Inferring future changes in gene flow under climate change in riverscapes: a pilot case study in fluvial sculpin

Souta Nakajima[1,2,*], Hiroaki Suzuki[3,4], Makoto Nakatsugawa[3], Ayumi Matsuo[5], Shun K. Hirota[5,6], Yoshihisa Suyama[5], Futoshi Nakamura[1]

[1] Graduate School of Agriculture, Hokkaido University, Kita-ku N9W9, Sapporo, Hokkaido 060-8589, Japan

[2] Present address: Water Environment Research Group, Public Works Research Institute, Minamihara 1-6, Tsukuba, Ibaraki 305-8516, Japan

[3] Graduate School of Engineering, Muroran Institute of Technology, Mizumoto-cho 27-1, Muroran, Hokkaido 050-8585, Japan

[4] Research Institute of Energy, Environment and Geology, Hokkaido Research Organization, Kita-ku N19W12, Sapporo, Hokkaido 060-0819, Japan

[5] Graduate School of Agricultural Science, Tohoku University, Yomogida 232-3, Naruko-onsen, Osaki, Miyagi 989-6711, Japan

[6] Present address: Botanical Gardens, Osaka Metropolitan University, Kisaichi 2000, Katano, Osaka 576-0004, Japan

* Corresponding author

Souta Nakajima: n.souta891@gmail.com

Watershed Restoration Team, Water Environment Research Group, Public Works Research Institute, Minamihara 1-6, Tsukuba, Ibaraki 305-8516, Japan

Phone: +81-29-879-6775, Fax: +81-29-869-5082

ORCID

Souta Nakajima: https://orcid.org/0000-0003-3701-5428

Shun K. Hirota: https://orcid.org/0000-0002-6104-1119

Yoshihisa Suyama: https://orcid.org/0000-0002-3136-5489

Futoshi Nakamura: https://orcid.org/0000-0003-4351-2578

Acknowledgments

We thank Nobuo Ishiyama for his cooperation in validating the hydrological model. This study is partly supported by the research fund for the Ishikari and Tokachi Rivers provided by the Ministry of Land, Infrastructure, Transport and Tourism of Japan.

Abstract

Context: Global climate change poses a significant threat to the habitat connectivity of cold-water-adapted organisms, leading to species extinctions. If gene flow can be modeled by landscape variables, changes in connectivity among populations could be predicted. However, in dendritic and heterogeneous stream ecosystems, few studies have estimated the changes in gene flow from genetic data, in part due to the difficulty in applying landscape genetics methods and accessing water temperature information.

Objectives: Inferring the determinants and future changes of the gene flow in the cold-water adapted fluvial sculpin *Cottus nozawae* using a recently developed model-based riverscape genetics technique and a hydrological model for estimating water temperature.

Methods: The strength of gene flow on each stream section was modeled by watershed-wide riverscape variables and genome-wide SNP data for *C. nozawae* in the upper reaches of the Sorachi River, Hokkaido, Japan. Future changes in gene flow were inferred by this model and hydrologically estimated water temperatures under the high greenhouse gas concentration scenario (IPCC RCP8.5).

Results: Stream order, water temperature, slope, and distance were selected as riverscape variables affecting the strength of gene flow in each stream section. In particular, the trend of greater gene flow in sections with higher stream order and lower temperature fluctuations or summer water temperatures was pronounced. The map from the model showed that gene flow is overall prevented in small tributaries in the southern area, where spring-fed environments are less prevalent. Estimating future changes, gene flow was predicted to decrease dramatically at the end of the 21st century.

Conclusions: Our results demonstrated that the connectivity of cold-water sculpin populations is expected to decline dramatically in a changing climate. Riverscape genetic modeling is useful for gaining information on population connectivity that does not fully coincide with habitat suitability.

Keywords

model-based riverscape genetics; cold-water fish; *Cottus*; water temperature; global warming

Introduction

Global climate change modifies water temperatures and flow regimes, the two key habitat factors affecting freshwater species, posing a critical threat to stream ecosystems (Barbarossa et al. 2021). The spatial distribution of species' suitable habitats shifts with environmental changes, and population fragmentation due to impassable environments may eventually result in local and/or species extinctions (Woodward et al. 2010). Numerous studies have predicted changes in species distributions and suitable habitats of stream organisms (Elith and Leathwick 2009; Comte et al. 2012; Ishiyama et al. 2023; Rahel et al. 1996), but how will the actual population connectivity and migration potential change?

Gene flow represents the functional connectivity among wild populations and is critical in species viability (Kottler et al. 2021; Manel and Holderegger 2013). The strength of gene flow is usually discussed individually from the observed genetic structure, but if gene flow could be modeled by landscape variables, the gained knowledge regarding gene flow could be generalized and used to predict its future changes (McRae and Beier 2007). The relationships between gene flow and landscape variables have been investigated in the field of landscape genetics (Balkenhol et al. 2015). However, most analytical techniques developed in landscape genetics exert only poor power in linear and dendritic stream ecosystems (Davis et al. 2018; Chafin et al. 2021), making it difficult to predict future changes in gene flow in riverscapes. Even in streams, regression models can be created by contrasting a genetic distance matrix against pairwise differences in local conditions (Grummer et al. 2019), but this approach fails to account for the network architecture and for the attributes in all the spaces that individuals must pass through when traveling between sampling sites (Davis et al. 2018; White et al. 2020; Escalante et al. 2020). Another versatile approach to investigating the effects of landscape elements on gene flow is defining "landscape resistance" surfaces and assessing the relationship between genetic distance and cumulative resistance between populations (isolation by resistance; IBR (McRae 2006)). Although this idea has been applied to studies on stream ecosystems in several cases (e.g., Inoue and Berg 2017; Oliveira et al. 2019; Landguth et al. 2016; Escalante et al. 2018), the landscape resistance must be parametrized *a priori* through expert opinion or other empirical methods (e.g., using the inverse of species distribution model estimates) (Spear et al. 2015;

92 Zeller et al. 2012). To understand the gene flow itself, its determinants should be identified directly
93 from genetic data (Sartor et al. 2022; Wasserman et al. 2010). Fortunately, alternative methods for
94 modeling gene flow from genetic data within a spatially explicit graph-theoretic framework have been
95 developed rapidly in recent years (White et al. 2020; Chafin et al. 2021). Although not yet practically
96 applied to predictions under environmental changes, we thought that these “riverscape genetics”-
97 dedicated methods are the key to determining landscape resistance and modeling current and future
98 gene flow.
99 Another theme that makes riverscape genetics challenging is the data availability of key
100 environmental elements such as water temperatures. For terrestrial organisms, globally available
101 climate data such as WorldClim (Hijmans et al. 2005) are commonly used to estimate the effects of
102 climate change. However, data on current and future water temperatures that are critical for stream
103 organisms are difficult to obtain as are data on flow rate. Although some studies have used air
104 temperature data as a surrogate of water temperatures (Almodóvar et al. 2012), water temperatures do
105 not actually coincide with air temperatures. In particular, local spatial heterogeneity in water
106 temperatures caused by groundwater discharge and other factors is truly a source of ecosystem
107 diversity and resilience to climate change that cannot be ignored (Koizumi and Maekawa 2004;
108 Nakajima et al. 2021; Ishiyama et al. 2023; Nakamura 2022). Therefore, it is critical in riverscapes to
109 utilize water temperature information considering the spatial heterogeneity generated by
110 hydrogeological factors.
111 *Cottus nozawae* is a cold-water-adapted sculpin inhabiting northern Japan. Since the
112 distribution and ecology of this species are highly influenced by summer water temperatures (Yagami
113 and Goto 2000), available habitats are expected to decrease significantly under climate change (Suzuki
114 et al. 2021). At a local scale, streams with low summer water temperatures characterized by spring-fed
115 environments have been shown to display high population densities and to be the source of individuals
116 in a watershed (Suzuki et al. 2021; Nakajima et al. 2021). Under ongoing climate change, the
117 migration of this species is expected to be frequently blocked by unsuitable habitats, resulting in
118 population fragmentation and shrinkage. To sustain the species into the future, it is critical to
119 accurately predict the relationship between population connectivity and climate-related variables. Such

predictions will contribute to the advancement of climate change adaptation measures for this species, such as by identifying sections where stream continuity should be ensured.

Considering the challenges of data availability and analysis in riverscape genetics, we thought that the recently developed model-based riverscape genetics approaches and physics-based hydrological model to estimate water temperatures would enable the modeling and future prediction of gene flow in cold-water fish. The aims of this study are (i) to identify the factors determining the gene flow of *C. nozawae* in the stream network, (ii) to model the strength of gene flow using riverscape variables and predict its future changes, and (iii) to discuss the applicability of riverscape genetic modeling in conservation ecology.

Material and Methods

Study sites and sampling

In 2019, small pieces of fin tissue were sampled from 376 individuals of *C. nozawae* caught by electrofishing (model 12-B Backpack Electrofisher; Smith-Root Inc.) at 13 sites located in the upstream section of the Sorachi River, Hokkaido, Japan (Fig. 1; Table S1). Because no river-crossing structures that would obviously prevent fish migration are present between sampling sites, this area is considered suitable for evaluating the effects of riverscape variables. Regarding the environmental conditions, the tributaries in the northern volcanic watersheds have spring-fed environments with stable water temperatures and flow regimes (García Molinos et al. 2022; Ishiyama et al. 2023). For riverscape genetic modeling, the stream network among sampling sites was viewed as a graph consisting of 24 “nodes” and 23 “edges” (Fig. 2a). We defined “nodes” as the sampling sites and major tributary confluences between them, and analysis was conducted with “edges”, the stream segments between adjacent nodes, as units.

Genetic data

Genomic DNA was extracted using the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN Inc.). In this study, we used the multiplexed ISSR genotyping by sequencing (MIG-seq) method (Suyama and Matsuki 2015; Suyama et al. 2022), a technique in which loci between two microsatellite

regions are amplified and neutral genome-wide single nucleotide polymorphisms (SNPs) are detected. A MIG-seq library preparation and read quality filtering were performed according to the protocol described in Suyama et al. (2022), with the modification that two runs were conducted and the obtained data were combined after quality filtering. In addition, quality filtering was performed on 71 bases with six 5'-end bases and three 3'-end bases removed. After quality filtering, SNP selection was performed using STACKS 2.41 (Catchen et al. 2013). First, the reads were grouped to each locus using the *ustacks*, *cstacks*, *sstacks*, *tsv2bam*, and *gstacks* commands with the following parameters recommended by Paris et al. (2017): minimum depth option creating a stack (m) = 3, maximum distance between stacks (M) = 2, maximum mismatches between loci when building the catalog (n) = 2, and number of mismatches allowed to align secondary reads (N) = 4. From the derived dataset of assembled loci, SNPs were detected using the *populations* commands under the following criteria: only loci present at a rate of more than 80% of individuals within all populations were extracted (-p 13 -r 0.8); the minimum minor allele frequency was 5% (--min-maf 0.05); sites showing excess heterozygosity were removed (--max-obs-het 0.6); and the output was limited to one SNP per locus (--write-single-snp). After filtering, 212 SNPs were obtained.

For populations in each sampling site, the expected heterozygosity (H_E) and fixation index (F_{IS}) were calculated using the *populations* command in STACKS. Significant deviations from Hardy–Weinberg equilibrium, as indicated by F_{IS} deviating from zero, were tested by 1000 randomizations using FSTAT 2.9.4 (Goudet 1995). Genetic differentiation among populations was assessed by G_{ST} (Nei 1973) and D_{PS} (Bowcock et al. 1994). D_{PS} is the genetic distance based on the dissimilarities of population allele pools and reflects gene flow over a shorter timescale (approximately 10 generations; Landguth et al. 2010; Leroy et al. 2018), whereas G_{ST} is assumed to reflect long-term gene flow (Holsinger and Weir 2009). G_{ST} was calculated using GenAlEx 6.51 (Peakall and Smouse 2012), and D_{PS} was calculated using the package graph4lg (Savary et al. 2021) in R 3.6.0 (R Core Team 2019). To understand the general patterns of population structure, STRUCTURE 2.3.4 (Pritchard et al. 2000) was performed in the setting of the admixture and allele frequency correlated model with previous sampling location information (LOCPRIOR; Hubisz et al. 2009). The algorithm was run 10 times for each K from 1 to 10 with a burn-in of 20,000 followed by 30,000 MCMC replicates. The program

CLUMPAK (Kopelman et al. 2015) was then used to summarize the results for each K. STRUCTURE HARVESTER (Earl and vonHoldt 2012) was employed to calculate the probability of the data for each K (LnP(D); Pritchard et al. 2000), the corresponding standard deviation, and the ΔK (Evanno et al. 2005).

Riverscape data

Riverscape variables were collected as a unit of edges. Edge length, slope, stream orders, and catchment area were calculated in ArcGIS 10.7.1 (ESRI Inc.) using National Land Numerical Information (nlftp.mlit.go.jp) from the Ministry of Land, Infrastructure, Transport and Tourism (MLIT) of Japan. Flow rate and water temperature were estimated by a hydrological model based on Suzuki et al. (2022), which considers differences in groundwater discharge depending on catchment geology (see Appendix 1 for details). Briefly, the daily flow rate on the stream in each 1 km mesh was reproduced by four-layered tank models (Sugawara 1979), and the flow and heat flux were tracked along the streamflow. Importantly, different tank parameters were given for the volcanic areas and other areas, based on validation using measured water temperature data from field surveys at multiple sites in the study area. Flow rate and water temperature from September 2018 to August 2019 were reproduced and used to calculate the riverscape variables. In future predictions, 1 km-downscaled data on meteorological elements (Ueda et al. 2020), calculated under the climate data projected in the representative concentration pathway scenario 8.5 (RCP8.5) in the IPCC 5th Assessment (IPCC 2014), was used to derive the input water amount to the tank model. We used the mean of the predicted variables for the years 2081 to 2100 (all from September to next August) as future riverscape variables.

Gene flow analysis

We probabilistically modeled the relative migration rate (edge passability) of each edge as a function of riverscape variables using the "BGR model" (White et al. 2020). This is a novel method that can model bidirectional gene flow in stream networks using genetic distance matrices as input data and riverscape variables as explanatory variables, rigorously accounting for the spatial autocorrelation

structure of stream networks using a graph-theoretical framework and a spatial autoregressive model. Specifically, the nearly homogeneous stream segments (delimited by nodes that are sampling sites or major tributary confluences) were defined as edges, and the relative migration rate (edge passability; w_{ij}) of each edge linking nodes i and j was estimated as a function of k riverscape variables (x_{ij1}, x_{ij2}, …, x_{ijk}) and the corresponding parameters (β_1, β_2, …, β_k) as:

$$w_{ij} = \exp\left(\beta_0 + \sum_k \beta_k x_{ijk}\right)$$

where β_0 is the intercept term. Here, all riverscape variables were normalized from 0 to 1. The posterior distribution of parameters β_0, β_1, …, β_k was estimated by a Markov Chain Monte Carlo (MCMC) sampler, to fit the input genetic data. The mathematics linking w_{ij} to genetic distance are described in Peterson et al. (2019).

The BGR model was run in R. We used G_{ST} and D_{PS} as genetic distances and 10 possible riverscape variables (Table 1; Fig. S1) as covariates x_{ijk}. All variables except direction are symmetric. For each summary statistic, forward selections were conducted based on the deviance information criterion (DIC). Variables were added until the DIC no longer decreased by 7 or more (Cain and Zhang 2019). At each step of the forward selection, the variables that were highly correlated (Pearson's $r > 0.7$) with other variables already included in the model were not added to the model. Models with fewer than four variables were run for 50,000 MCMC iterations and parameters were estimated after 25,000 burn-in. Models with four or more variables were run for 100,000 iterations including 50,000 burn-in. After the final model was identified, we conducted a long run with 500,000 iterations including 200,000 burn-in, to accurately estimate the β values and 95% credible intervals. Landscape resistance, calculated as the inverse of w_{ij} of each edge, was estimated and mapped from the selected models. To evaluate the estimates, the correlations between genetic distances and estimated landscape resistance (sum of edges between populations) were calculated by Mantel tests with 9999 permutations, and compared to the correlations between genetic distances and waterway geographical distance. The Mantel tests were conducted using the package VEGAN 2.5.6 (Oksanen et al. 2019) in R. Future landscape resistance was inferred by substituting the future water temperature variable into the final BGR model. At this stage, we used the model derived from D_{PS} because G_{ST}

displays long-term patterns and D_{PS} is more likely to reflect current changes in gene flow.

Results

The level of H_E was similar across the watershed (ranged from 0.241–0.272), and F_{IS} ranged from -0.012–0.023 with no populations deviating significantly from zero (Table S1). The average G_{ST} was 0.029 (ranged from 0.000–0.047; Table S2) and D_{PS} was 0.087 (ranged from 0.050–0.135). In the STRUCTURE, while LnP(D) for each K increased progressively, ΔK was highest at K = 2, and locally maximum at K = 6 (Fig. S2). Populations in the southern area were grouped into distinct clusters from low K, and as K increased, populations in other tributaries were also mixed with geographically uneven clusters. The strength of population structure differed geographically (Fig. 2a), but the factors determining this difference are not known by the STRUCTURE. From the forward selection of the model explaining the strength of gene flow, Shreve's stream order, water temperature fluctuation, slope, and edge length were selected for G_{ST}, and Strahler's stream order and summer water temperature were selected for D_{PS}, in this order (Tables 2 and S3). In both cases, the first and second variables added to the model were the stream order and water temperature, respectively. While different types of variables were selected for G_{ST} and D_{PS} (Shreve's or Strahler's; summer water temperature or water temperature fluctuation), these results show the importance of the stream order and water temperature on the strength of gene flow. The stream orders had a positive effect on gene flow, while the water temperature fluctuation or summer water temperature had a negative effect. The effect (β) of the water temperature on gene flow was higher in D_{PS} than in G_{ST}. In G_{ST}, the slope and edge length were also selected and had negative effects, indicating their relevance to long-term gene flow. Geographically, the southern upstream area had generally higher landscape resistance (lower gene flow) than the main stream, while in the northern volcanic area, landscape resistance was not so high even upstream (Fig. 2). The Mantel tests between the genetic distances and estimated landscape resistance suggested significant relationships ($r = 0.46$, $p < 0.05$ for G_{ST}; $r = 0.60$, $p < 0.01$ for D_{PS}), and the correlations were much higher than those between the genetic and geographic distances (Fig. 3). The future prediction indicated that the landscape resistance would increase overall from the current levels. Some sections in the main stream and in the upper reaches in the volcanic area were

estimated to exhibit as high landscape resistance levels as the present southern upstream area. The southern upstream area was projected to display very high resistance.

Discussion

In this study, we succeeded in modeling and future predicting of gene flow of *C. nozawae* in the stream network. Although there are still challenges in the modeling technique (e.g., simplicity of model assumptions, difficulty of model evaluation, etc.), the modeled landscape resistance explained the genetic distances well (Fig. 3); the strength of gene flow could be largely explained by riverscape variables.

It was a somewhat unexpected result that the stream order was identified as the variable with the strongest effect on gene flow. Previous studies of cold-water fish have reported both higher and lower gene flow in streams with higher stream orders (Aunins et al. 2015; Escalante et al. 2018; White et al. 2020). Within the arbitrary study areas, main streams tend to have higher water temperatures and are often unsuitable environments for cold-water fish. On the other hand, in dendritic stream structures, confluences are often known to be stable gene accumulation and source points for stream organisms (Grant et al. 2007; Paz-Vinas and Blanchet 2015), and the downstream passage of those organisms may result in higher gene flow in higher-order streams. In addition, as the main stream tends to be more severely affected by flooding (Han et al. 2007; Koizumi et al. 2013), individuals, especially those of low-mobility species, may have a greater chance of being flushed. There may be differences regarding which processes are predominant depending on the studied species or areas. Summer water temperature (or water temperature fluctuation in G_{ST}) negatively affected gene flow. This is probably because streams with high summer water temperatures and large fluctuations are not suitable environments for *C. nozawae* (Suzuki et al. 2021), making successful dispersal difficult. While it is not uncommon for studies of cold-water fishes to implicate an association between gene flow and water temperature-related variables (Kanno et al. 2011; Escalante et al. 2018; Hand et al. 2016), the present study was able to represent this pattern using more realistic water temperature information. The model from G_{ST} also included the slope and edge length, but the model from D_{PS} did not. We found that topography and distance affected the formation of the long-term population

structure as in many other systems (Kanno et al. 2011; Caldera and Bolnick 2008), but that most of the current gene flow can be explained by the stream order and water temperature. The upstream-downstream direction did not affect gene flow, probably because environmental conditions influence the direction of gene flow (Nakajima et al. 2021).

Maps displaying modeled landscape resistance from G_{ST} and D_{PS} were visually similar (Fig. 2), indicating that the pattern has probably been maintained for a long time. Overall, gene flow is prevented in small tributaries in the southern area. This area displays higher water temperature fluctuations than the northern volcanic area where spring-fed environments are more prevalent (Ishiyama et al. 2023); gene flow in the southern area is probably suppressed by the effects of water temperature. When comparing this geographical pattern with the STRUCTURE barplots, the upper reaches in the non-volcanic area where gene flow is prevented roughly corresponded to the areas where a strong population structure was observed. While the reason for the heterogeneity in the strength of population structure could not be known by the STRUCTURE, a possible explanation was explicitly presented in the gene flow analysis.

Under the RCP8.5 scenario, reduced gene flow and increased landscape resistance across the watershed were predicted (Fig. 2d). Since the studied species exhibited a clear genetic structure only in the southern area, the prediction that the northern area will have the same level of gene flow as the present southern area indicates that each tributary within the watershed may experience genetic fragmentation in the future. Nevertheless, gene flow in the northern area was expected to be maintained spatially continuously to some extent, indicating that streams with volcanic watersheds are important for ensuring population connectivity under climate change. A previous study suggested that streams with low summer temperatures behave as source habitats in the watershed (Nakajima et al. 2021). Our study showed that these streams may serve not only as source habitats but also as migration pathways in the watershed. As a scenario analysis, Inoue and Berg (2017) considered landscape resistance to be the inverse of the species distribution model (SDM) estimates and predicted that an increased landscape resistance would reduce the gene flow of freshwater bivalves in the future. This is a valuable study that attempts to predict future changes in gene flow; however, it is known that the habitat suitability maps created by SDMs provide poor estimates of genetic resistance, because of

the conceptual differences between habitat selection and entire gene flow (Wasserman et al. 2010, 2012; Sator et al. 2022; Mateo-Sánchez 2015). Actually, in *C. nozawae*, the SDM created in Suzuki et al. (2021) indicated that the catchment area, analogous to the stream order, had a negative effect on the occurrence of this species, in contrast to the gene flow characteristics estimated in our study. Therefore, genetic population connectivity should be considered separately from habitat suitability.

The present study is novel in that gene flow was modeled using riverscape variables identified from genetic data and including water temperature. Our results showed that gene flow in the cold-water sculpin is expected to decrease dramatically in response to a changing climate. Therefore, under ongoing climate change, it is important to maintain habitat continuity within the distribution ranges. In particular, it is necessary to consider that the risk is high in sections where water temperature fluctuations are large (such as non-volcanic watersheds). Additionally, while main streams may be less suitable as habitats, they are important as migration corridors. No structures such as weirs should be installed so that drifted individuals can quickly enter suitable habitats.

To obtain more robust results, it would be desirable to increase the number of sampling populations. This study has the potential for further development. For example, demography simulations using inferred landscape resistance (Landguth et al. 2010, 2016) could reveal population viability. Also, combined with habitat quality analyses such as SDMs, population connectivity could be quantified for more detailed predictions from the viewpoint of habitat availability (Saura and Pascual-Hortal 2007). We hope that riverscape genetic modeling will be applied to predict the consequences of environmental changes on a variety of freshwater organisms.

References

Almodóvar A, Nicola GG, Ayllón D, Elvira B (2012) Global warming threatens the persistence of Mediterranean brown trout. Glob Chang Biol 18:1549–1560. https://doi.org/10.1111/j.1365-2486.2011.02608.x

Aunins AW, Petty JT, King TL et al (2015) River mainstem thermal regimes influence population structuring within an appalachian brook trout population. Conserv Genet 16:15–29. https://doi.org/10.1007/s10592-014-0636-6

Balkenhol N, Cushman SA, Storfer A, Waits LP (2015) Introduction to landscape genetics - concepts, methods, applications. In: Balkenhol N, Cushman SA, Storfer A, Waits LP (ed) Landscape Genetics. Wiley, New York, pp 1–8

Barbarossa V, Bosmans J, Wanders N, et al (2021) Threats of global warming to the world's freshwater fishes. Nat Commun 12:1701. https://doi.org/10.1038/s41467-021-21655-w

Bowcock AM, Ruiz-Linares A, Tomfohrde J, et al (1994) High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368:455–457. https://doi.org/10.1038/368455a0

Cain MK, Zhang Z (2019) Fit for a Bayesian: An evaluation of PPP and DIC for structural equation modeling. Struct Equ Model 26:39–50. https://doi.org/10.1080/10705511.2018.1490648

Caldera EJ, Bolnick DI (2008) Effects of colonization history and landscape structure on genetic variation within and among threespine stickleback (*Gasterosteus aculeatus*) populations in a single watershed. Evol Ecol Res 10:575–598

Catchen J, Hohenlohe PA, Bassham S, et al (2013) Stacks: An analysis tool set for population genomics. Mol Ecol 22:3124–3140. https://doi.org/10.1111/mec.12354

Chafin TK, Mussmann SM, Douglas MR, Douglas ME (2021) Quantifying isolation-by-resistance and connectivity in dendritic ecological networks. bioRxiv. https://doi.org/10.1101/2021.03.25.437078

Comte L, Buisson L, Daufresne M, Grenouillet G (2012) Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. Freshw Biol 58:625–639. https://doi.org/10.1111/fwb.12081

Davis CD, Epps CW, Flitcroft RL, Banks MA (2018) Refining and defining riverscape genetics: How rivers influence population genetic structure. WIREs Water 5:e1269. https://doi.org/10.1002/wat2.1269

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361. https://doi.org/10.1007/s12686-011-9548-7

Elith J, Leathwick JR (2009) Species distribution models: Ecological explanation and prediction across space and time. Annu Rev Ecol Evol Syst 40:677–697.

https://doi.org/10.1146/annurev.ecolsys.110308.120159

Escalante MA, García-De León FJ, Ruiz-Luna A et al (2018) The interplay of riverscape features and exotic introgression on the genetic structure of the Mexican golden trout (*Oncorhynchus chrysogaster*), a simulation approach. J Biogeogr 45:1500–1514. https://doi.org/10.1111/jbi.13246

Escalante MA, Perrier C, García-De León FJ et al (2020) Genotyping-by-sequencing reveals the effects of riverscape, climate and interspecific introgression on the genetic diversity and local adaptation of the endangered Mexican golden trout (*Oncorhynchus chrysogaster*). Conserv Genet 21:907–926. https://doi.org/10.1007/s10592-020-01297-z

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14:2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

García Molinos J, Ishiyama N. Sueyoshi M, Nakamura F (2022) Timescale mediates the effects of environmental controls on water temperature in mid- to low-order streams. Sci Rep 12:12248. https://doi.org/10.1038/s41598-022-16318-9

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486. https://doi.org/10.1093%2Foxfordjournals.jhered.a111627

Grant EHC, Lowe WH, Fagan WF (2007) Living in the branches: population dynamics and ecological processes in dendritic networks. Ecol Lett 10:165–175. https://doi.org/10.1111/j.1461-0248.2006.01007.x

Grummer JA, Beheregaray LB, Bernatchez L (2019) Aquatic landscape genomics and environmental effects on genetic variation. Trends Ecol Evol 34:641–654. https://doi.org/10.1016/j.tree.2019.02.013

Han CC, Tew KS, Fang LS (2007) Spatial and temporal variations of two cyprinids in a subtropical mountain reserve – a result of habitat disturbance. Ecol Freshw Fish 16:395–403. https://doi.org/10.1111/j.1600-0633.2007.00227.x

Hand BK, Muhlfeld CC, Wade AA et al (2016) Climate variables explain neutral and adaptive variation within salmonid metapopulations: the importance of replication in landscape genetics.

399 Mol Ecol 25:689–705. https://doi.org/10.1111/mec.13517
400 Hijmans RJ, Cameron SE, Parra JL, et al (2005) Very high resolution interpolated climate surfaces for
401 global land areas. Int J Climatol 25:1965–1978. https://doi.org/10.1002/joc.1276
402 Holsinger KE, Weir BS (2009) Genetics in geographically structured populations: Defining,
403 estimating and interpreting F_{ST}. Nat Rev Genet 10:639–650. https://doi.org/10.1038/nrg2611
404 Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the
405 assistance of sample group information. Mol Ecol Resour 9:1322–1332.
406 https://doi.org/10.1111/j.1755-0998.2009.02591.x
407 Inoue K, Berg DJ (2017) Predicting the effects of climate change on population connectivity and
408 genetic diversity of an imperiled freshwater mussel, *Cumberlandia monodonta* (Bivalvia:
409 Margaritiferidae), in riverine systems. Glob Chang Biol 23:94–107.
410 https://doi.org/10.1111/gcb.13369
411 IPCC (2014) Summary for policymakers. https://www.ipcc.ch/report/ar5/wg2/
412 Ishiyama N, Sueyoshi M, García Molinos J, et al (2023) Underlying geology and climate interactively
413 shape climate change refugia in mountain streams. Ecol Monogr.
414 https://doi.org/10.1002/ecm.1566
415 Kanno Y, Vokoun JC, Letcher BH (2011) Fine-scale population structure and riverscape genetics of
416 brook trout (*Salvelinus fontinalis*) distributed continuously along headwater channel networks.
417 Mol Ecol 20:3711–3729. https://doi.org/10.1111/j.1365-294X.2011.05210.x
418 Koizumi I, Kanazawa Y, Tanaka Y (2013) The fishermen were right: experimental evidence for
419 tributary refuge hypothesis during floods. Zool Sci 30:375–379.
420 https://doi.org/10.2108/zsj.30.375
421 Koizumi I, Maekawa K (2004) Metapopulation structure of stream-dwelling Dolly Varden charr
422 inferred from patterns of occurrence in the Sorachi River basin, Hokkaido, Japan. Freshw Biol
423 49:973–981. https://doi.org/10.1111/j.1365-2427.2004.01240.x
424 Kopelman NM, Mayzel J, Jakobsson M, et al (2015) Clumpak: A program for identifying clustering
425 modes and packaging population structure inferences across K. Mol Ecol Resour 15:1179–1191.
426 https://doi.org/10.1111/1755-0998.12387

427 Kottler EJ, Dickman EE, Sexton JP, et al (2021) Draining the swamping hypothesis: little evidence
428 that gene flow reduces fitness at range edges. Trends Ecol Evol 36:533–544.
429 https://doi.org/10.1016/j.tree.2021.02.004
430 Lamphere BA, Blum MJ (2012) Genetic estimates of population structure and dispersal in a benthic
431 stream fish. Ecol Freshw Fish 21:75–86. https://doi.org/10.1111/j.1600-0633.2011.00525.x
432 Landguth EL, Bearlin A, Day CC, Dunham J (2016) CDMetaPOP: an individual-based, eco-
433 evolutionary model for spatially explicit simulation of landscape demogenetics. Methods Ecol
434 Evol 8:4–11. https://doi.org/10.1111/2041-210X.12608
435 Landguth EL, Cushman SA, Schwartz MK, et al (2010) Quantifying the lag time to detect barriers in
436 landscape genetics. Mol Ecol 19:4179–4191. https://doi.org/10.1111/j.1365-294X.2010.04808.x
437 Leroy G, Carroll EL, Bruford MW et al (2018) Next-generation metrics for monitoring genetic erosion
438 within populations of conservation concern. Evol Appl 11:1066–1083.
439 https://doi.org/10.1111/eva.12564
440 Manel A, Holdergger R (2013) Ten years of landscape genetics. Trends Ecol Evol 28:614–621.
441 https://doi.org/10.1016/j.tree.2013.05.012
442 Mateo-Sánchez MC, Balkenhol N, Cushman S, et al (2015) A comparative framework to infer
443 landscape effects on population genetic structure: are habitat suitability models effective in
444 explaining gene flow? Landsc Ecol 30:1405–1420. https://doi.org/10.1007/s10980-015-0194-4
445 McRae BH (2006) Isolation By Resistance. Evolution 60:1551–1561. https://doi.org/10.1554/05-321.1
446 McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. Proc
447 Natl Acad Sci U S A 104:19885–19890. https://doi.org/10.1073/pnas.0706568104
448 Nagasaka A, Sugiyama S (2010) Factors affecting the summer maximum stream temperature of small
449 streams in northern Japan. Bull Hokkaido For Res Inst 47:35–43. (In Japanese with English
450 abstract)
451 Nakajima S, Sueyoshi M, Hirota SK, et al (2021) A strategic sampling design revealed the local
452 genetic structure of cold-water fluvial sculpin: a focus on groundwater-dependent water
453 temperature heterogeneity. Heredity 127:413–422. https://doi.org/10.1038/s41437-021-00468-z
454 Nakamura F (2022) Riparian forests and climate change: interactive zone of green and blue

455 infrastructure. In: Nakamura F (ed) Green Infrastructure and Climate Change Adaptation.
456 Springer, Singapore, pp 73–91
457 Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci U S A
458 70:3321–3323. https://doi.org/10.1073/pnas.70.12.3321
459 Oksanen JF, Blanchet G, Friendly M et al (2019) vegan: community ecology package. R package
460 version 2.5-6. https://CRAN.R-project.org/package=vegan
461 Oliveira J dos A, Farias IP, Costa GC, Werneck FP (2019) Model-based riverscape genetics:
462 disentangling the roles of local and connectivity factors in shaping spatial genetic patterns of two
463 Amazonian turtles with different dispersal abilities. Evol Ecol 33:273–298.
464 https://doi.org/10.1007/s10682-019-09973-4
465 Paris JR, Stevens JR, Catchen JM (2017) Lost in parameter space: a road map for stacks. Methods
466 Ecol Evol 8:1360–1373. https://doi.org/10.1111/2041-210X.12775
467 Paz-Vinas I, Blanchet S (2015) Dendritic connectivity shapes spatial patterns of genetic diversity: A
468 simulation-based study. J Evol Biol 28:986–994. https://doi.org/10.1111/jeb.12626
469 Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for
470 teaching and research-an update. Bioinformatics 28:2537–2539.
471 https://doi.org/10.1093/bioinformatics/bts460
472 Peterson EE, Hanks EM, Hooten MB, et al (2019) Spatially structured statistical network models for
473 landscape genetics. Ecol Monogr 89:e01355. https://doi.org/10.1002/ecm.1355
474 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus
475 genotype data. Genetics 155:945–959. https://doi.org/10.1093/genetics/155.2.945
476 Rahel FJ, Keleher CJ, Anderson JL (1996) Potential habitat loss and population fragmentation for cold
477 water fish in the North Platte River drainage of the Rocky Mountains: Response to climate
478 warming. Limnol Oceanogr 41:1116–1123. https://doi.org/10.4319/lo.1996.41.5.1116
479 R Core Team (2019) R: a language and environment for statistical computing. R Foundation for
480 Statistical Computing, Vienna, Austria. https://www.R-project.org/
481 Sartor CC, Wan HY, Pereira JA, et al (2022) Landscape genetics outperforms habitat suitability in
482 predicting landscape resistance for congeneric cat species. J Biogeogr 49:2206–2217.

https://doi.org/10.1111/jbi.14498

Saura S, Pascual-Hortal L (2007) A new habitat availability index to integrate connectivity in landscape conservation planning: Comparison with existing indices and application to a case study. Landsc Urban Plan 83:91–103. https://doi.org/10.1016/j.landurbplan.2007.03.005

Savary P, Foltête JC, Moal H, et al (2021) graph4lg: A package for constructing and analysing graphs for landscape genetics in R. Methods Ecol Evol 12:539–547. https://doi.org/10.1111/2041-210X.13530

Spear SF, Cushman SA, McRae BH (2015) Resistance Surface Modeling in Landscape Genetics. In: Balkenhol N, Cushman SA, Storfer A, Waits LP (ed) Landscape Genetics. Wiley, New York, pp 129–148

Sugawara M (1979) Automatic calibration of the tank model. Hydrological Sciences Bulletin 24:375–388. https:/doi.org/10.1080/02626667909491876

Suyama Y, Hirota SK, Matsuo A, et al (2022) Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny. Ecol Res 37:171–181. https://doi.org/10.1111/1440-1703.12270

Suyama Y, Matsuki Y (2015) MIG-seq: An effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. Sci Rep 5:16963. https://doi.org/10.1038/srep16963

Suzuki H, Nakatsugawa M, Ishiyama N (2022) Climate change impacts on stream water temperatures in the snowy cold region according to geological conditions. Water 14:2166. https://doi.org/10.3390/w14142166

Suzuki K, Ishiyama N, Koizumi I, Nakamura F (2021) Combined effects of summer water temperature and current velocity on the distribution of a cold-water-adapted sculpin (*Cottus nozawae*). Water 13:975. https://doi.org/10.3390/w13070975

Ueda S, Nakatsugawa M, Usutani T (2020) Estimation of high-resolution downscaled climate information based on verification of water balance in watershed of Hokkaido. Journal of Japan Society of Civil Engineers, Ser. B1 (Hydraulic Engineering) 76:I_25–I_30. https:/doi.org/10.2208/jscejhe.76.2_I_25. (In Japanese with English abstract)

Wasserman TN, Cushman SA, Schwartz MK, Wallin DO (2010) Spatial scaling and multi-model inference in landscape genetics: Martes americana in northern Idaho. Landsc Ecol 25:1601–1612. https://doi.org/10.1007/s10980-010-9525-7

Wasserman TN, Cushman SA, Shirk AS, et al (2012) Simulating the effects of climate change on population connectivity of American marten (*Martes americana*) in the northern Rocky Mountains, USA. Landsc Ecol 27:211–225. https://doi.org/10.1007/s10980-011-9653-8

White SL, Hanks EM, Wagner T (2020) A novel quantitative framework for riverscape genetics. Ecol Appl 30:e02147. https://doi.org/10.1002/eap.2147

Woodward G, Perkins DM, Brown LE (2010) Climate change and freshwater ecosystems: Impacts across multiple levels of organization. Philos Trans R Soc B Biol Sci 365:2093–2106. https://doi.org/10.1098/rstb.2010.0055

Wright S (1943) Isolation by distance. Genetics 28:114–138. https://doi.org/10.1093/genetics/28.2.114

Yagami T, Goto A (2000) Patchy distribution of a fluvial sculpin, *Cottus nozawae*, in the Gakko River system at the southern margin of its native range. Ichthyol Res 47:277–286

Zeller KA, McGarigal K, Whiteley AR (2012) Estimating landscape resistance to movement: a review. Landsc Ecol 27:777–797. https://doi.org/10.1007/s10980-012-9737-0

Statements & Declarations

Funding: This study is partly supported by the research fund for the Ishikari and Tokachi Rivers provided by the Ministry of Land, Infrastructure, Transport and Tourism of Japan.

Competing Interests: The authors declare no competing interests.

Author Contributions: Conceptualization, S.N. and F.N.; Data curation, S.N. and S.K.H.; Formal analysis, S.N.; Funding acquisition; F.N.; Investigation, S.N., H.S., A.M., and S.K.H. ; Methodology, S.N., H.S., M.N., and Y.S.; Project administration, F.N.; Resources, S.N., M.N., A.M., and Y.S.; Software, S.N. and H.S., A.M., and S.K.H.; Supervision, M.N., Y.S. and F.N.; Validation, S.N. and F.N.; Visualization, S.N.; Writing – original draft, S.N. and H.S. ; Writing – review & editing, S.N., H.S., M.N., A.M., S.K.H., Y.S., and F.N.

Data Availability: Genetic and environmental data generated in this study were deposited at Figshare

539 (doi: 10.6084/m9.figshare.19694989).

540

541 **Table 1** Riverscape variables considered in the present study.

Variable	Description	Hypothesis / Ecological importance	Ranges
Summer water temperature [A]	Mean water temperature from July to August (July 2019 to August 2019) [°C]	Streams with low summer water temperatures are suitable for *C. nozawae* occupancy/survival (Yagami and Goto 2000; Suzuki et al. 2021) and therefore migration may also occur frequently in these streams.	8.32–14.2
Water temperature fluctuation [A]	Standard deviation of the water temperature in one year (September 2018 to August 2019)	Thermally stable streams can be suitable for migration.	1.97–5.09
Drought water discharge [BC]	Flow rate on the day when the flow is 355th highest in one year [m^3/s] (September 2018 to August 2019)	Drought water discharges, which particularly reflect the environmental heterogeneity created by groundwater (Nagasaka and Sugiyama 2010), ensure opportunities to colonize throughout the year.	0.07–6.24
Flow fluctuation [AB]	Coefficient of variation in the daily flow rate in one year (September 2018 to August 2019)	Hydrologically stable streams can be suitable for migration.	0.31–0.65
Edge length	Length of edges [km]	Isolation by distance (Wright 1943)	0.05–7.19
Slope	Mean gradient of the edge, i.e., the elevation range divided by edge length	Fish movement and migration are often impeded on steep slopes (Kanno et al. 2011).	4.15–56.5
Strahler's stream order [C]	Strahler's stream order of the edge	Even in cold-water fish, the mainstem may function as a corridor that facilitates connectivity among populations (White et al. 2020).	1–4
Shreve's stream order [BC]	Shreve's stream order (link magnitude), i.e., the numbers of confluence points upstream, at the midpoint of the edge	Given the dendritic arrangement and asymmetry of stream networks, sections with more confluence points upstream may increase the number of migrants passing through (Paz-Vinas and Blanchet 2015).	1–62
Catchment area [BC]	Cumulative area of the catchment calculated at the midpoint of the edge [km^2]	Sections with larger catchment areas may have more migrants passing through, based on the same principle as that of the stream order. Or, conversely, streams with larger catchment areas have been identified to have lower *C. nozawae* occupancies (Suzuki et al. 2021) and therefore it is also possible that less migration occurs in sections with larger catchment areas.	6.4–296.3
Direction	Whether the gene flow is toward the upstream (0) or downstream (1) direction	Most stream organisms have higher migration rates in the downstream direction than in the upstream direction (Lamphere and Blum 2012).	0 or 1

542 Variables with the same letters (A, B, C) have high correlations ($|r| > 0.7$); these variables were not included in the same model.

Table 2 Selected models explaining the strength of gene flow on the edges. Estimated β values (median) and their 95% credible intervals (95% CI) are displayed.

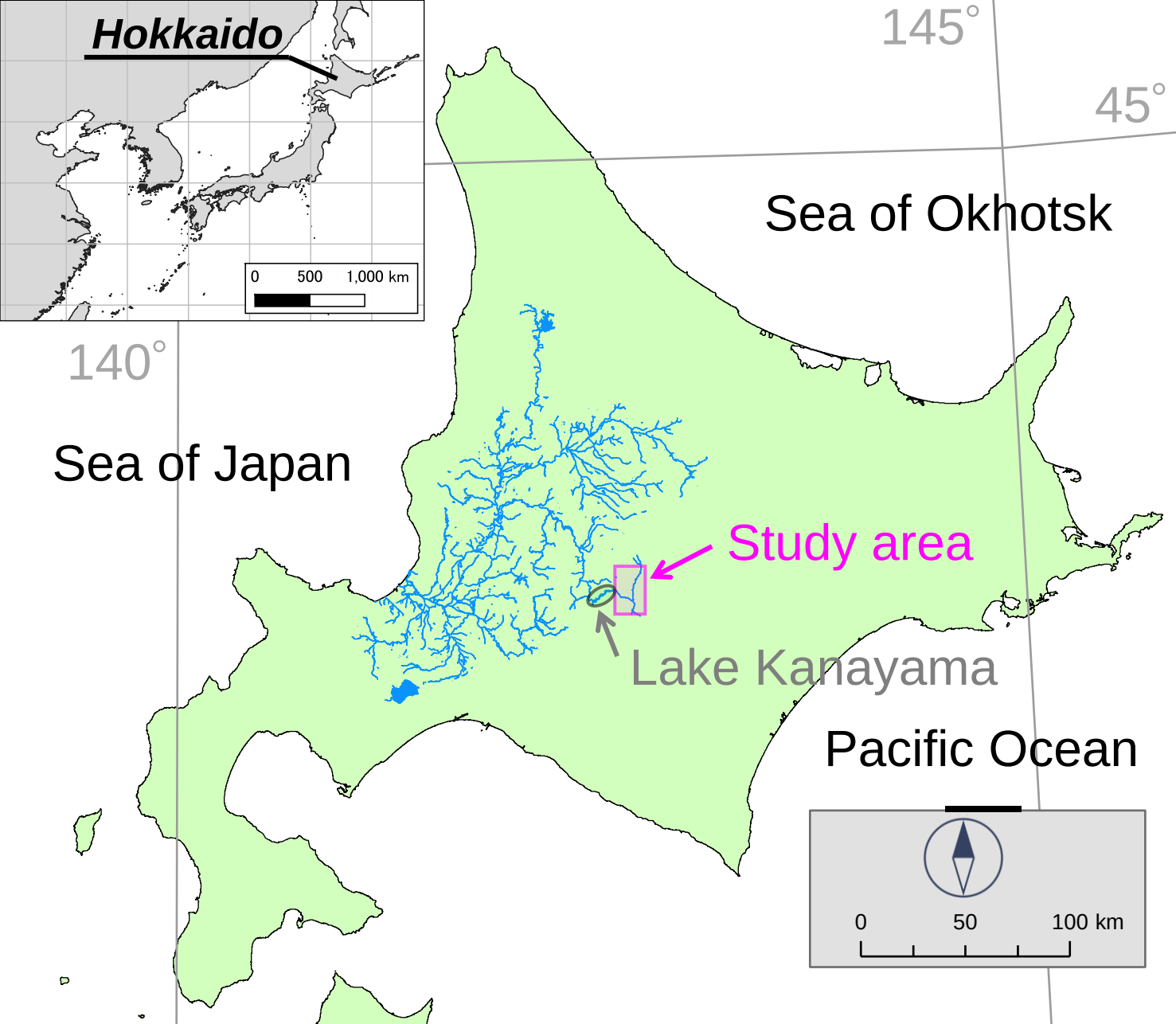
Variables	β	95% CI
(A) G_{ST}		
(Intercept)	5.84	5.65, 5.98
Shreve's stream order	4.51	3.96, 5.19
Water temperature fluctuation	-0.48	-0.61, -0.35
Slope	-0.94	-1.15, -0.67
Edge length	-0.42	-0.54, -0.32
(B) D_{PS}		
(Intercept)	6.06	4.94, 6.85
Strahler's stream order	2.50	1.72, 3.15
Summer water temperature	-1.42	-2.49, -0.38

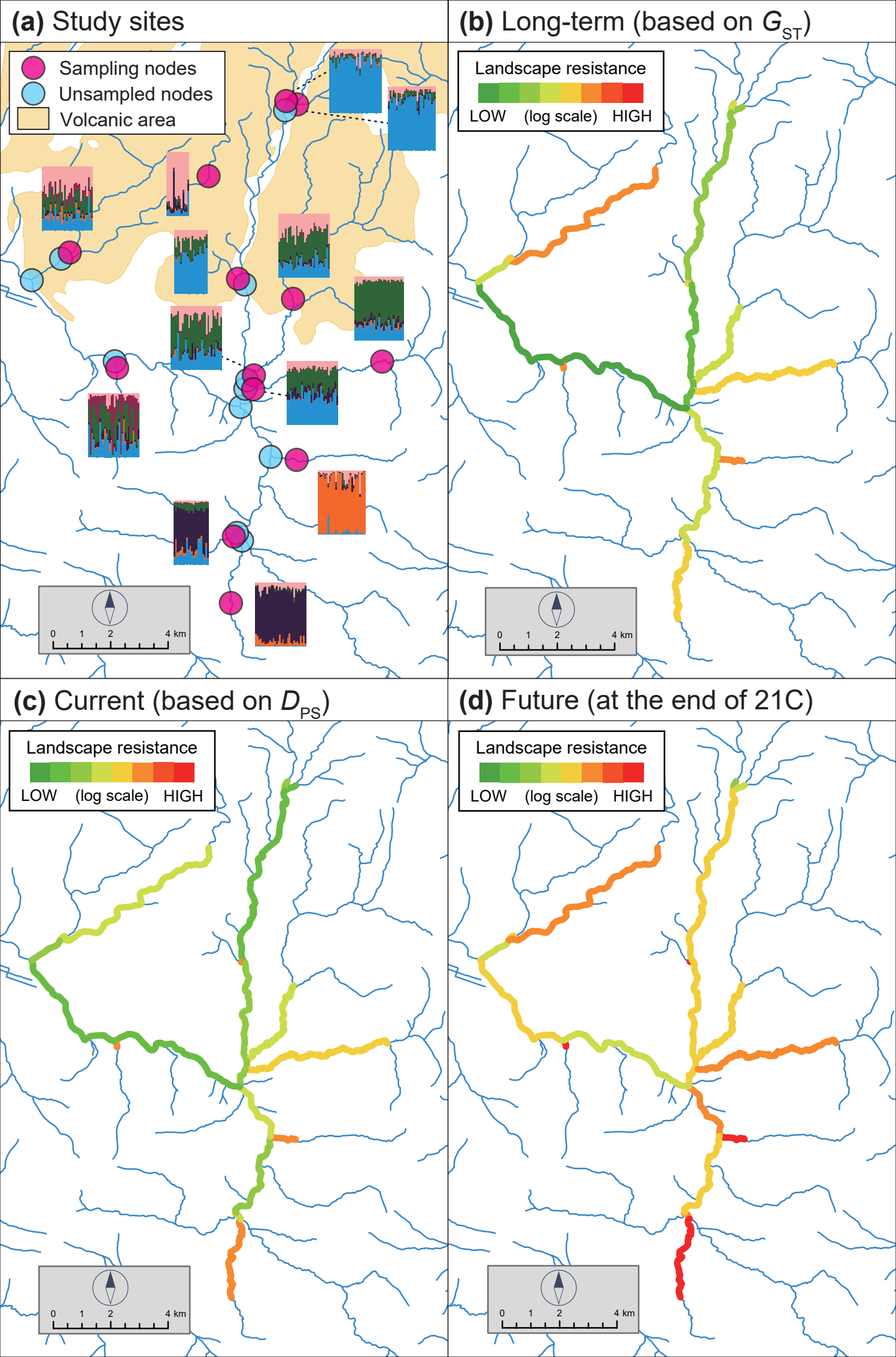
Figure Legends

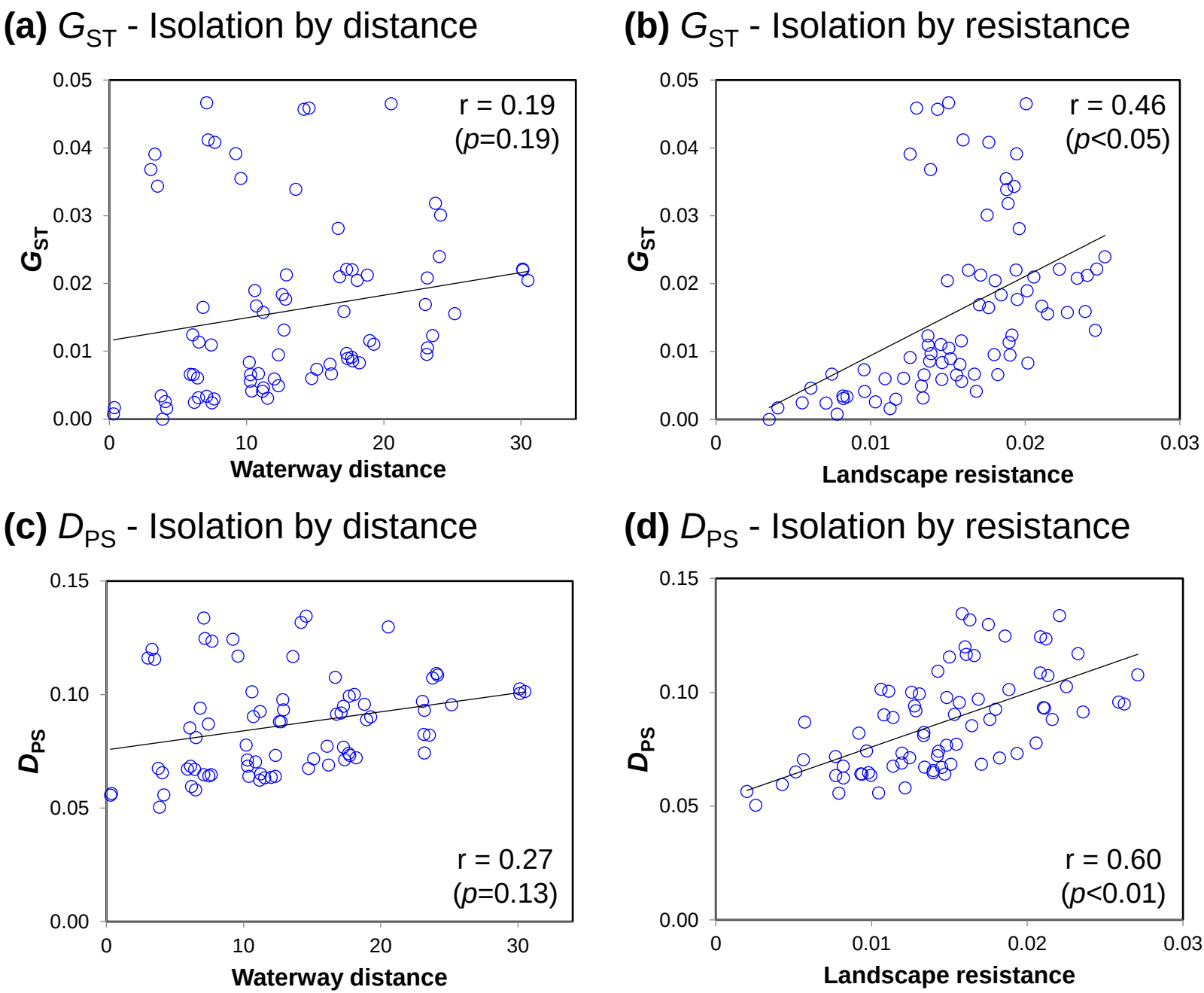
Fig. 1 Location of the study area. The blue network indicates the rivers belonging to the Ishikari River system, which has the second largest watershed in Japan and includes the Sorachi River.

Fig. 2 Maps of the Sorachi River watershed showing the study area (a) and landscape resistance estimated by BGR models (b–d). In panel (a), sampling nodes (sampling sites) and unsampled nodes (major confluences between them), which are delimitations of edges (stream sections), are denoted. Barplots with each sampling node indicate the population structure inferred by STRUCTURE (K = 6). Landscape resistance is shown in three patterns: long-term gene flow modeled by G_{ST} (b), recent gene flow modeled by D_{PS} (c), predicted gene flow at the end of the 21st century derived by substituting future water temperatures into the model derived by D_{PS} (d).

Fig. 3 Isolation by distance and isolation by resistance. The relationship between pairwise genetic distance and cumulative landscape resistance between populations (b, d) is compared to the relationship with simple waterway geographic distance (a, c). The cases of G_{ST} (a, b) and D_{PS} (c, d) are shown.


Hokkaido
0 500 1,000 km
145°
45°
140°
Sea of Okhotsk
Sea of Japan
Study area
Lake Kanayama
Pacific Ocean
0 50 100 km


(a) Study sites
Sampling nodes
Unsampled nodes
Volcanic area
0 1 2 4 km
(b) Long-term (based on G_{ST})
Landscape resistance
LOW (log scale) HIGH
0 1 2 4 km
(c) Current (based on D_{PS})
Landscape resistance
LOW (log scale) HIGH
0 1 2 4 km
(d) Future (at the end of 21C)
Landscape resistance
LOW (log scale) HIGH
0 1 2 4 km


(a) G_{ST} - Isolation by distance
0.05
0.04
0.03
0.02
0.01
0.00
G_{ST}
r = 0.19
(p=0.19)
0
10
20
30
Waterway distance
(b) G_{ST} - Isolation by resistance
0.05
0.04
0.03
0.02
0.01
0.00
G_{ST}
r = 0.46
(p<0.05)
0
0.01
0.02
0.03
Landscape resistance
(c) D_{PS} - Isolation by distance
0.15
0.10
0.05
0.00
D_{PS}
r = 0.27
(p=0.13)
0
10
20
30
Waterway distance
(d) D_{PS} - Isolation by resistance
0.15
0.10
0.05
0.00
D_{PS}
r = 0.60
(p<0.01)
0
0.01
0.02
0.03
Landscape resistance