Genetic structure and diversity of amphidromous sculpin in Shiretoko, a mountainous peninsula in Japan

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Abstract

Despite the global crisis facing migratory benthic fishes, conservation genetic knowledge of these species remains scarce. In this study, we conducted a population genetic analysis using seven microsatellite loci to obtain basic information for determining conservation units and priorities of *Cottus hangiongensis* in Shiretoko, a mountainous peninsula where sculpin habitats are thought to be in decline throughout the region. The genetic structure was clearly divided between west and east coastal populations, and there was little recent migration between them. The western populations, which are closer to the center of the species' range, had significantly higher genetic diversity than the eastern populations. However, a bottleneck analysis and the inference of demographic history using approximate Bayesian computation showed that only the west group had experienced a significant recent bottleneck, probably due to recent habitat losses. These results suggest that the western and eastern populations should be different conservation units and that the western populations should be prioritized for conservation despite their high genetic diversity. This study contributes to the conservation genetics of diadromous sculpin and reiterates the importance of analyzing not only the current levels but also temporal changes in assessing genetic diversity.

Keywords: Diadromous benthic fish, *Cottus hangiongensis*, Bottleneck, Central–marginal hypothesis, Approximate Bayesian skyline plots, Microsatellite

Introduction

In a global biodiversity crisis, understanding the genetic structure and diversity of wild populations prior to planning conservation measures is effective in strategically preventing the loss of species and intraspecific diversity (Spielman et al. 2004; Hohenlohe et al. 2021). This is because genetic structure provides the basis for deciding management units based on population connectivity (Moritz 2002), and genetic diversity is an indicator of effective population size related to population vulnerability and adaptability to change (Reusch et al. 2005; Willi et al. 2022). Since natural and human interference with the environment will also change genetic diversity, investigating recent changes in population size is also considered to be effective for understanding the current health and viability of populations in a changing environment (Nunziata et al. 2017; Nakajima et al. 2020). Currently, it is required to investigate genetic structure and diversity in a wide range of organisms to assess extinction risks of species and genetically defined populations (Laikre et al. 2020).

Fishes with diadromous (migration between marine and freshwater) life histories are in rapid global decline due to river fragmentation and subsequent loss of available habitats (Limburg and Waldman 2009; Mota et al. 2016). The impact of such habitat loss has been recognized mainly in salmonids, and habitat restoration through environmental improvement or fishway installation has been carried out for these species (Roni et al. 2008; Nakamura and Komiyama 2010), but to a lesser extent for benthic fishes typified by sculpin. The lack of focus on benthic fishes may be due not only to their low economic and commercial value (Lucek et al. 2018) but also to a lack of understanding of the vulnerability of populations. In the case of the genus *Cottus* (Teleostei: Cottidae), a highly diversified sculpin group that comprises more than 60 species worldwide (Goto et al. 2015), although genetic diversity in species with fluvial life-history has been studied in relation to dams (Hänfling and Weetman 2006; Junker et al. 2012) or environments (Ruppert et al. 2017; Nakajima et al. 2021), there are not many studies pertaining to species with diadromous life history. Additionally, a few studies treating genetic data of diadromous species have usually focused on macroevolutionary processes such as speciation, and have not assessed and discussed recent changes in genetic diversity (e.g., Tsukagoshi et al. 2013; Dennenmoser et al. 2014, 2017; Kanno et al. 2018; but see Baek et al. 2018), even though benthic fish are particularly susceptible to river-crossing structures (Terui and Miyazaki

2017). Considering that diadromous fish usually exhibit high mobility and connectivity between neighboring tributaries, it will be necessary to identify the group of strongly connected populations (subpopulation structure) and to evaluate the genetic diversity of each subpopulation unit. In areas where check dams are often placed near the mouth of rivers to prevent coastal residential areas from sediment-related disasters, the decline in genetic diversity is expected to be more pronounced.

Cottus hangiongensis is a diadromous benthic fish that is distributed along the Sea of Japan in Russia, Korea, and northern Japan (Baek et al. 2018). In Japan, this species is distributed on the Sea of Japan side from Central Honshu to Hokkaido Island, with a discontinuous distribution on the Sea of Okhotsk side of Hokkaido and around the Tsugaru Strait (Goto 1981). Korean and Honshu populations are locally threatened with extinction in part due to river construction pressures and a decrease in available habitat (National Institute of Biological Resources of Korea 2011; Ministry of the Environment Government of Japan 2020). Hatched juveniles of this species migrate downstream to the ocean, and after 4–6 weeks they return to the river to begin their benthic life (i.e., a life history called ”amphidromous” among diadromous; Goto 1981, 1984), while it is still not known whether the juveniles of *C. hangiongensis* return to the same rivers where they hatched. From the perspective of conservation, although studies have been conducted on what kind of environment should be improved to increase suitable habitat (Goto 1981; Kitagawa et al. 2021), research on conservation genetics is scarce. The only population genetic study of *C. hangiongensis* using microsatellite loci was conducted by Baek et al. (2018), who evaluated the genetic structure and diversity of this species on the east coast of Korea and revealed the absence of genetic structure and highly maintained genetic diversity. However, since this is an example of one region with a relative abundance of rivers where fish can run up, we may not be able to generalize this pattern. For instance, patterns may be different in regions where the habitat is decreasing at the regional level. Although it is obvious that the habitat of this species has been decreasing in recent years in some regions, our knowledge about the current status and genetic demography of this species is lacking, as it is for other diadromous sculpin species.

Given the need to understand the current status of diadromous sculpin populations where the habitat is decreasing, we conducted a population genetic study of *C. hangiongensis* in the Shiretoko Peninsula, a mountainous peninsula where the habitat of this species is thought to be shrinking at the

regional level. The aims of the present study are (i) to identify the connectivity and substructure of the *C. hangiongensis* populations in the Shiretoko Peninsula and (ii) to evaluate patterns of genetic diversity in this species, considering recent temporal changes. This study will provide a basis for determining conservation units and priorities for this species as a case study in the conservation genetics of diadromous sculpin whose habitat is decreasing.

Methods

Sample collection

This study was conducted in the rivers of the Shiretoko Peninsula, which is located at the northeastern tip of Hokkaido Island (Japan) and protrudes into the Sea of Okhotsk, characterized by a branch of the Tsushima Current (Fig. 1; Table 1). The Shiretoko Peninsula has a mountainous topography, and most of the streams empty directly into the ocean with the appearance of mountain streams, so it is not uncommon for check dams to be installed only a few hundred meters from the sea (Nakamura and Komiyama 2010); the space available for migratory fishes to inhabit would have been correspondingly decreased. A total of 165 *Cottus hangiongensis* were caught from nine rivers using electrofishing (Model 12-B Backpack Electrofisher; Smith-Root, Vancouver, USA) in October 2020. Three of nine populations were sampled from the Okhotsk (west) side and the other six were from the Nemuro Strait (east) side. All sampling sites are downstream of the lowest dam of each river, as we could not capture any individuals above the dams. From the captured fish, small pieces of fin tissue were clipped, placed in 99.5% ethanol, and stored at −20 °C in the laboratory until DNA extraction.

DNA extraction, amplification, and sequencing

Total DNA was extracted using the QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The nine nuclear microsatellite loci (Englbrecht et al. 1999; Nolte et al. 2005), where the 5’ end of each forward primer was labeled with either a HEX, 6-FAM, NED, or PET fluorescent dye, were amplified in two multiplex sets A and B (Table 2). PCR was conducted using the Type-it Microsatellite PCR Kit (Qiagen), with each 10.0 µl reaction containing 5.0 µl of 2× Type-it Multiplex PCR Master Mix, 1.0 µl of primer mix with 0.2 µM of each of forward and reverse primer, 2.0 µl of

RNase-free water, and 2.0 µl of extracted genomic DNA. For multiplex set A, the PCR process consisted of an initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 90 s, and extension at 72 °C for 30 s; and a final extension at 60 °C for 30 min. For multiplex set B, an initial denaturation at 95 °C for 5 min; 27 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 90 s, and extension at 72 °C for 30 s; and a final extension at 60 °C for 30 min. Fragment sizes were determined using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, USA) in the DNA Sequencing Facility of the Graduate School of Agriculture (Hokkaido University) and GeneMarker software (SoftGenetics, State College, USA) with GeneScan 500 LIZ dye Size Standard (Applied Biosystems). All samples were scored blindly (i.e., with sample names removed) and repeated multiple times to ensure accuracy and consistency. The two loci, Cott207 and Cgo1016, did not allow for accurate scoring in multiple samples; these loci were excluded from the analysis.

Genetic diversity and differentiation

To evaluate the intrapopulation genetic diversity, allelic richness (El Mousadik and Petit 1996), expected heterozygosity (H_E), and the fixation index (F_{IS}) were calculated for each population using FSTAT 2.9.4 (Goudet 1995). To examine regional trends in genetic diversity, the differences in allelic richness and H_E between the western and eastern populations were tested by Welch's t-test. Significant deviations from Hardy–Weinberg equilibrium (HWE), as indicated by F_{IS} deviation from zero, were tested by 1,000 randomizations using FSTAT. Genetic differentiation between populations was evaluated by calculating global/pairwise F_{ST} (Weir and Cockerham 1984) and its standardized value, F'_{ST} (Meirmans and Hedrick 2011), with 9,999 randomizations using GenAlEx 6.5 (Peakall and Smouse 2012).

Population structure

The population structure was examined using STRUCTURE 2.3.4 (Pritchard et al. 2000), which implements a Bayesian clustering method using multi-locus allele frequency data. The STRUCTURE settings were the admixture and allele frequency correlated model with previous sampling location

information (LOCPRIOR; Hubisz et al. 2009). The algorithm was run 20 times for each K from 1 to 9 with a burn-in of 20,000 followed by 30,000 Markov chain Monte Carlo (MCMC) replicates. The program CLUMPAK (Kopelman et al. 2015) was used to summarize the results and to generate barplots for each K. STRUCTURE HARVESTER (Earl and vonHoldt 2012) was employed to calculate the probability of the data (LnP(D); Pritchard et al. 2000), the corresponding standard deviation, and Evanno's delta K (ΔK; Evanno et al. 2005).

Gene flow between subpopulations

Because STRUCTURE divided western and eastern individuals distinctly (see Results), we pooled individuals of western and eastern populations into their respective population groups (hereafter, subpopulation) and estimated recent gene flow between the two subpopulations. We used BA3MSAT (Mussmann et al. 2019), a reconstructed version of BayesAss (Wilson and Rannala 2003) that estimates dual-direction pairwise migration rates over a few generations, for the two subpopulations. The setting was 10,000,000 MCMC iterations, including a burn-in period of 5,000,000. The inferred migration rates with 95% credible intervals (estimates ± 1.96 × SD [SD: standard deviation]) that did not include zero were regarded as significant migration.

Temporal changes in genetic diversity indicated by population demography

To evaluate whether each subpopulation had experienced recent bottlenecks, heterozygosity excess was tested using Wilcoxon's signed rank test in software BOTTLENECK 1.2.02 (Piry et al. 1999) with 1,000 iterations. The program was run under the infinite alleles model (IAM), the stepwise mutation model (SMM), and the 2-phase model (TPM; assuming 70% single step mutations and a variance among mutation steps of 30). In addition to the bottleneck analysis, we inferred the shape of the demographic trajectories of each subpopulation using DIYABCskylineplot 1.0.1 (Navascués et al. 2017), which implements a relatively novel approach that introduces appropriate Bayesian computation (ABC) methods into the model-flexible demographic inferences. We set the following parameter values for DIYABCskylineplot analysis: num_of_points = 100 (number of points to draw skyline plot), prior_THETA_min = 0.1 (THETA is the population size and is measured by $4N\mu$, where

176 N is the effective population size and μ is the mutation rate per generation), prior_THETA_max = 10,
177 prior_GSM_min = 0.1 (GSM is the generalized stepwise mutation model for microsatellites),
178 prior_GSM_max = 0.8, prior_T_max = 250 (maximum time, measured in number of generations),
179 prior_MUTRATE_min = 0.0001 (MUTRATE is the μ value; μ for microsatellites generally ranges
180 from 10^{-4} to 10^{-3} [Estoup and Angers 1998; Yue et al. 2007]), prior_MUTATE_max = 0.0010, and the
181 repeat size for each locus was specified (2 for all). All other options and priors were set to default
182 values.
183
184 **Results**
185 *Summary statistics*
186 F_{IS} showed negative and positive values (ranged from -0.126–0.151) but did not deviate significantly
187 from zero in all populations, suggesting that HWE could be assumed in all populations. The allelic
188 richness and H_E ranged from 1.748–2.420 and 0.287–0.463, respectively (Table 1). Across the nine
189 populations, the global F_{ST} value was 0.121, and its standardized value, F'_{ST}, was 0.206, indicating
190 moderate population differentiation. Pairwise F_{ST} and F'_{ST} values ranged from 0.000–0.284 and
191 0.000–0.455, respectively (Table 3). The F'_{ST} values were 0.159–0.455 and significant in all pairs
192 between populations on different seaward sides, but up to 0.141 between populations on the same side
193 and were not significant in most pairs, with no differentiation observed in some of the especially close
194 populations (Pop5, 6, and 7; Pop7 and 8; Pop8 and 9).
195
196 *Population structure and gene flow*
197 In the STRUCTURE analysis, ΔK showed the highest value at K = 2, and LnP(D) increased until K =
198 4 (Fig. 1B). The population structure at K = 2 was quite clear, and each cluster corresponded with
199 actual western and eastern populations (Fig. 1C). In K = 4, individuals of Pop4 were grouped into
200 another single cluster, but this cluster was also mixed in with individuals of other eastern populations,
201 especially individuals of Pop8 and 9. Bayesian estimate of contemporary migration rate from west to
202 east subpopulations was 0.014 (SD = 0.012) and that from east to west subpopulations was 0.011 (SD
203 = 0.008), indicating that no significant migration occurred between the two subpopulations.

Genetic diversity and demographic histories in west and east subpopulations

From the comparison of genetic diversity, western populations have significantly higher genetic diversity than eastern populations with respect to both allelic richness ($p < 0.05$; Fig. 2A) and H_E ($p < 0.001$; Fig. 2B). BOTTLENECK software detected a recent population bottleneck owing to significant heterozygosity excess under all mutation models in the west subpopulation (Table 4), but not for the east subpopulation. DIYABCskylineplot estimated simple trajectories with overall constant population sizes, except that the west subpopulation experienced a rapid population contraction from tens of generations ago (Figs. 2C and 2D).

Discussion

Genetic differentiation and structure

This study revealed the patterns of population structure and genetic diversity of *C. hangiongensis* in the mountainous Shiretoko Peninsula. Genetic differentiation was low on the same seaward side, and there was no differentiation in some of the especially close populations (Table 3). This means that the tributaries on the same side are strongly connected by gene flow and that this species is not constrained to return to the same river. In the STRUCTURE analysis, Pop4 may appear to be grouped in a different cluster at high K. This population is a bit distant from the other eastern sampling sites and has low genetic diversity (Table 1), which may have led to greater genetic drift. However, individuals of other eastern populations also have a mixture of the cluster found in Pop4, and the F_{ST} between Pop4 and the majority of eastern populations was not significant; this population should be considered as being part of the east subpopulation.

Contrary to the low genetic differentiation between populations on the same side of the ocean, there was significant genetic differentiation and little gene flow between the western and eastern populations. Based on the distribution of *C. hangiongensis*, dispersal of this species is thought to be related to the Tsushima Current, which flows northward across the Sea of Japan and partly into the Sea of Okhotsk around the Shiretoko Peninsula (Goto 1981; Fig. 1A). Typically, the genetic differentiation of coastal fish inhabiting along ocean currents is low even if they are in distant locations (>500 km)

because the constitutive individuals are potentially dispersed by ocean currents (Mukai et al. 2009; Xiao et al. 2011; Yamakawa et al. 2019). In the case of *Cottus* species, although a previous study pertaining to diadromous populations in a gulf region (no currents) has shown clear genetic variation between different tributaries (Dennenmoser et al. 2014), Baek et al. (2018), studying the same species as the present study along the Sea of Japan, reported the absence of genetic structure in a maximum distance of 140 km between sites, a spatial scale almost identical to that of our study (the maximum coastal distance is 127 km in the present study). Therefore, a clear genetic structure within a narrow region may represent an unusual case and the genetic differentiation between the western and eastern populations may be due to the geographical or environmental conditions of the Shiretoko Peninsula. For example, genetic exchange with the eastern populations may be less likely to occur because the majority of the Tsushima Current coming from the west exits to the northeast instead of turning to the south (east coastal) direction (Takizawa 1982; Fig. 1).

Genetic diversity and its temporal change

Genetic diversity varied among the populations (Table 1), and there was a clear pattern of higher genetic diversity in the western populations than in the eastern populations (Fig. 2). It is known that wild organisms have higher genetic diversity in populations at the center of their distribution and lower in the peripheral (edge) populations (central–marginal hypothesis; Micheletti and Storfer 2015; Takahashi et al. 2016). In *C. hangiongensis*, the center of the distribution is around the Sea of Japan, and populations on the Sea of Okhotsk coast are considered to have been dispersed and established by the Tsushima Current (Goto 1981). Thus, the detected patterns of genetic diversity are consistent with the central–marginal hypothesis.

In contrast to the level of genetic diversity, BOTTLENECK and demographic analysis consistently showed that only the west subpopulation experienced recent bottlenecks (Fig. 2; Table 4). While genetic diversity often maintains equilibrium at low levels, experiencing a bottleneck indicates that genetic diversity is in decline and population viability is threatened (Teixeira and Huber 2021). In this case, although current genetic diversity in western populations is still high, it may be in the process of decreasing with shrinking population. The rapid population contraction was estimated to

have begun tens of generations ago, although the uncertainty of the DIYABCskylineplot somewhat increases in bottleneck patterns (Navascués et al. 2017). What BOTTLENECK is inferring is also a recent population shrinkage because this analysis detects heterozygosity excess based on the principle that allelic diversity is reduced faster than heterozygosity in recently bottlenecked populations. These results suggest that the population contraction of the west subpopulation is probably related to recent habitat loss due to anthropogenic impacts. In particular, the rapid installation of check dams in rivers of the Shiretoko Peninsula began approximately 60 years ago (Takahashi et al. 2005), and the inferred time in the demographic analysis is consistent with this period if the generation time of the species is assumed to be 2.5 years based on Goto (1981). However, there is no evidence to indicate that the habitat loss along the west coastal side was particularly severe than in the east, and it is also possible that the bottleneck was caused by reduced gene flow from unsampled populations in more central parts of the species' distribution. To understand the reasons why only the west subpopulation is declining, it is necessary to elucidate the relationship with the local environment or to sample more extensively. Since the purpose of this study is to identify patterns of genetic structure and diversity, understanding the details of its process is left for future work.

Implication for conservation genetics

While most applications of conservation genetics are limited to calculating current genetic diversity and differentiation, examining recent changes in genetic diversity may lead to different conclusions. With respect to the conservation of the local genetic diversity of *C. hangiongensis* in the Shiretoko Peninsula, we first found that the western and eastern populations are genetically distinct and should be considered different management units. We then investigated the genetic diversity of each subpopulation and found that the genetic diversity of the western populations was significantly higher than that of the eastern populations. However, the recent bottleneck was more pronounced in the west subpopulation. In terms of the level of genetic diversity alone, the conservation priority appears to be higher in the eastern region, but the lower genetic diversity in the eastern populations is probably a natural state in line with the central–marginal hypothesis. From the perspective of reducing human

impacts, the conservation priority would be rather higher in the west region which has experienced a recent bottleneck.

There are a lot of sculpin species in the world, some of which are diadromous species or have polymorphisms displaying diadromous life history (Goto et al. 2015). Even though diadromous populations have not been well studied, it should be recognized that there may be unknown evolutionarily significant units in regions where genetic studies have not yet been conducted. Additionally, this is probably the first study to actually show evidence of a bottleneck in the genetic data of diadromous *Cottus* species. We present that other populations that appear to have high genetic diversity may also have been potentially impacted in this era of global habitat loss for migratory fishes.

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Statements & Declarations

Competing Interests: The authors declare no competing interests.

Author Contributions: Y.A., S.N., and F.N. conceived and designed the study. Y.A. performed the field sampling. Y.A. and S.N. performed the laboratory work. S.N. analyzed the data and wrote the manuscript, with contributions from Y.A. and F.N. All authors read and approved the final manuscript.

Data Availability

Genotype data generated in this study is available at Figshare (doi: 10.6084/m9.figshare.17429417).

460 **Tables**

461 **Table 1** Sampling localities and genetic diversity of each population.

Pop ID	Name of river	Latitude (N°)	Longitude (E°)	N	AR	H_E	F_{IS}
Pop1	Osyamappu	43.987	144.882	35	2.410	0.463	-0.025
Pop2	Kanayama	43.992	144.886	17	2.392	0.425	0.151
Pop3	Opekepu	44.027	144.928	18	2.420	0.452	-0.124
Pop4	Rusa	44.139	145.265	20	1.748	0.287	0.139
Pop5	Matsunori	43.987	145.162	22	2.051	0.371	-0.126
Pop6	Tachikariusu	43.968	145.142	9	2.127	0.342	-0.051
Pop7	Chashibetsu	43.902	145.102	11	2.343	0.375	0.091
Pop8	Ponrikushibetsu	43.881	145.096	9	2.221	0.327	-0.067
Pop9	Rikusibetu	43.878	145.097	24	1.993	0.343	-0.015

462 N, number of individuals; AR, allelic richness; H_E, expected heterozygosity; F_{IS}, fixation index

463

464 **Table 2** Characteristics of the nine microsatellite loci used in this study.

Locus	Motif size	Allele size	Multiplex set	Fluorescent dye	References
Cgo18	2	242–250	B	6-FAM	Englbrecht et al. 1999
Cgo22	2	203–213	B	HEX	Englbrecht et al. 1999
Cgo33	2	164–168	B	6-FAM	Englbrecht et al. 1999
Cgo56	2	232–240	A	HEX	Englbrecht et al. 1999
Cgo1016[a]	2	-	B	PET	Englbrecht et al. 1999
Cgo1114	2	145–163	A	NED	Englbrecht et al. 1999
Cott112	2	166–170	A	HEX	Nolte et al. 2005
Cott138	2	270–274	A	6-FAM	Nolte et al. 2005
Cott207[a]	2	-	B	NED	Nolte et al. 2005

465 [a] Loci amplified but not included in the dataset

466

Table 3 Pairwise F_{ST} (below diagonal) and F'_{ST} (above diagonal) matrices among the nine populations of *C. hangiongensis*.

	West			East					
	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9
Pop1		0.035	0.008	0.439	0.310	0.340	0.262	0.308	0.298
Pop2	0.019		0.028	0.455	0.274	0.332	0.224	0.303	0.271
Pop3	0.004	0.016		0.369	0.221	0.259	0.159	0.191	0.197
Pop4	0.252**	0.284**	0.225**		0.163	0.107	0.141	0.094	0.077
Pop5	0.170**	0.162**	0.127**	0.104**		0.000	0.000	0.028	0.058
Pop6	0.181**	0.191**	0.145**	0.068	0.000		0.000	0.013	0.063
Pop7	0.136**	0.125**	0.086**	0.087*	0.000	0.000		0.000	0.019
Pop8	0.173**	0.186**	0.114*	0.063	0.018	0.008	0.000		0.000
Pop9	0.170**	0.167**	0.119**	0.051	0.036	0.039	0.012	0.000	

Asterisks in below diagonal denote significant differentiations; *$p < 0.05$ and **$p < 0.01$ after Bonferroni correction

Table 4 BOTTLENECK results under infinite alleles model (IAM), stepwise mutational model (SMM), and 2-phase models (TPM). *P*-values are shown.

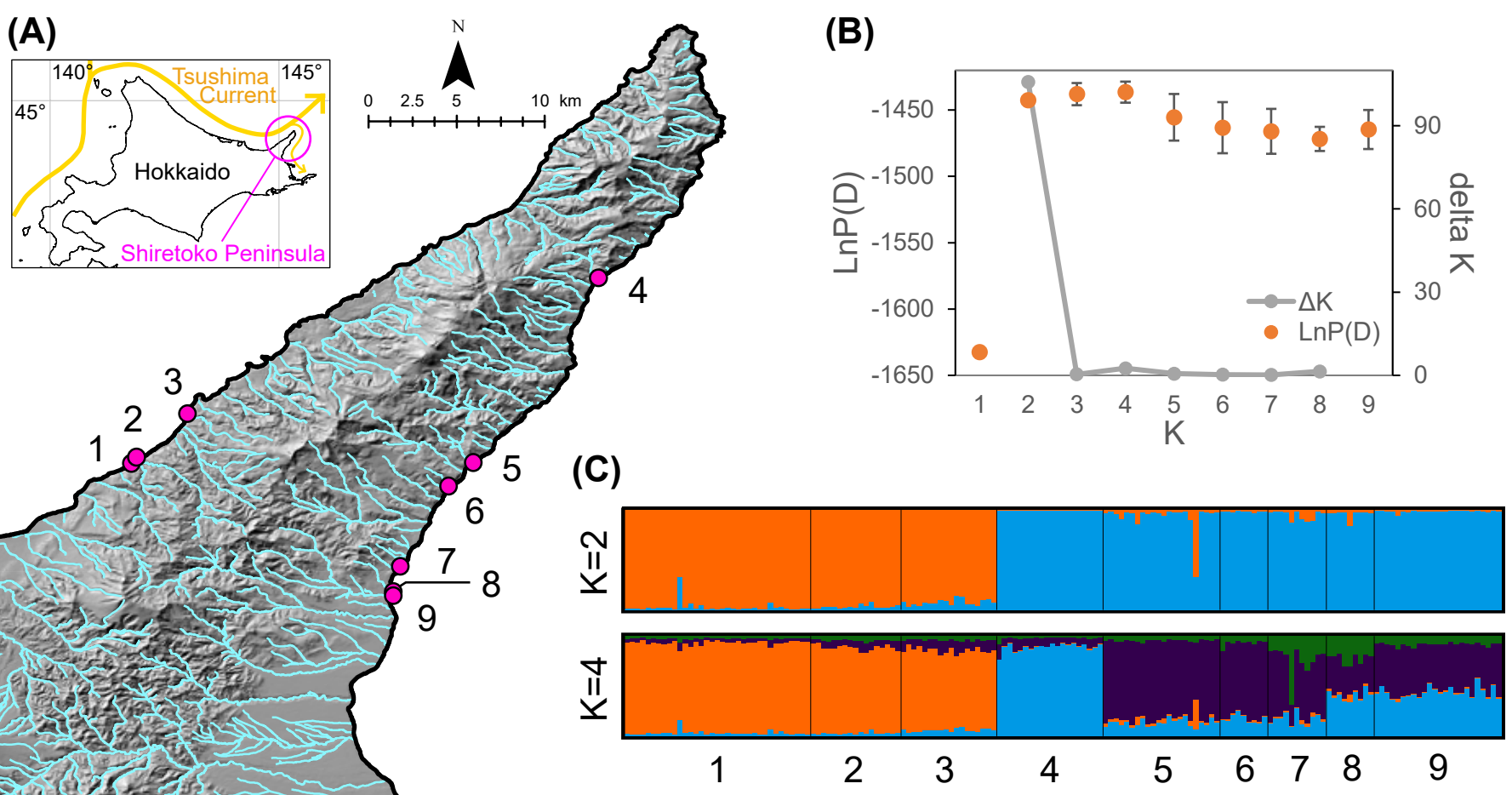
	Mutation model		
Subpopulation	IAM	TPM	SMM
West	<0.01	<0.01	<0.05
East	0.08	0.50	0.92

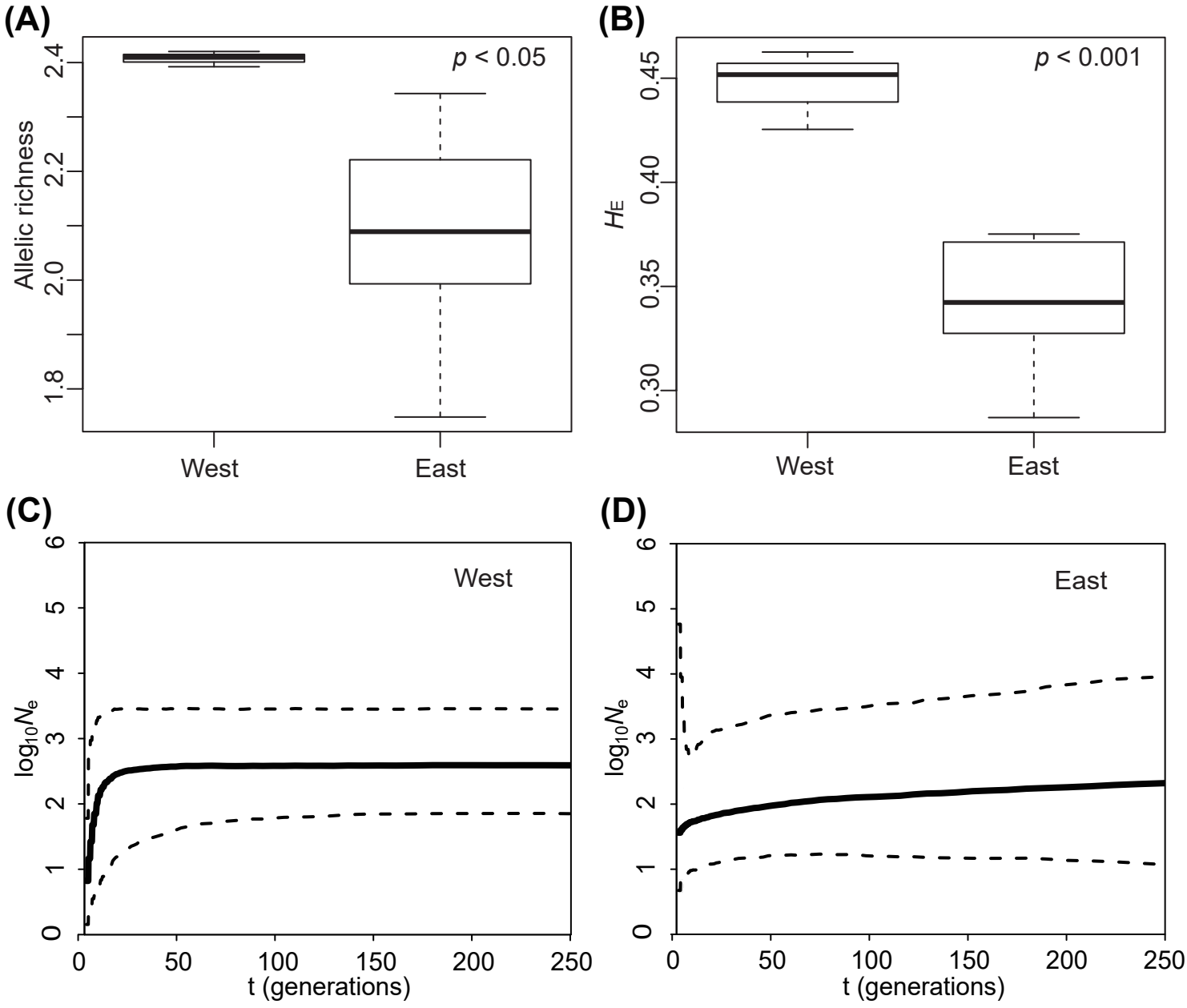
Figure legends

Fig. 1 Study area and genetic structure. (A) Sampling localities. Labels correspond to population IDs (Pop1, 2, …, 9) in Table 1. The broad-area map shows the location of the Shiretoko Peninsula and routes of the Tsushima Current, including its branches. (B) The values of posterior probability of the data (LnP(D)) from 20 runs for each value of K (1–9) and Evanno's delta K in the STRUCTURE analysis. (C) Population structure estimated in STRUCTURE. Barplots display the proportion of the membership coefficient in the inferred clusters at K = 2 and 4 for all individuals, and the numbers indicate the population IDs.

Fig. 2 Comparison of genetic diversity between west and east subpopulations. (A, B) Boxplots showing differences in the level of allelic richness (A) and expected heterozygosity (B) between the

487 western and eastern populations. (C, D) Approximate Bayesian skyline plots showing the demographic
488 history of *C. hangiongensis* for west (C) and east (D) subpopulations. The solid line shows the median
489 estimates of historical effective population size (N_e), and the dashed lines show the 95% highest
490 posterior density estimates of the N_e.
491


(A)
140°
145°
45°
Tsushima Current
Hokkaido
Shiretoko Peninsula
N
0 2.5 5 10 km
1
2
3
4
5
6
7
8
9
(B)
LnP(D)
-1450
-1500
-1550
-1600
-1650
delta K
90
60
30
0
ΔK
LnP(D)
1 2 3 4 5 6 7 8 9
K
(C)
K=2
K=4
1 2 3 4 5 6 7 8 9


(A)
Allelic richness
2.4
2.2
2.0
1.8
p < 0.05
West
East
(B)
H_E
0.45
0.40
0.35
0.30
p < 0.001
West
East
(C)
West
$\log_{10}N_e$
0
1
2
3
4
5
6
0
50
100
150
200
250
t (generations)
(D)
East
$\log_{10}N_e$
0
1
2
3
4
5
6
0
50
100
150
200
250
t (generations)