	Baro	2.92	2.67	2.75	0.45	0.45	0.44	0.45	0.45	0.41	0.00	0.05	-0.03	1.45	1.47	1.43	0.36	0.35	0.38	0.17	0.14	0.19
7	Kuratomizawa	3.50	3.08	2.83	0.42	0.43	0.40	0.40	0.40	0.37	-0.03	-0.02	-0.03	1.40	1.43	1.39	0.29	0.31	0.31	0.13	0.11	0.15
8	Hobetsuhiraoka	3.92	3.17	3.58	0.57	0.58	0.56	0.52	0.50	0.49	-0.10	-0.10	-0.08	1.52	1.52	1.52	0.30	0.33	0.33	0.10	0.09	0.09
9	Nioi	4.50	3.58	3.50	0.50	0.53	0.46	0.50	0.50	0.46	0.00	-0.02	0.05	1.50	1.52	1.49	0.31	0.31	0.36	0.12	0.11	0.12
10	Biu	4.50	3.92	3.58	0.55	0.58	0.52	0.55	0.53	0.51	-0.02	-0.06	0.04	1.55	1.55	1.54	0.29	0.29	0.34	0.08	0.06	0.09
11	Toyonukabashi	4.33	3.50	3.75	0.49	0.48	0.50	0.50	0.47	0.47	0.02	0.05	0.01	1.50	1.50	1.50	0.27	0.28	0.31	0.07	0.07	0.08
12	Shizunai-North	4.75	4.58	4.00	0.51	0.50	0.51	0.52	0.51	0.50	0.02	0.04	0.02	1.52	1.52	1.52	0.27	0.28	0.31	0.08	0.06	0.09
13	Shizunai-South	4.00	3.67	3.25	0.55	0.52	0.58	0.50	0.51	0.45	-0.09	0.01	-0.23	1.50	1.53	1.48	0.29	0.29	0.34	0.10	0.07	0.13
	Average (SD)	3.96	3.53	3.37	0.52	0.52	0.52	0.51	0.49	0.48	-0.02	-0.01	-0.02	1.51	1.51	1.51	0.31	0.32	0.35	0.11	0.10	0.12
		(0.56)	(0.55)	(0.45)	(0.06)	(0.07)	(0.08)	(0.05)	(0.04)	(0.06)	(0.05)	(0.07)	(0.08)	(0.05)	(0.04)	(0.07)	(0.03)	(0.03)	(0.03)	(0.03)	(0.04)	(0.04)

Note 1: *A* = mean number of alleles per locus; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = fixation index within population; *R*, allelic richness after rarefaction to 2; *D*c = Cavalli-Sforza and Edwards' (1967) chord distance (average of 12 pairwise distances to other populations); F_{ST} = fixation index (average of 12 pairwise values to other populations).

Note 2: The genetic statistics were calculated over the 12 microsatellite loci.

Table 3. Results of multiple regression on distance matrices (MRM) and comparisons of landscape models by AIC for fit of pairwise genetic distances (*D*c) on geographic and resistance distances in 13 natural populations of *Acer miyabei* in Hokkaido, Japan.

Sample group [1)]	Landscape model [2)]	Resistance surface (pixel size)	Coefficient	r^2	*p*	AIC [3)]
All	Isolation by Distance	N/A	3.626×10^{-4}	0.034	0.0995	-233.65
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (500 m)	3.708×10^{-7}	0.102	0.0215	-239.40
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (1000 m)	4.406×10^{-7}	0.162	0.0105	-244.76
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (2000 m)	3.809×10^{-7}	0.217	0.0045	-250.09
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (3000 m)	3.984×10^{-7}	0.207	0.0140	-249.08
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (500 m)	2.879×10^{-7}	0.148	0.0030	-243.49
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (1000 m)	3.906×10^{-7}	0.168	0.0095	-245.27
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (2000 m)	4.013×10^{-7}	0.243	0.0025	-252.65
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (3000 m)	3.889×10^{-7}	0.201	0.0170	-248.47
	Isolation by Resistance (CT)	Forest vs. Non-Forest (500 m)	2.613×10^{-2}	0.200	0.0470	-248.34
	Isolation by Resistance (CT)	Forest vs. Non-Forest (1000 m)	2.768×10^{-2}	0.337	0.0105	**-263.06**
	Isolation by Resistance (CT)	Forest vs. Non-Forest (2000 m)	2.708×10^{-2}	0.343	0.0100	**-263.77**
	Isolation by Resistance (CT)	Forest vs. Non-Forest (3000 m)	2.564×10^{-2}	0.346	0.0070	**-264.07**
	Isolation by Resistance (CT)	Riparian Forest vs. Others (500 m)	1.629×10^{-2}	0.131	0.0575	-241.95
	Isolation by Resistance (CT)	Riparian Forest vs. Others (1000 m)	2.252×10^{-2}	0.298	0.0095	-258.61
	Isolation by Resistance (CT)	Riparian Forest vs. Others (2000 m)	2.646×10^{-2}	0.325	0.0100	-261.67
	Isolation by Resistance (CT)	Riparian Forest vs. Others (3000 m)	2.550×10^{-2}	0.348	0.0095	**-264.27**
Large trees	Isolation by Distance	N/A	2.328×10^{-4}	0.013	0.3170	-228.44
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (500 m)	2.999×10^{-7}	0.064	0.0810	-232.55
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (1000 m)	3.787×10^{-7}	0.114	0.0255	-236.86
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (2000 m)	3.452×10^{-7}	0.171	0.0075	-241.98
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (3000 m)	3.831×10^{-7}	0.183	0.0180	-243.16
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (500 m)	2.526×10^{-7}	0.109	0.0145	-236.40
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (1000 m)	3.511×10^{-7}	0.129	0.0140	-238.19
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (2000 m)	3.603×10^{-7}	0.187	0.0105	-243.52
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (3000 m)	3.715×10^{-7}	0.175	0.0250	-242.42
	Isolation by Resistance (CT)	Forest vs. Non-Forest (500 m)	2.235×10^{-2}	0.140	0.1125	-239.11
	Isolation by Resistance (CT)	Forest vs. Non-Forest (1000 m)	2.493×10^{-2}	0.261	0.0315	-251.01
	Isolation by Resistance (CT)	Forest vs. Non-Forest (2000 m)	2.652×10^{-2}	0.315	0.0110	-256.85
	Isolation by Resistance (CT)	Forest vs. Non-Forest (3000 m)	2.567×10^{-2}	0.331	0.0125	**-258.77**
	Isolation by Resistance (CT)	Riparian Forest vs. Others (500 m)	1.282×10^{-2}	0.078	0.1665	-233.69
	Isolation by Resistance (CT)	Riparian Forest vs. Others (1000 m)	1.939×10^{-2}	0.211	0.0455	-245.89
	Isolation by Resistance (CT)	Riparian Forest vs. Others (2000 m)	2.270×10^{-2}	0.229	0.0510	-247.67
	Isolation by Resistance (CT)	Riparian Forest vs. Others (3000 m)	2.555×10^{-2}	0.334	0.0080	**-259.04**

Small trees	Isolation by Distance	N/A	4.189×10^{-4}	0.053	0.0375	-247.09
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (500 m)	3.847×10^{-7}	0.129	0.0080	-253.60
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (1000 m)	4.318×10^{-7}	0.181	0.0030	-258.47
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (2000 m)	3.606×10^{-7}	0.227	0.0015	-262.95
	Isolation by Resistance (LCP)	Forest vs. Non-Forest (3000 m)	3.621×10^{-7}	0.199	0.0135	-260.21
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (500 m)	2.795×10^{-7}	0.163	0.0070	-256.74
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (1000 m)	3.657×10^{-7}	0.171	0.0045	-257.51
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (2000 m)	3.798×10^{-7}	0.253	0.0025	-265.64
	Isolation by Resistance (LCP)	Riparian Forest vs. Others (3000 m)	3.555×10^{-7}	0.196	0.0075	-259.86
	Isolation by Resistance (CT)	Forest vs. Non-Forest (500 m)	2.808×10^{-2}	0.269	0.0100	-267.27
	Isolation by Resistance (CT)	Forest vs. Non-Forest (1000 m)	2.563×10^{-2}	0.337	0.0025	-274.90
	Isolation by Resistance (CT)	Forest vs. Non-Forest (2000 m)	2.492×10^{-2}	0.339	0.0035	**-275.14**
	Isolation by Resistance (CT)	Forest vs. Non-Forest (3000 m)	2.173×10^{-2}	0.289	0.0140	-269.52
	Isolation by Resistance (CT)	Riparian Forest vs. Others (500 m)	1.891×10^{-2}	0.206	0.0060	-260.87
	Isolation by Resistance (CT)	Riparian Forest vs. Others (1000 m)	2.227×10^{-2}	0.340	0.0040	**-275.23**
	Isolation by Resistance (CT)	Riparian Forest vs. Others (2000 m)	2.555×10^{-2}	0.354	0.0020	**-276.91**
	Isolation by Resistance (CT)	Riparian Forest vs. Others (3000 m)	2.158×10^{-2}	0.290	0.0075	-269.60

1) All, sample set using all trees (n = 290). Large trees, sample set consisting of the larger trees (n = 149). Small trees, sample set consisting of the smaller trees (n = 141).

2) LCP, least-cost path; CT, circuit theory.

3) The lowest AIC value in each sample group and similar ones (≤2.0) are marked in bold.

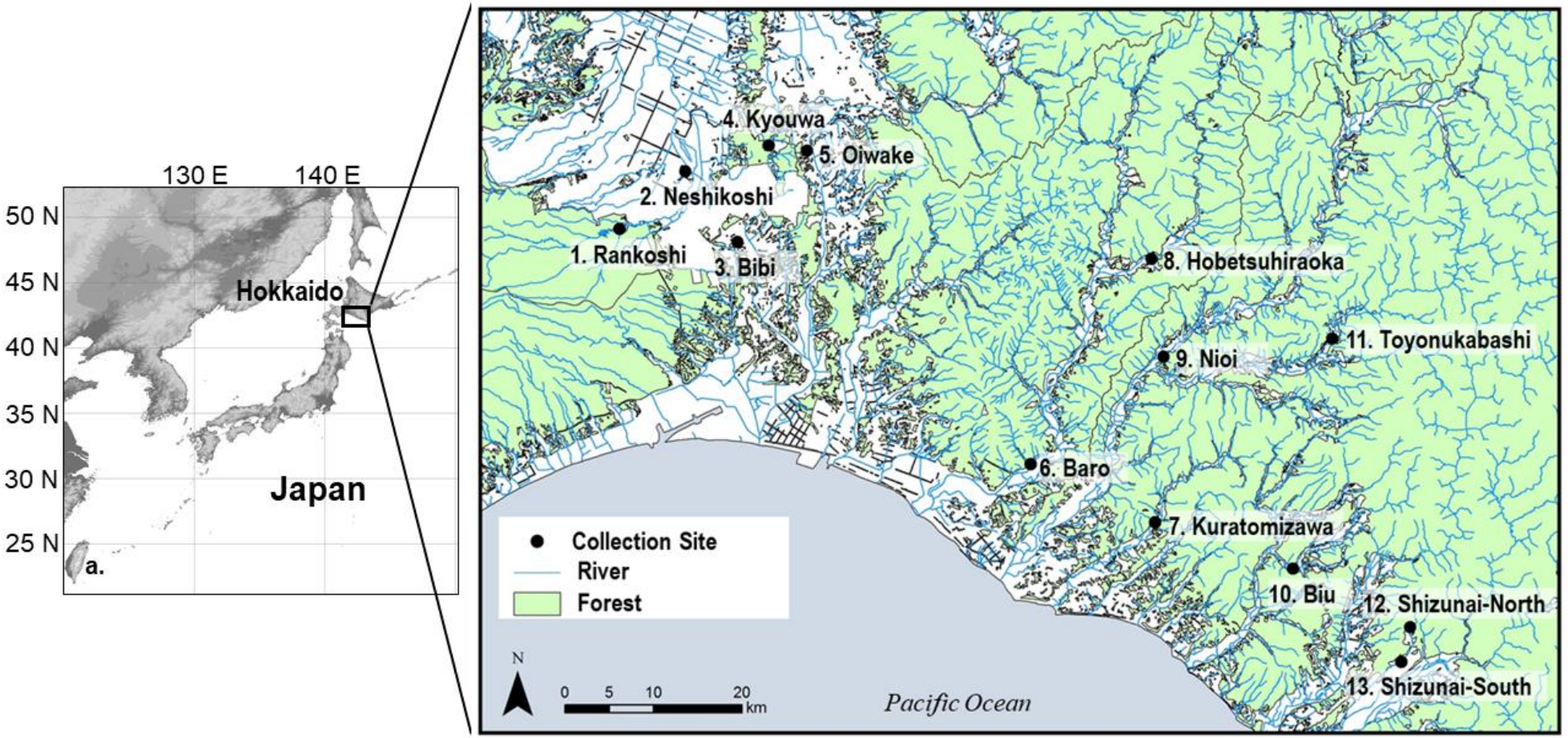
Figure legends

Fig. 1. Map of Japan (left) and inset of southern Hokkaido showing the 13 *Acer miyabei* populations sampled.

Fig. 2. Resistance surfaces used for least-cost path and circuit theory modeling and corresponding cumulative current maps. The resistance surfaces were created by a combination of two models and four cell sizes: (a–d) Forest vs. Non-Forest and (e–h) Riparian Forest vs. Other with a pixel width of (a, e) 500 m, (b, f) 1000 m, (c, g) 2000 m, (d, h) 3000 m. Black lines indicates rivers. Dark gray areas, high resistance values; light gray areas, low resistance values. The cumulative current maps were created in Circuitscape v. 4.0 software (McRae et al., 2013) based on circuit theory. Warmer colors indicate areas with higher current density or that are expected to contribute more to connectivity between the populations (McRae et al., 2008).

Fig. 3. Comparisons of the Cavalli-Sforza and Edwards' (1967) chord distance (*D*c) among neighboring populations of *Acer miyabei* between the large (above) and small (below) tree groups. Neighbors were identified by Voronoi algorithms. Line width represents degree of genetic differentiation: thinner lines indicate greater differentiation. The diagram for the small tree group illustrates the proportional change in genetic differentiation from the corresponding *D*c in the large tree group. Colored lines indicate population pairs with an increase in genetic differentiation: red, >30%; yellow, from 15% to 30%.

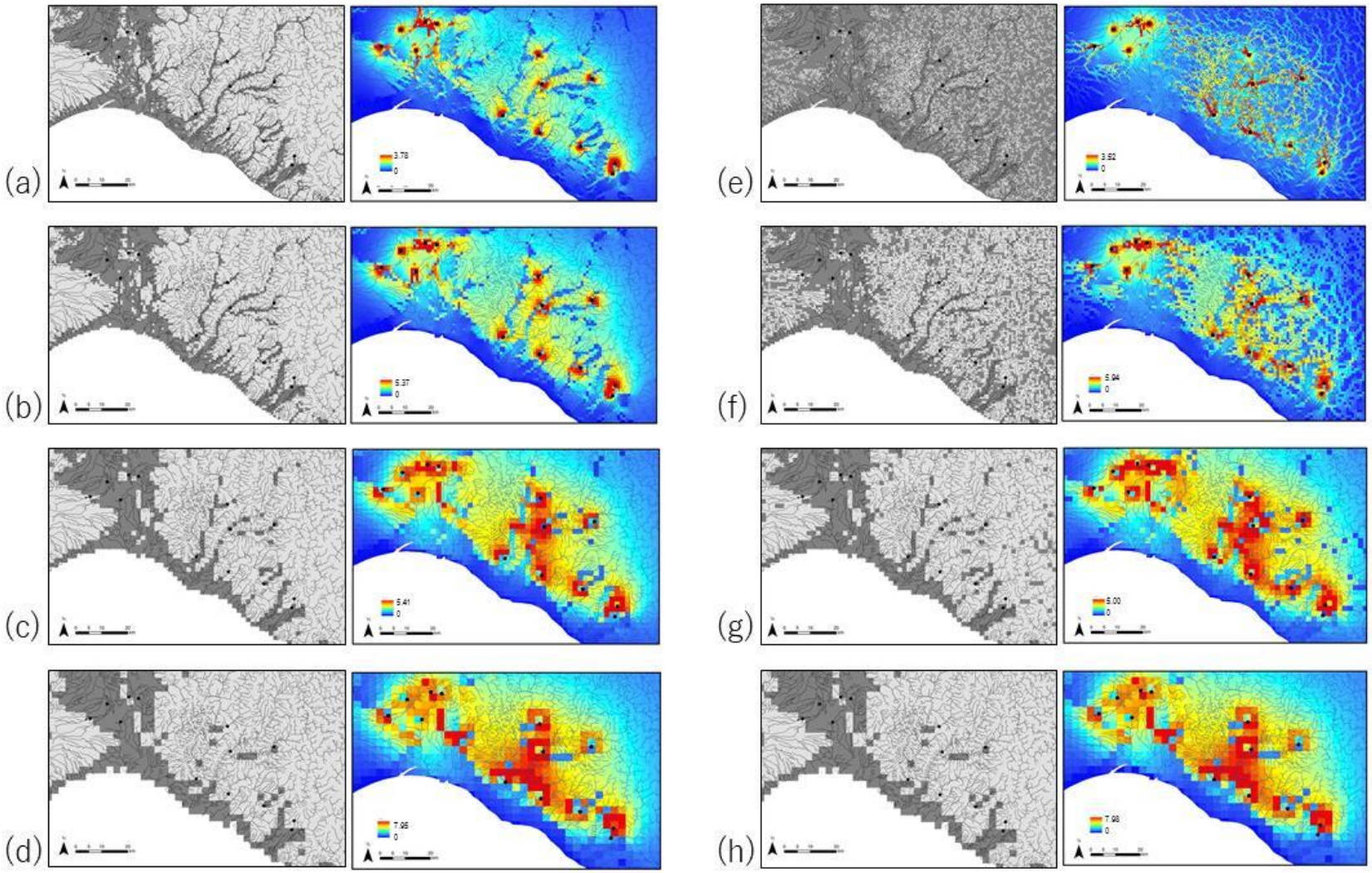
1 Fig. 1


130 E
140 E
50 N
45 N
40 N
35 N
30 N
25 N
Hokkaido
Japan
a.
4. Kyouwa
5. Oiwake
2. Neshikoshi
1. Rankoshi
3. Bibi
8. Hobetsuhiraoka
11. Toyonukabashi
9. Nioi
6. Baro
7. Kuratomizawa
10. Biu
12. Shizunai-North
13. Shizunai-South
Collection Site
River
Forest
N
0 5 10 20
km
Pacific Ocean

2

3

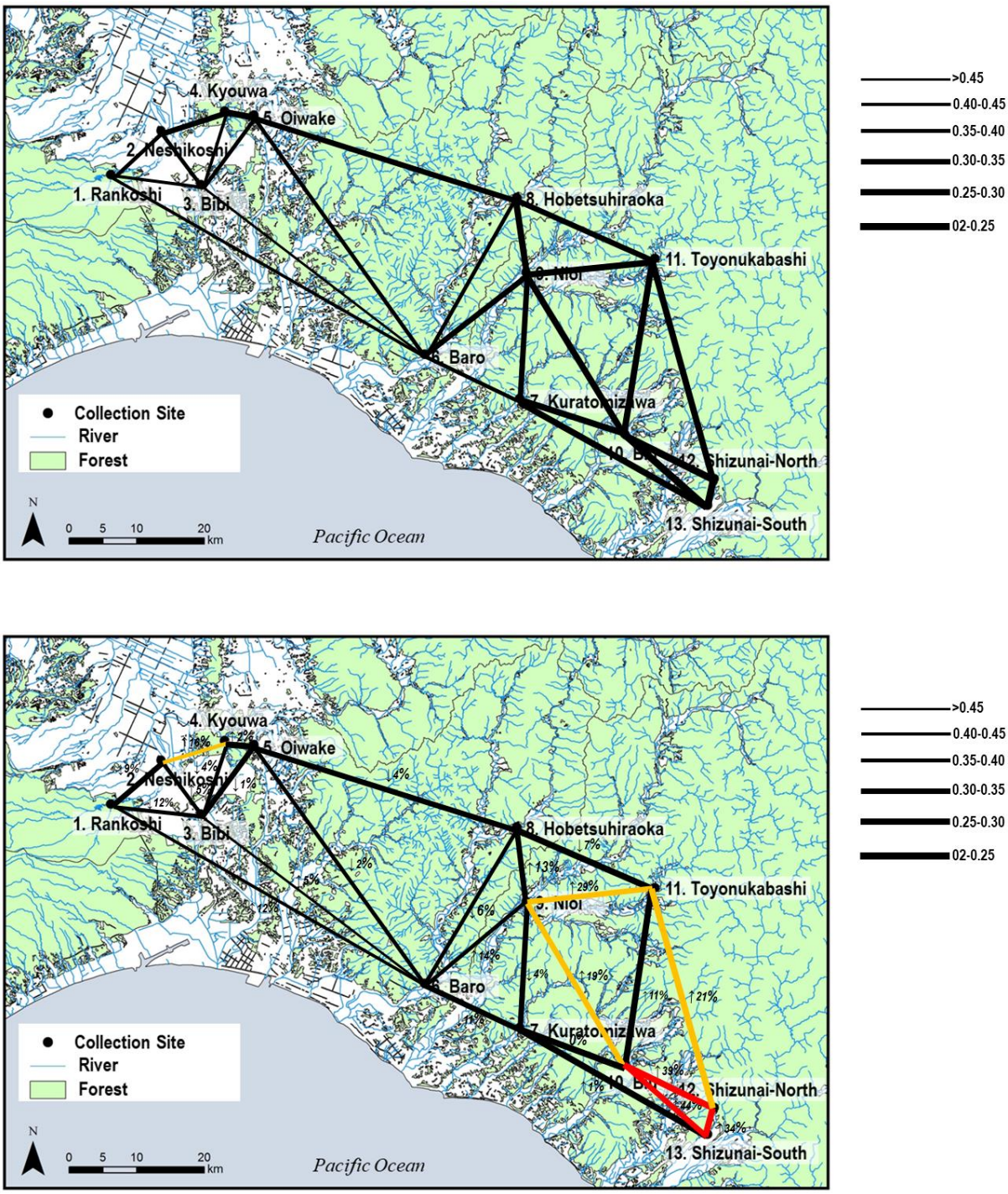
1 Fig. 2.



2

3

Fig. 3.


4. Kyouwa
5. Oiwake
2. Neshikoshi
1. Rankoshi
3. Bibi
8. Hobetsuhiraoka
11. Toyonukabashi
9. Nioi
6. Baro
7. Kuratomizuwa
12. Shizunai-North
13. Shizunai-South
Collection Site
River
Forest
N
0 5 10 20 km
Pacific Ocean
>0.45
0.40-0.45
0.35-0.40
0.30-0.35
0.25-0.30
02-0.25

Appendix 1. Voucher information of representative samples of *Acer miyabei* analyzed in this study. Voucher specimens are stored at Makino Herbarium (MAK), Tokyo Metropolitan University, Japan.

Sample ID	**Population name**	**Location**	**Lat.**	**Long.**	**Voucher no.**
272	Rankoshi	Chitose, Hokkaido	42.81	141.59	IOS10396
441	Neshikoshi	Chitose, Hokkaido	42.86	141.66	IOS10397
001-015, 017-023	Bibi	Chitose, Hokkaido	42.80	141.72	IOS10138-IOS10159
025-036, 038-047	Kyouwa	Chitose, Hokkaido	42.88	141.76	IOS-10160-IOS10181
049	Oiwake	Abira, Hokkaido	42.87	141.81	IOS10398
292	Baro	Mukawa, Hokkaido	42.60	142.05	IOS10399
353	Kuratomizawa	Hidaka, Hokkaido	42.55	142.19	IOS10400
314	Hobetsuhiraoka	Mukawa, Hokkaido	42.77	142.20	IOS10401
138	Nioi	Biratori, Hokkaido	42.69	142.21	IOS10402
180	Biu	Niikappu, Hokkaido	42.50	142.35	IOS10403
157	Toyonukabashi	Biratori, Hokkaido	42.70	142.41	IOS10404
395	Shizunai-North	Shinhidaka, Hokkaido	42.45	142.48	IOS10405
377	Shizunai-South	Shinhidaka, Hokkaido	42.42	142.47	IOS10406

Appendix 2. Comparisons of F_{ST} among the neighboring populations of *Acer miyabei* between large tree (above) and small tree (below) groups. The neighbors were identified by Voronoi algorithms. Line width represents degree of genetic differentiation: thinner lines indicate greater differentiation. The diagram for small tree group illustrates the proportional change in genetic differentiation from the corresponding F_{ST} in large tree group. Colored lines indicate population pairs with an increase in genetic differentiation: red, >100%; yellow, from 50% to 100%.